Cancer Chemopreventive Potential of Apples, Apple Juice, and Apple Components

Author **Clarissa Gerhauser**

Affiliation Division of Toxicology and Cancer Risk Factors, German Cancer Research Center (DKFZ), Heidelberg, Germany

Key words
- apples
- *Malus* sp.
- Rosaceae
- cancer chemoprevention
- oligomeric procyanidins
- polyphenols

Dedication
In memory of
Prof. B. L. Pool-Zobel

received May 25, 2008
revised July 17, 2008
accepted July 31, 2008

Bibliography
DOI 10.1055/s-0028-1088300
Planta Med 2008; 74: 1608–1624

Published online October 14, 2008
ISSN 0032-0943

Correspondence
Dr. Clarissa Gerhäuser
Deutsches Krebsforschungszentrum (DKFZ)
Toxikologie und Krebsrisikofaktoren
Im Neuenheimer Feld 280
9120 Heidelberg
Germany
Tel.: +49-6221-42-3306
Fax: +49-6221-42-3359
c.gerhauser@dkfz.de

Abstract

Apples (*Malus* sp., Rosaceae) are a rich source of nutrient as well as non-nutrient components and contain high levels of polyphenols and other phytochemicals. Main structural classes of apple constituents include hydroxycinnamic acids, dihydrochalcones, flavonols (quercetin glycosides), catechins and oligomeric procyanidins, as well as triterpenoids in apple peel and anthocyanins in red apples. Several lines of evidence suggest that apples and apple products possess a wide range of biological activities which may contribute to health beneficial effects against cardiovascular disease, asthma and pulmonary dysfunction, diabetes, obesity, and cancer (reviewed by Boyer and Liu, Nutr J 2004). The present review will summarize the current knowledge on potential cancer preventive effects of apples, apple juice and apple extracts (jointly designated as apple products). In brief, apple extracts and components, especially oligomeric procyanidins, have been shown to influence multiple mechanisms relevant for cancer prevention in *in vitro* studies. These include antimutagenic activity, modulation of carcinogen metabolism, antioxidant activity, anti-inflammatory mechanisms, modulation of signal transduction pathways, antiproliferative and apoptosis-inducing activity, as well as novel mechanisms on epigenetic events and innate immunity. Apple products have been shown to prevent skin, mammary and colon carcinogenesis in animal models. Epidemiological observations indicate that regular consumption of one or more apples a day may reduce the risk for lung and colon cancer.

Abbreviations

AC:	aberrant crypts
ACF:	aberrant crypt foci
AE02/-03/-04:	apple juice polyphenol extract
AIS:	`alcohol-insoluble substance'
AP-1:	activator protein 1
Apaf-1:	apoptotic protease activating factor 1
APE:	apple polyphenol extract
B(a)P:	benzo[*a*]pyrene
bw:	body weight
CI:	confidence interval
COBRA:	combined bisulfite restriction analysis
Cox-1:	cyclooxygenase-1
Cyp1A:	cytochrome P450 1A
DEP-1:	density-enhanced protein-tyrosine phosphatase-1
DMBA:	7,12-dimethylbenz[*a*]anthracene
DMH:	1,2-dimethylhydrazine
DNMT:	DNA methyltransferase
DPPH:	1,1-diphenyl-2-picrylhydrazyl
EGCG:	(–)-epigallocatechin 3-gallate
EGF:	epidermal growth factor
EGFR:	epidermal growth factor receptor
FCS:	fetal calf serum
Fr.P:	procyanidin-enriched fraction P
GJIC:	gap-junctional intracellular communication
GSK3β:	glycogen synthase kinase 3β
GST:	glutathione *S*-transferase
HDAC:	histone deacetylase
IL-2Rα:	interleukin-2 receptor α-chain
IQ:	2-amino-3-methylimidazo [4,5-*f*]quinoline
MAP-kinase:	mitogen-activated protein kinase
NSP:	non-starch polysaccharides
NHS:	Nurses Health Study
ODC:	ornithine decarboylase
OPC:	oligomeric procyanidins
OR:	odds ratio
ORAC:	oxygen radical absorbance capacity
PARP:	poly(ADP-ribose)polymerase
PBMC:	peripheral blood mononuclear cells

PGs:	prostaglandins
PTM:	permeability transition pore
PKC:	protein kinase C
ROS:	reactive oxygen species
RR:	relative risk
SCFA:	short-chain fatty acids
TNF-α:	tumor necrosis factor-α
TPA:	12-*O*-tetradecanolyphorbol 13-acetate
TRAIL:	TNF-related apoptosis-inducing ligand
TSG:	tumor suppressor gene

Introduction

During the past years, we have made tremendous progress in our understanding of the carcinogenic process at the cellular and molecular level. This has led to the development of a promising new approach to cancer prevention, termed "chemoprevention" [1], which aims to block, inhibit or reverse the development and progression of precancerous cells through use of non-cytotoxic nutrients and/or pharmacological agents [2]. Carcinogenesis is generally a slow process and often takes decades from tumor initiation to diagnosis, offering a considerable time frame for chemopreventive approaches. Accordingly, the validation and utilization of dietary components, natural products, or their synthetic analogs as potential cancer chemopreventive agents in the form of functional foods or nutraceuticals has become an important issue in current health- and cancer-related research.

Apples (*Malus* sp., Rosaceae) and apple juice are the most consumed fruit and fruit juice in Germany, with an average annual per capita consumption of 18.4 kg and 12.8 L, respectively [3], [4]. Several lines of evidence suggest that apples and apple products possess a wide range of biological activities which may contribute to health beneficial effects against cardiovascular disease, asthma and pulmonary dysfunction, diabetes, obesity and cancer [5]. This review will summarize the present knowledge on potential cancer preventive effects of apples, apple juice and apple extracts (jointly designated as apple products). After a short summary of nutrient and phytochemical composition, the manuscript gives an overview of results from bio-availability studies and *in vitro* investigations of apple polyphenols, triterpenoids and apple pectin on cancer preventive mechanisms. Studies with apple products in animal models of skin, mammary and colon carcinogenesis and in xenograft models for tumor growth and invasion are described, followed by a brief compilation of animal studies with apple-derived dietary fiber and apple pectin. Subsequently, short-term human intervention studies on the modulation of antioxidant parameters with apples and apple juice are summarized. Finally, the current evidence on links between apple consumption and human cancer occurrence based on epidemiological studies is presented.

Apples and Apple Juice: Nutrient and Phytochemical Composition

Apples and apple juice contain nutrient as well as non-nutrient components, including dietary fiber, minerals, and vitamins, as summarized in **Table 1**. With the exception of protein levels, fiber, and natural vitamin C contents, the average nutrient composition of apples and apple juice is quite similar [6]. Apples are a rich source of phenolic constituents, which are distributed in the peel, core and pulp [7], [8]. Content and composition of phenolic compounds vary strongly in dependence of the apple variety, area of cultivation, and time and year of harvest [9], [10], [11], [12]. The total polyphenol content of apples represents about 0.01 to 1 % of the fresh weight. Main structural classes include hydroxycinnamic acids, dihydrochalcones, flavonols (quercetin glycosides), catechins and oligomeric procyanidins, as well as anthocyanins in red apples (selected chemical structures in **Fig. 1**) [13], [14], [15], [16], [17], [18]. Cider apples are particularly rich in polyphenols [19], [20], [21]. Consequently, freshly squeezed apple juice from cider apples has highest levels of the four major classes of apple constituents, whereas commercially available clear juice (often made from concentrates) is very poor in polyphenols (summarized from [12] in **Table 2**). Apples and apple juice are good sources of oligomeric procyanidins (OPC) composed of (epi)catechin units [22], [23], [24], [25], [26], which have recently gained interest because of potential health promoting effects (review in [27]). In apples, 63 – 77 % of all polyphenols were attributed to OPC [13]. Similar to the differences in polyphenol levels in apple juices, polyphenolic extracts prepared by enrichment on adsorber resins from cloudy apple juice contained higher amounts of OPC (48 – 61 %) than extracts from clear juice (28 – 49 %) [28].

In addition to polyphenols, apple peel contains considerable amounts of lipophilic triterpenoids, which are concentrated in the cuticular wax layer. The most abundant triterpene, ursolic acid, was isolated in amounts up to 50 mg per medium size fruit [29]. A series of thirteen triterpenes with antiproliferative activity was isolated from apple peel by extraction with organic solvents [30].

Absorption, Bioavailability and Metabolism of Apple Juice Constituents

Apple components may influence multiple mechanisms contributing to cancer prevention as outlined below. However, to do so *in vivo* they must be absorbed and achieve effective concentrations at the target site in the correct metabolic form [31]. Several earlier reviews have summarized evidence on absorption, bioavailability and metabolism of polyphenolic compounds, including all major classes of apple and apple juice constituents [32], [33], [34]. Boyer and Liu summarized data related to bioavailability and metabolism of apple components, covering

Table 1 Average nutrient content in apples and apple juice (per 100 g fresh weight) [6]

	Apples	Apple juice
Water (g)	85.3	88.1
Energy (kcal/kJ)	54/227	48/203
Protein (g)	0.3	0.07
Fat (g)	0.6	n. a.
Carbohydrates (g)	11.4	11.1
Fibre (g)	2.0	0.77
• Pectin (g)	0.5	0.032
Potassium (mg)	144	116
Calcium (mg)	7.0	4.2
Magnesium (mg)	6.0	6.9
Phosphorus (mg)	12.0	7.0
Vitamin C (mg)	12.0	1.4
Organic fruit acids (g)	0.5	0.74

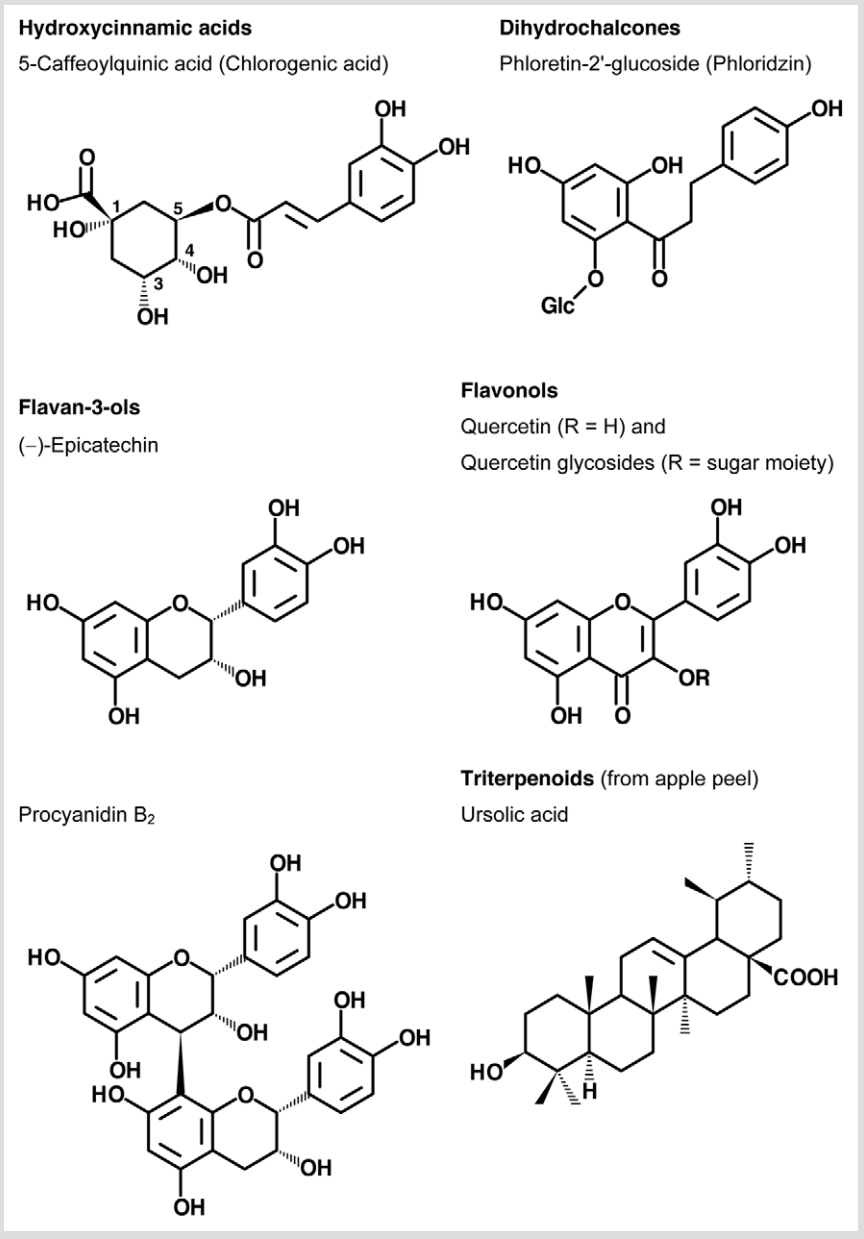

Fig. 1 Chemical structures of selected typical apple juice components belonging to the structural classes of hydroxycinnamic acids, dihydrochalcones, flavan-3-ols (catechins and procyanidins), flavonols (quercetin-glycosides) and triterpenoids.

Table 2 Polyphenol content of apples and apple juice (mg/kg fresh weight or mg/L) (adapted from [12], [13])

	Apples[a]	Fresh juice[b]		Commercial juice[b]	
	(n = 8)	from dessert apples (n = 4)	from cider apples (n = 7)	clear (n = 3)	cloudy (n = 21)
Total polyphenols	662 – 2119	154 – 178	261 – 970	110 – 173	152 – 459
• Hydroxycinnamic acids	45 – 384	57 – 68	134 – 593	69 – 122	74 – 259
• Dihydrochalcones	20 – 155	10 – 35	34 – 171	9 – 54	14 – 87
• Flavan-3-ols: Mono- and dimers	116 – 411	50 – 95	70 – 393	14 – 32	46 – 124
Oligomeric procyanidins	388 – 1622	n. d.	n. d.	n. d.	n. d.
• Flavonols (quercetin-glycosides)	34 – 83	0.4 – 4	0.4 – 27	4 – 7	2 – 14
• Anthocyanins (in red apples)	0 – 37	n. d.	n. d.	n. d.	n. d.

[a] From [13]. Values were multiplied × 10 to adjust for mg/kg.

[b] From [12].

n. d. not determined

studies performed until 2003 [5]. They concluded that flavonoid aglycones apparently pass through epithelial cells where they will be further conjugated. Flavonoid glycosides may be absorbed intact in low levels. Mostly, they will be absorbed after hy-

drolysis by small intestinal hydrolases such as β-glucosidases or lactase phloridzin hydrolase, and also conjugated [5], [35], [36]. According to a comprehensive review by Manach et al. on 97 bioavailability studies with polyphenols in humans [34], metabolites present in blood result from digestive and hepatic activity. Plasma concentrations of total metabolites range from 0 to 4 μM after an intake of 50 mg aglycone equivalents, with relative urinary excretion up to 43 % of the ingested dose, depending on the polyphenol. Overall, gallic acid and isoflavones (which are not present in apples) are the best absorbed polyphenols, followed by catechins and quercetin glucosides, but with different kinetics. The least well absorbed apple polyphenols are the procyanidins and anthocyanins (if present). It was concluded that data are still too limited for assessment of hydroxycinnamic acids and other polyphenols. Most of the studies were performed with purified compounds, and more research needs to address questions of bioavailability from whole foods such as apples, which includes effects of food matrix, processing, digestion, and interactions between different food components [5].

Kahle et al. performed an apple juice intervention study with ileostomy patients [37], [38]. Colonic degradation is minimal in these patients, therefore they represent an interesting study collective to investigate which portion of ingested polyphenols is absorbed and how much would reach the colon after ingestion. Eleven volunteers drank 1 L of cloudy apple juice after an overnight fast. Ileostomy bags were collected immediately before and 1 – 8 h after apple juice consumption. Most of the ingested polyphenols were absorbed from or metabolized in the small intestine. Maximum total recovery in the bags was after 2 h. Only 0 – 33 % of the consumed hydroxycinnamic acids, and 10 % (~11 mg) of chlorogenic acid in particular, were found in ileostomy bags. In an earlier study conducted by Olthof et al., in which a dose of 1000 mg purified chlorogenic acid (representative of chlorogenic acid intake in coffee drinkers) was applied to ileostomy patients, about 67 % were excreted in ileostomy bags within 24 h [39]. These differences may be due to the 10-fold higher absolute amount applied in this earlier study. The same group reported that in humans with intact colon, 50 % of ingested chlorogenic acid was extensively metabolized to hippuric acid (*N*-benzoylglycine) by colonic microorganisms [40]. Similar results were observed in rats [41].

In Kahle's ileostomy study with apple juice, recovery of quercetin glycosides in the ileostomy bags was extremely low [37], [38]. Only two of five derivatives present in the juice, i. e., quercetin 3-*O*-arabinoside and quercetin 3-*O*-rhamnoside, were detected in the bags, and recovery was only 6 % and 10 % of the ingested dose. This was in line with earlier studies by Walle et al. Their data from an ileostomy study suggested that quercetin mono- and diglucosides provided by an onion meal were efficiently hydrolyzed in the small intestine by β-glucosidases to quercetin which was then absorbed [42]. Interestingly, 23 % - 81 % of ^{14}C-labeled quercetin applied *p. o.* or *i. v.* to healthy volunteers was exhaled as $^{14}CO_2$, indicating a complex metabolism of quercetin in humans [43].

The metabolic fate of OPC is of particular interest due to high abundance in apple juice and other dietary sources [44], [45]. Based on *in vitro* incubations to mimic gastric passage, Spencer et al. suspected that cleavage of higher OPC to mixtures of monomers and dimers in the stomach may enhance their absorption in the small intestine [46]. However, Rios et al. demonstrated in a human intervention study that OPC from cocoa, which are chemically very similar to apple OPC, were stable during gastric passage [47]. This observation was confirmed by Kahle et al., who detected about 90 % of the ingested OPC in ileostomy bags with a maximum 2 h after consumption of 1 L cloudy apple juice [38]. Still, the mean degree of OPC polymerization was reduced from 5.7 (juice) to 3.4 within 2 h and further declined with time. Interestingly, polyphenols present in ileostomy bags still demonstrated antioxidant activity against peroxyl radicals and potently scavenged DPPH radicals after passage through the gastrointestinal tract [48]. *In vitro* incubations with human colonic microflora suggest that OPC will be catabolized into low molecular weight phenolic acids when they reach the colon [49]. Biological properties of these metabolites should therefore be considered.

Overall, these studies suggest that low molecular weight constituents in apple juice are likely to be absorbed and metabolized. OPC are stable in the stomach and will reach the colon, were they may exert a local effect before they are degraded by the microflora.

Bioactivities Indicative of Cancer Chemopreventive Potential: *In Vitro* Investigations

Carcinogenesis evolves through a network of events with multiple pathways which virtually all can be modulated exogenously [50]. Cancer preventive strategies include mechanisms to trap and remove carcinogens from the organism, thus minimizing their contact with DNA and reducing their genotoxic and promutagenic action. Another approach is to prevent the activation of procarcinogens to ultimate carcinogens and to enhance their detoxification through modulation of xenobiotic metabolism (anti-initiating mechanisms). Further, chemopreventive agents may target cellular alterations associated with tumor promotion and progression by antioxidant and anti-inflammatory activity, inhibition of signaling pathways which result in enhanced cell proliferation, cell growth inhibitory mechanisms, and induction of programmed cell death. Recent research also indicates epigenetic mechanisms and modulation of immune functions as novel targets of chemoprevention. Apple components have been shown to influence all of these mechanisms as summarized in **Fig. 2**.

Antimutagenic potential

Some dietary fibers can act as scavengers of exogenous and endogenous mutagens [51]. Apple pectin and pectin from other sources were effective against the direct-acting standard mutagen 1-nitropyrene, which represents the predominant nitrated polyaromatic hydrocarbon emitted in diesel exhaust [52]. In the classical Ames test, preincubation of *Salmonella* strains with apple pectin before addition of the mutagen dose-dependently decreased the mutagenic activity. Pectins are characterized by a rhamnogalacturonan backbone structure with neutral side chains. Removal of the side chains by hydrolysis removed the antimutagenic potential, indicating that side chains are a prerequisite for antimutagenic polysaccharides. Polymers with acidic side chains were equally effective, whereas linear neutral polymers or highly branched compounds were inactive [52].

Besides direct scavenging of mutagens, dietary fibers are supposed to act as antimutagens in the intestinal tract by increasing fecal mass through their water-binding capacity, therefore diluting mutagen concentrations and increasing fecal transit time. Ferguson et al. and Kestell et al. compared antimutagenic prop-

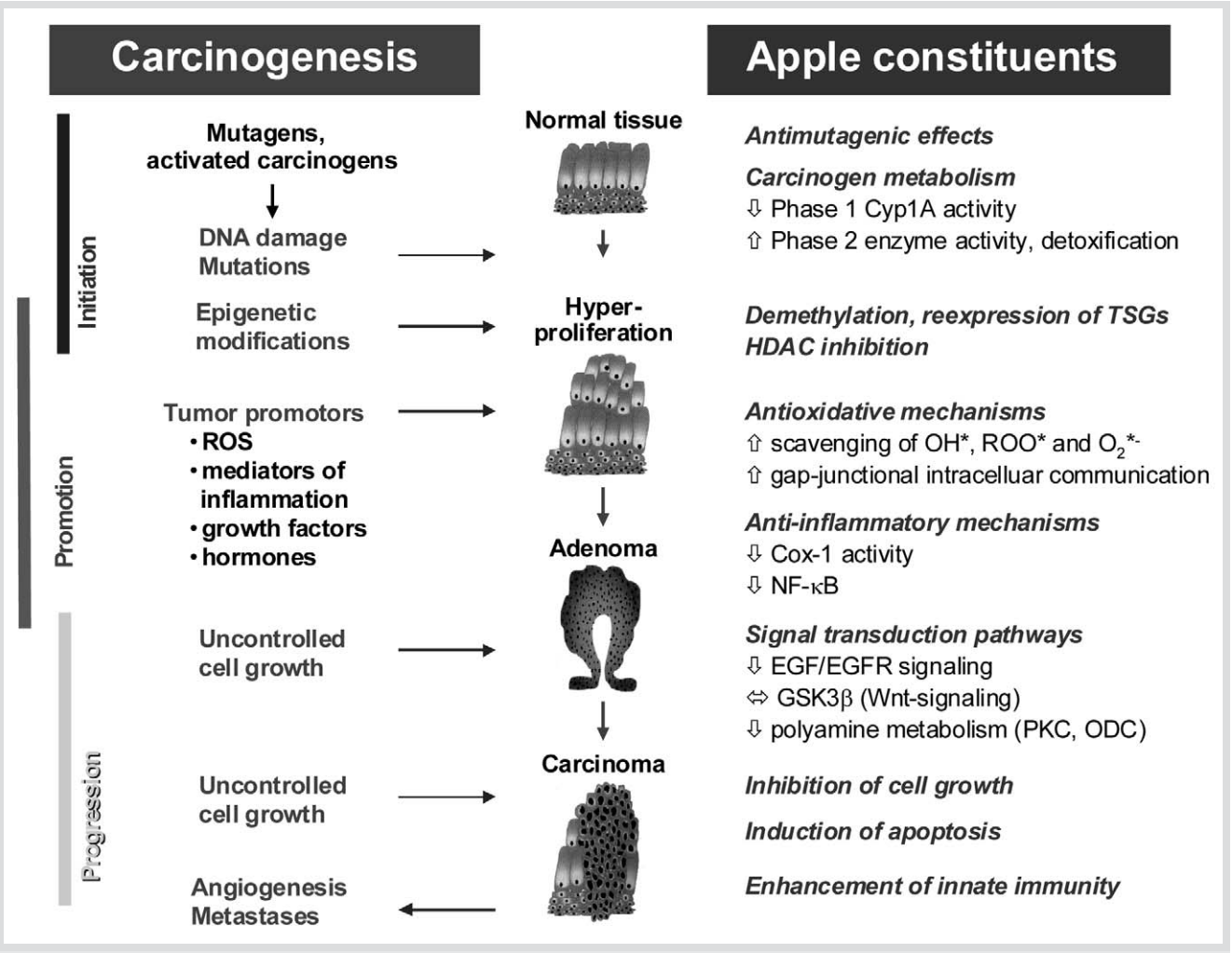

Fig. 2 Schematic presentation of colon carcinogenesis (left) and mechanisms relevant for the cancer preventive potential of apple constituents (right). $OH^{\bullet}$, hydroxyl radicals; $ROO^{\bullet}$, peroxyl radicals; $O_2^{\bullet -}$, superoxide anion radicals; TSG, tumor supressor gene; HDAC, histone deacetylase.

erties of resistant starch *vs.* non-starch polysaccharides (NSP) against the food-derived heterocyclic aromatic amine IQ (2-amino-3-methylimidazo[4,5-*f*]quinoline). Apple pectin (10% in the diet) was tested as an example of soluble dietary fiber of the NSP group. Both types of fiber significantly enhanced fecal bulk and transit times. However, whereas resistant starch significantly *elevated* IQ carcinogen bioavailability, non-starch polysaccharides including apple pectin *reduced* IQ bioavailability and *enhanced* fecal excretion, thereby reducing the risk of mutagenicity. These differences were associated with distinct effects on the expression of enzymes involved in the metabolism of IQ [53], [54].

Modulation of phase 1 and phase 2 carcinogen metabolism

Enzymes of the phase 1 of drug metabolism (cytochromes P450) activate xenobiotics by addition of functional groups which render these compounds more water-soluble. Phase 1 functionalization may be required to efficiently detoxify carcinogens. However, carcinogens similar to benzo[α]pyrene [B(a)P], a planar polycyclic aromatic hydrocarbon, are capable of inducing the activity of phase 1 enzymes such as cytochrome P450 1A (Cyp1A). This may further increase the risk to produce ultimate carcinogens capable of reacting with DNA and thus initiating carcinogenesis [55]. Phase 2 enzymes such as glutathione *S*-transferases (GST) and sulfotransferases conjugate activated phase 1 metabolites and xenobiotics to endogenous ligands like glutathione, glucuronic, acetic, or sulfuric acid and enhance excretion and detoxification in form of these conjugates. Reduction of elevated phase 1 enzyme activities to physiological levels and enhancing excretion of carcinogens *via* upregulation of phase 2 enzymes is considered a logical strategy in chemoprevention.

Pohl et al. investigated the effect of apple juice extracts on Cyp1A expression and activity in the Caco-2 colon cancer cell line and demonstrated a strong reduction at the mRNA, protein and activity level [56]. Zessner et al. identified the flavonoid quercetin as the most potent inhibitor of Cyp1A activity from apple juice extracts, with inhibitory potential in the nM range. The aglycone was orders of magnitude more potent than its glycosides, but overall, these were still more potent than hydroxycinnamates and dihydrochalcones ([57], [58] and unpublished results). Quercetin or 4-week intervention with quercetin-rich fruit juice reduced DNA adduct formation with B(a)P-diol epoxide, an active metabolite of B(a)P formed *via* Cyp1A activity, in human lymphocytes *in vitro* and *ex vivo* [59], [60]. Besides quercetin, fractions of apple juice extracts containing OPC demonstrated

potent Cyp1A-inhibitory activity when enzyme activity was tested in a cell-free assay with crude cell homogenates as enzyme source [58]. This may however be due to unspecific protein binding effects.

Veeriah et al. focused on the induction of phase 2 enzymes by apple juice constituents. Using a microarray-based approach, treatment of the HT29 adenocarcinoma cell line with a mixture of apple juice polyphenols for 24 h resulted in 1.6- to 2.1-fold induction of mRNA levels of GSTP1, GSTT2 and MGST2, as well as of sulfotransferases CHST5, CHST6, and CHST7. At the same time, mRNA expression of epoxide hydrolase, which contributes to the metabolic activation of B(a)P and other carcinogens, was significantly reduced by 50% [61]. Similarly, treatment of the preneoplastic colon adenoma cell line LT97 with an apple juice extract induced mRNA expression (measured by microarray analyses and confirmed by quantitative RT-PCR) of selected phase 2 enzymes (GSTP1, GSTT2, GSTA4, UGT1A1, UGT2B7) and total enzyme activities, indicating potential protection of the cells against toxicological insults [62]. Using induction of NAD(P)H: quinone oxidoreductase in Hepa1c1c7 mouse hepatoma cells as a simple colorimetric test system to detect inducers of Phase 2 enzymes, we recently identified apple aroma compounds as novel inducers of phase 2 enzymes in polyphenolic apple juice extracts ([58] and manuscript in preparation].

Antioxidant activities

Manifestation of oxidative stress by infections, immune diseases and chronic inflammation has been associated with carcinogenesis [63]. Overproduction of reactive oxygen species (ROS) may lead to the formation of highly reactive oxidation products, activation of carcinogens, formation of oxidized DNA bases and DNA strand breaks. These then cause mistakes during DNA replication and genetic alterations, increased transformation frequencies, induced transcription of redox-regulated proteins and ultimately result in enhanced cell proliferation and tumor promotion/progression [63].

Eberhardt et al. demonstrated that radical scavenging activity of fresh apples was mainly attributed to the phytochemical content rather than to that of vitamin C [64]. Interestingly, several groups reported that highest antioxidant activity was associated with apple peel rather than pulp, and identified quercetin-glycosides as active principle, which are mainly found in the skin [65], [66], [67], [68], [69]. A comprehensive comparison of radical scavenging activity of apple extracts, fractions and subfractions with their phytochemical composition revealed that all major classes of apple phytochemicals contribute to antioxidant activity against peroxyl radicals measured in the ORAC assay, whereas DPPH (1,1-diphenyl-2-picrylhydrazyl) and superoxide anion radicals were potently scavenged by more lipophilic fractions containing quercetin-glycosides and OPC [58]. Several other studies demonstrated potent *in vitro* peroxyl radical scavenging potential and consequent inhibition of lipid peroxidation. Apple extracts and individual polyphenols inhibited copper-induced oxidation of human low density lipoproteins, lipid peroxidation induced in rat liver microsomes by ascorbic acid and $FeSO_4$, and peroxidation of linoleic acid in a micellar system [18], [70], [71]. In cell culture, apple juice extracts and polyphenols from apple pomace also reduced oxidative DNA damage induced by menadione or H_2O_2 treatment in colon cancer cell lines, and reduced *tert*-butylhydroperoxide-induced intracellular ROS measured by 2′,7′-dichlorofluorescin oxidation [72], [73]. Apple polyphenol extracts protected from H_2O_2-induced cytotoxicity in Caco-2 colon cancer cells [74], and prevented Cr(VI)-induced lipid peroxidation, DNA damage and NF-κB activation in human lung epithelial A549 cells [75].

It has been suggested that H_2O_2 and other non-genotoxic factors may contribute to tumor promotion by inhibition of gap-junctional intracellular communication (GJIC) [76]. Lee et al. reported that apple extracts significantly prevented H_2O_2-mediated inhibition of GJIC measured with WB-F344 rat liver epithelial cells by a scrape loading/dye transfer technique [77]. Treatment with 500 μM H_2O_2 reduced the number of communicating cells by about 90%. Simultaneous treatment with increasing concentrations of apple extract equivalent to 15 – 25 mg/mL fresh apples increased cell-cell communication to control levels. Similar effects were obtained with apple-derived flavonols (quercetin), flavan-3-ols [(–)-epicatechin, procyanidin B_2], and vitamin C, whereas chlorogenic acid and phloretin were inactive.

Anti-inflammatory mechanisms

Prostaglandins (PGs) are hormone-like endogenous mediators of inflammation and are formed from arachidonic acid by cyclooxygenase-1 (Cox-1) and the inducible form Cox-2, which is often elevated in tumor tissue. Excessive production of PGs is thought to be a causative factor of cellular injury and may ultimately lead to carcinogenesis by inhibition of apoptosis (programmed cell death) as well as stimulation of cell proliferation, formation of new blood vessels (angiogenesis) and tumor invasiveness [78].

For activity-guided fractionation of apple juice extracts we used sheep seminal vesicle microsomes as a source of Cox-1 to detect anti-inflammatory activity. Inhibition of Cox-1 activity was highest with all fractions containing (–)-epicatechin, which dose-dependently inhibited Cox-1 activity by 50% at a concentration of 2.2 μg/mL (7.5 μM). OPC may also contribute to the anti-inflammatory potential [58].

The transcription factor NF-κB plays an important role in the induction of proinflammatory enzymes including Cox-2 and the inducible nitric oxide synthase. NF-κB is inducible by the proinflammatory cytokine tumor necrosis factor-α (TNF-α), bacterial lipopolysaccharides, tumor promoter 12-*O*-tetradecanolyphorbol 13-acetate (TPA) and other factors [79]. In a study by Davis et al., NF-κB was induced in human umbilical vein endothelial cells transfected with a NF-κB-driven reporter construct. Pretreatment with apple polyphenol extracts for 24 h significantly reduced TNF-α-mediated expression of the reporter gene [80]. Similarly, reduction of nuclear NF-κB was observed when MCF-7 cells were pretreated with apple extracts for 2 h and stimulated with TNF-α for 30 min [81]. Inhibition of proteasomal activity was identified as the underlying mechanisms, which is important to release NF-κB from a complex with its inhibitor IκB in the cytosol.

Inhibition of signaling pathways

Uncontrolled cell proliferation often involves disorganization of signaling pathways. Binding of growth factors (e.g., epidermal growth factor EGF) to their receptors located in the cell membrane (e.g., epidermal growth factor receptor EGFR) stimulates signal transduction cascades. Growth-stimulating signals are transduced to the nucleus *via* phosphorylation/activation steps mediated by protein kinase cascades (e.g., Ras/Raf/MAP kinase cascade), resulting in activation or repression of gene transcription and consequently up- or down-regulated protein expression. Consequently, overexpression of hormone/growth factors and their receptors might present a growth advantage to preneoplastic cells [82], [83], [84].

Kern et al. [85] and Friedrich et al. [86] investigated the potential of apple juice extracts and apple juice polyphenols to influence EGF signaling. Apple juice extract effectively inhibited protein tyrosine kinase activity of EGFR and suppressed EGFR autophosphorylation and the subsequent MAP kinase cascade. Procyanidin dimers B_1 and B_2 as well as two quercetin glycosides possessed substantial EGFR-inhibitory properties [85], [86]. Apple juice polyphenols also blocked the signaling cascade leading to the induction of ornithine decarboxylase (ODC), which is essential for cellular proliferation by formation of polyamines, but is often overexpressed in tumor cells [87]. An OPC-rich fraction P (Fr. P) potently inhibited protein kinase C (PKC) activity by 70% in human colon cancer-derived SW620 cells. This was associated with downregulation of polyamine biosynthesis and activation of apoptosis [87]. Inhibition of cytosolic PKC activity in a cell-free system, but not in intact HT29 cells was also shown by Kern et al. [88].

Kern et al. also investigated the effect of apple juice extracts on key elements of the Wnt signaling pathway, which is often activated in colon carcinogenesis through mutation of the tumor suppressor gene Apc (adenomatous polyposis coli). Apc protein forms a complex with GSK3β (glycogen synthase kinase 3β) and other factors and regulates levels of β-catenin, which otherwise accumulates, translocates to the nucleus and activates transcription of growth promoting proteins. Mutation of Apc or inhibition of GSK3β by Wnt signaling disrupts this regulatory process [82]. Apple juice polyphenols inhibited GSK3β kinase activity in a cell-free system as well as in intact HT29 cells, but induced GSK3β protein expression measured by Western blotting. Overall, the extract did not influence downstream signaling parameters regulated by the Wnt signaling pathway [89].

Inhibition of cell proliferation by native and fermented apple juice extracts

Multiple studies have demonstrated cell growth inhibitory potential of apple juice components in cultured cancer cell lines. These results should be interpreted with some caution. Similar to green tea polyphenols [90], [91], apple polyphenols have been shown to artefactually induce formation of hydrogen peroxide in cell culture medium which could account for some or all of the reported effects in cell culture. H_2O_2 formation by apple phenolics was first described by Lapidot et al. in 2002 [92]. The effect was particularly high in serum-free incubations, since serum was able to decompose H_2O_2 due to residual enzyme activity [92]. Fridrich et al. confirmed H_2O_2 production by apple juice extracts in cell culture media [86]. Janzowski et al. recently revealed that H_2O_2 formation only occurred in bicarbonate-buffered solutions (C. Janzowski, personal communication). This observation may explain why H_2O_2 was not detected in a study on polyphenolic apple extracts by Liu and Sun, who added 10 mM HEPES to cell culture media [93]. Overall, these findings should be taken into consideration when polyphenol extracts from apples or other sources are investigated in cell culture.

Veeriah et al. compared antiproliferative activity of three apple extracts in HT29 and the adenoma cell line LT97 [61], [94]. Extracts were prepared either from apple juice (AE02 and AE04) or apple pomace after enzyme treatment to release cell wall bound compounds (AE03), respectively, and had distinct polyphenol profiles (compare [28], AE02 = AS02, AE03 = AS03B and AE04 = AS04). The pomace extract AE03 contained only 1/8 of the hydroxycinnamic acids and less total polyphenols, but about 10-fold higher flavonol levels than the two juice extracts. Overall, the adenoma cell line was more sensitive to the antiproliferative action of the apple extracts than the HT29 cell line. The growth inhibitory potential increased with incubation time with both cell lines. As anticipated by the higher content in flavonols, AE03 was more growth inhibitory than AE02 and AE04 [94]. When a native extract was compared with a composed mixture of low molecular weight apple polyphenols (including flavan-3-ol mono- and dimers, but no OPC), the native extract was about twice as potent as the mixture in inhibiting HT29 cell growth. This indicated that OPC contribute to a substantial part to the antiproliferative activity of apple extracts [61].

Anaerobic fermentation of the three apple extracts with human fecal slurries for 24 h resulted in the generation of mM concentration of short-chain fatty acids (SCFA) (also compare [95]). SCFA production from AE02 was in the order of acetate (max. 29 mM) > propionate (max. 6 mM) > butyrate (max. 5 mM). Although SCFA and particularly butyrate have been associated with inhibition of cell proliferation by induction of cell differentiation and apoptosis [96], the antiproliferative potential of all three fermented extracts was considerably reduced in both cell lines after 24 – 48 h of incubation, and fermentation led to an almost complete degradation of apple polyphenols. Low amounts of two metabolites, phloroglucinol and 3,4-dihydroxyphenylpropionic acid, were detected [94].

Butyrate-mediated effects on cell proliferation have been associated with histone hyperacetylation due to inhibition of histone deacetylase (HDAC) [97]. Waldecker et al. compared the potential of apple juice extracts AJE03B and AJE04 (= AE03 and AE04), fermented in the presence or absence of apple pectin, or pectin alone, to inhibit proliferation and nuclear HDAC activity in HeLa Mad 38, HT29 and Caco-2 cells [95]. HeLa Mad 38 cells are stably transfected with an HDAC inhibition-inducible reporter construct. Significant induction of reporter gene activity was observed with fermentation supernatants of all samples. Fermentation supernatants of the extract + pectin combinations were most active, although the butyrate content was lower than in the fermented pectin sample. This indicated that additional HDAC inhibitors may have been formed during fermentation of the apple juice extracts. Similar conclusions were drawn when HDAC activity was directly measured with nuclear extracts of all three cell lines treated with increasing concentrations of fermentation supernatants [95].

Induction of programmed cell death (apoptosis)

Programmed cell death or apoptosis is a physiological process involved in the maintenance of multi-cellular organisms and plays an important role in development, metamorphosis, hormonal atrophy and chemical-induced cell death [98]. Generally, apoptosis can be induced by two major pathways: the extrinsic, death receptor-mediated pathway and the intrinsic, mitochondrial-mediated activation [99]. Stimulation of the death receptor pathway leads to receptor aggregation, which then initiates recruitment and activation of initiator caspase-8. Caspase-8 activation subsequently triggers apoptosis by cleavage of downstream effector caspases. The mitochondrial pathway of cell death is mediated by Bcl-2 family proteins, which are a group of anti- (e.g., Bcl-2, Bcl-x_L) and proapoptotic proteins (e.g., Bax, Bak). Bcl-2 family proteins regulate the passage of small molecules like cytochrome c through the mitochondrial permeability transition pore (PTM). Release of cytochrome c then activates Apaf-1 (apoptotic protease activating factor 1), allowing it to assemble the multiprotein caspase-activating complex `apopto-

some' and to bind to and activate procaspase-9 and the downstream effector caspase cascade. Caspases are proteolytic enzymes that are synthesized as enzymatically inert zymogens and act in a self-amplification cascade. Initiator caspases-8 and -9 are characterized by longer pro-domains that mediate transduction of death signals and assembly of activating complexes. The major effector caspases-3, -6 and -7 execute apoptosis by cleavage of key cellular proteins that cause the typical morphological changes observed in cells undergoing apoptosis. Cleavage of the DNA repair-associated enzyme poly(ADP-ribose)polymerase (PARP) is accepted as a major marker of apoptosis induction. The 26S proteasome system is a large protease complex that also plays an important role in the regulation of cell growth and cell death [100]. The proteasome controls the turnover of a variety of intracellular regulatory proteins involved in cell cycle and apoptosis. Short-term exposure to proteasome inhibitors protects cells from toxic stimuli; long-term exposure however is toxic to nearly all cells and is associated with induction of apoptosis. Based on earlier results with (–)-epigallocatechin 3-gallate (EGCG) from green tea (review in [101]), Chen et al. investigated the influence of apples and other fruit and vegetables on the 26S proteasome as a mechanism to inhibit cell proliferation [102]. In a cell-free system, an apple "extract" consisting of freshly prepared, centrifuged, and sterile filtered apple juice, inhibited chymotrypsin-like activity of the proteasome when added at 1 – 10% (v/v) concentrations to the incubation mixture. Grape "extract" was about equally active, whereas green tea was more potent with up to 96% inhibition at the same test concentrations. In a cellular system with intact leukemic Jurkat T cells, 5% green tea, apple or grape "extract" all resulted in the accumulation of ubiquitinated proteins (as a sign of intracellular proteasome inhibition) concomitant with activation of effector caspase-3/-7 and induction of PARP cleavage. These results indicate that inhibition of chymotrypsin-like activity of the proteasome can be regarded as an interesting novel mechanism of apple and grape "extract" contributing to apoptosis induction [102].

Kern et al. analyzed the potential of apple juice polyphenol extract AE02 (see *Inhibition of cell proliferation*) to induce apoptosis in HT29 cells [88]. The AE02 extract potently induced caspase-3 activity and DNA fragmentation measured by an ELISA assay, although at relatively high concentrations. Since these experiments were performed under serum-free conditions without addition of catalase, formation of H_2O_2 may be responsible for part of these results. Apoptosis induction by AE02 was also detected by PARP cleavage under normal cell culture conditions in the presence of 10% fetal calf serum (FCS). PAPR cleavage was dose-dependent and increased up to 72 h [88]. Quercetin and phloretin dose-dependently induced both caspase-3 activity and DNA cleavage under serum-free conditions, whereas phloridzin (phloretin-2'-glucoside), which is present in apples and apple juice/extract (**Fig. 1**), was basically inactive. The apoptosis inducing potential of the aglycone phloretin in HT29 cells was further investigated by Park et al. [103]. Under serum-deprived conditions (1% FCS), phloretin at 100 μM induced both the death-receptor as well as the mitochondrial pathway of apoptosis induction, detected by activation of the initiator caspases-8 and -9 and the effector caspases-3 and -7 as well as by PARP cleavage. Activation of caspase-9 was accompanied by release of cytochrome c and the mitochondrial protein Smac/Diablo from the mitochondria to the cytoplasm, and upregulation of proapoptotic Bax levels [103].

Several investigators analyzed the cell growth inhibitory potential of apple-derived OPC in various cancer cell lines. Raul and colleagues utilized the human cell line SW620 derived from a lymph node metastasis of a colon adenocarcinoma patient to identify links between polyamine metabolism and inhibition of cell proliferation by OPC. Fraction P (see *Inhibition of signaling pathways*) at 50 μg/mL accumulated SW620 cells in G_2/M phase of the cell cycle after 24 and 48 h of incubation, increased the sub-G1 fraction indicative of apoptosis induction after 72 h, and activated caspase-3 activity [87]. Modulation of enzymes of the polyamine pathway led to an accumulation of N^1-acetyl-polyamines. Cotreatment with an inhibitor of polyamine oxidase, which metabolizes N^1-acetyl-polyamines to polyamines, sensitized cells to Fr. P-induced cell growth inhibition [104]. The authors observed a significant reduction in total cellular polyamine pool, up-regulation of TRAIL (TNF-related apoptosis-inducing ligand)-responsive death receptors DR4/DR5, and inhibition of nuclear HDAC activity [105]. This was of interest as SW620 cells are usually TRAIL resistant. In contrast, apoptosis induction by Fr. P alone involved depolarization of the mitochondrial membrane potential and induction of the intrinsic mitochondrial-mediated apoptosis pathway [105]. Similarly, Miura et al. reported an increase in mitochondrial membrane permeability, release of cytochrome c, and activation of caspase-9 and -3 by apple procyanidins in B16 mouse melanoma cells and BALB-MC.E12 mouse mammary tumor cells [106]. Procyanidin di- and trimers induced DNA laddering as a sign of apoptosis in KATO III human stomach cancer cells, although at extremely high concentrations of up to 5 mg/mL [107]. Since DNA fragmentation was caspase-independent and prevented by co-treatment with the antioxidant *N*-acetylcysteine, these results may be caused by artifactual H_2O_2 formation as outlined above.

He and Liu reported that in addition to polyphenols [64], triterpenoids isolated from apple peel inhibited proliferation of HepG2 human hepatoma cells, MCF-7 human breast cancer cells, and Caco-2 human colon cancer cells and may contribute to the anticancer activity of apples [30]. Overall, 2α-hydroxyursolic acid had the highest anti-proliferative activity and was more active than the most abundant ursolic acid (**Fig. 1**). Of note, ursolic acid and other pentacyclic triterpene acids have been associated with cancer preventive mechanisms at all stages of tumorigenesis and may have antimetastatic potential by inhibition of angiogenesis and tumor invasion [108].

Recent novel mechanisms of apple polyphenols

Density-enhanced protein-tyrosine phosphatase-1 (DEP-1) has been recognized as a candidate tumor suppressor protein in colon epithelium and reduces cell proliferation and cell migration [109]. Apple juice extract induced DEP-1 mRNA and protein expression in LT97 colon adenoma cells, whereas protein expression was reduced in HT29 and Caco-2 cells. In contrast, butyrate and green tea extract stimulated DEP-1 expression in all three cell lines. It was postulated that DEP-1-mediated regulation of cell proliferation might represent a hitherto unrecognized mechanism of cell growth inhibition by dietary nutrients [109].

Epigenetic events such as the methylation of CpG rich sequences in gene promoter regions increase with increasing stage of malignancy in various human cancers, and often result in silencing of tumor suppressor genes [110], [111]. Promoter hypermethylation has been identified as a very early event in carcinogenesis. Consequently, development of agents or food components that prevent or reverse the hypermethylation-induced inactivation

of tumor suppressor genes is an attractive approach for cancer prevention. EGCG from green tea and genistein from soybean have been demonstrated to inhibit DNA methyltransferases DNMT *in vitro*. This was associated with the reactivation of methylation-silenced genes such as $p16^{INK4a}$[112]. Fini et al. recently discovered that treatment of the human colon cancer cell line RKO with an apple polyphenol extract resulted in demethylation of the DNA repair gene hMLH1, analyzed by methylation specific PCR. This was confirmed at the mRNA and protein level. Similarly, reversal of methylation in promoter regions of the tumor supresssor genes $p14^{ARF}$ and $p16^{INK4a}$ was demonstrated by COBRA (combined bisulfite restriction analysis). Re-expression of mRNA of both genes was detected in RKO and SW48 cells. Post-translational inhibition of expression of the two main DNA methyltransferases, DNMT-1 and DNMT-3b, was postulated as an underlying mechanism for the inhibitory effect of the apple polyphenol extract [113].

$\gamma\delta$T cells are innate immune cells that participate in host responses against many pathogens and cancers. Recent evidence has accumulated that dietary factors may strengthen the immune system by activation of these cells, and that this may contribute to cancer preventive potential [114]. Human $\gamma\delta$T cells can be categorized into two distinct populations, Vδ1 and Vδ2 T cells, based on the expression of cell surface receptors. Vδ2 T cells constitute the majority of $\gamma\delta$T cells in peripheral blood and the lymphatic system and are potent antimicrobial and antitumor effector cells, whereas Vδ1 T cells are located preferentially in skin epithelium and in the intestine and have immune regulatory functions [115]. Holderness et al. screened a library of >100.000 natural products including nutritional supplements to identify novel agonist of $\gamma\delta$T cells, based on up-regulation of IL-2Rα (interleukin-2 receptor α-chain) as a marker of $\gamma\delta$T cell activation. Treatment of bovine and human peripheral blood mononuclear cells (PBMC) with a water-soluble extract from peels of unripe apples at a concentration of 10 μg/mL induced IL-2Rα immune-positivity. The activity was linked to a polymeric fraction of condensed tannins (= oligo- and polymeric proanthocyanidins). In human PBMC, both Vδ1 and Vδ2 T cells were equally activated by the tannin fraction, in addition to non-$\gamma\delta$T cells including natural killer cells, natural killer T-cells and $\alpha\beta$T cells, but not B cells. Interestingly, immune-modulatory response was not limited to lymphocytes *in vitro*. Akiama et al. demonstrated that mucosal $\gamma\delta$T cells in the small intestine of mice expanded in response to polyphenols from unripe apples. In this study, the results were discussed in relation to prevention of food allergies [116]. (Over)-activation of immune cells may not always improve health. As an example, over-activated $\gamma\delta$T cells have been associated with inflammatory or celiac bowel disease [114]. In line with these observations, it was shown recently that the apple tannin fraction increased expression of the cell surface receptor CD11b, which is involved in leukocyte adhesion and migration [117]. This may be related with a *pro*-inflammatory rather than an anti-inflammatory response to the extract. Overall, the physiological consequences of these novel observations need further investigation.

Apples and Cancer Prevention: *In Vivo* Investigations in Animal Models

In vitro studies on molecular mechanisms will provide a hint to potential cancer preventive effects *in vivo*. Chemopreventive efficacy can only be demonstrated in animal models or human intervention studies with tumor incidence and multiplicity (e.g., number of tumors per animal) as endpoints. When considering application of a compound or product for prevention of cancer in humans, toxicological and safety issues also have to be considered.

One study has addressed toxicology and safety of a polyphenol-rich extract from unripe apples (Applephenon®) which is sold in Japan as a food additive and nutritional supplement. The product contains high levels of OPC (64%, dimers to 15-mers), 12% flavan-3-ol monomers, 7% flavonoids, and 18% non-flavonoids [118]. One gram of extract was reported to contain polyphenols equivalent to approximately four apples [119]. In the Ames mutagenicity test, only one out of five bacterial strains showed a slight increase in revertants indicative of mutagenic potential. No signs of mutagenicity were detected in the chromosomal aberration test in Chinese hamster lung cell culture and the micronucleus test in Sprague-Dawley rats. Also, no signs of toxicity at a dose of 2000 mg/kg body weight (bw) were observed in an acute and subchronic toxicity test. The extract was therefore regarded as safe [120].

As a first indication of cancer chemopreventive efficacy *in vivo*, apple products have been tested in experimental animal models for chemically- or genetically-induced tumors of the skin, breast, and colon, as well as in xenograft models for solid tumors and melanoma.

Oral administration of aqueous apple peel extracts (derived from 1 mL water/g apple peel, given *ad libitum*) significantly reduced the number of 7,12-dimethylbenz[*a*]anthracene (DMBA)-initiated and TPA-induced mouse skin papillomas by 55%. The effect was explained by antioxidant properties which may block ROS-mediated signal transduction pathways via MAP-kinase cascade and transcription factor AP-1 [121]. Liu et al. investigated the potential of a polyphenol-enriched apple extract on DMBA-induced mammary carcinogenesis in rats. Application by gavage of 9 – 54.4 mg extract (equivalent to 3.3 – 20 g apples) per kg bw two weeks prior to and for 24 weeks after carcinogen treatment lowered the number of tumor-bearing animals dose-dependently by 17%, 39%, and 44%. Tumor numbers per animal were also reduced by 25%, 25%, and 61% after 24 weeks [122]. In a rat xenograft model for tumor invasion and metastases with AH109A rat ascites hepatoma cells, a commercially available apple polyphenol extract from unripe apples was tested. Intervention with the extract (added to the chow at 0.3 and 1% concentrations) for 21 days after tumor cell inoculation significantly reduced the weight of solid tumors by 64% and 58%. In addition, numbers of lung and lymphatic node metastases were strongly reduced from 17 per 10 rats to 1 per 10 rats in the two extract groups [123]. Apple polyphenols and OPC (both applied at 1% in drinking water) also inhibited the growth of transplanted B16 mouse melanoma cells *in vivo*, and increased the survival rate of the host mice transplanted with B16 cells [106].

Several groups investigated the potential of various apple products [clear and cloudy apple juice, apple polyphenol extract (APE), OPC, apple pectin] to prevent colon carcinogenesis (**Table 3**). Barth et al. compared the effects of clear and cloudy apple juice in a rat model for chemically-induced colon carcinogenesis using 1,2-dimethylhydrazine (DMH) as a carcinogen [124]. After intervention for eight weeks, cloudy apple juice was more potent in inhibiting carcinogen-induced epithelial cell proliferation and DNA damage than clear apple juice. Also, cloudy apple juice reduced the number of aberrant crypt foci (ACF) as a pre-neoplastic marker for colon

Table 3 Summary of colon cancer preventive effects of apple products in animal models

Model (carcinogen)[a]	Intervention, dose	Duration[b]	Endpoint, results[c]	Ref.
F344 rats (DMH) (male, 118 g bw)	• clear apple juice (*ad lib.*) (37.9 mg polyphenols[d] and 22.6 mg pectin/kg bw/day)	7 w (b-p)	• genetic damage 21 % ↓, proliferation 45 % ↓	[124]
	• cloudy apple juice (*ad lib.*) (40.7 mg polyphenols[d] and 91.4 mg pectin/kg bw/day)	7 w (b-p)	• genetic damage 72 % ↓*, proliferation 72 % ↓*, ACF/colon 19 % ↓, AC/colon 29 % ↓	
F344 rats (DMH) (male, 100 g bw)	• cloudy apple juice (*ad lib.*) (39.3 mg polyphenols[d] and 88 mg pectin/kg bw/day)	7 w (b-p)	• genetic damage 77 % ↓*, proliferation 74 % ↓*, ACF/colon 15 % ↓, large ACF/colon 35 % ↓	[126]
	• APE (39.5 mg polyphenols[d]/kg bw/day)	7 w (b-p)	• genetic damage 20 % ↓, proliferation 34 % ↓	
	• cloud fraction (dissolved at 0.75 g/L)	7 w (b-p)	• genetic damage 32 % ↓, proliferation 45 % ↓	
	• APE and cloud fraction combined	7 w (b-p)	• genetic damage 53 % ↓, proliferation 34 % ↓	
Wistar rats (AOM) (male, 230 – 245 g bw)	• procyanidin fraction P with 78.4 % OPC (0.01 % in drinking water)	6 w (p)	• ACF/colon 50 % ↓*	[87]
Donryu rats (AOM) (male, 200 g bw, 4 w)	• apple pectin (20 % in the diet)	32 w (b-p)	• carcinoma incidence 75 % ↓*, carcinoma multiplicity 72 % ↓* • body weight (at 30 w) 15 % ↓*, fecal weight 48 %ñ*	[127]
C57BL/6 $APC^{Min/+}$ mice (male, 7 w)	• cloudy apple juice (*ad lib.*) (14 mg polyphenols[d]/kg bw/day)	10 w	• small intestinal adenoma 38 % ↓*	[128]
	• APE (0.2 % in drinking water) (70 mg polyphenols[d]/kg bw/day)	10 w	• small intestinal adenoma 40 % ↓*, hematocrit ñ*, spleen weights ↓*	
C57BL/6 $APC^{Min/+}$ mice (female, 4 w)	• dehydrated apple pomace (20 % in diet)	8 w	• small intestinal adenoma 132 %, small intestinal polyp burden 111 % ↑*, colon polyp diameter 40 % ↑*, colon polyp burden 150 % ↑*	[131]

[a] DMH, 1,2-dimethylhydrazine; AOM, azoxymethane; bw, body weight; w, age in weeks.

[b] w, weeks; dietary intervention before (b), during (d), post (p) carcinogen treatment.

[c] inhibition (↓), induction (↑), *significant results. ACF, aberrant crypt foci; AC, aberrant crypts. Endpoints without effect of intervention are not shown.

[d] OPC content was not considered.

carcinogenesis, whereas clear apple juice was ineffective. These results may be explained by a higher content in procyanidins in cloudy apple juice than in clear juice, as shown by Oszmianski et al. [125] and Hümmer et al. [28], who recently also determined that the cloud fraction responsible for the turbidity of cloudy apple juice contained up to 60% of oligomeric procyanidins (personal communication). Results obtained with cloudy apple juice were reproduced in a second investigation by Barth et al. [126]. In this study, neither isolated APE nor the cloud fraction alone or in combination significantly reduced the number of ACF. The potentially important role of OPC for colon cancer prevention was demonstrated by Gosse et al. [87], [104], [105]. In the AOM (azoxymethane)-induced rat model, a procyanidin-enriched fraction P from apples at a very low dose (0.01 % in drinking water) significantly reduced the number of ACF/colon by 50 % [87]. In an earlier study by Ohkami et al., addition of 20 % apple pectin to the diet significantly reduced the incidence and multiplicity of AOM-induced colonic adenomas and carcinomas in rats [127]. Concomitantly, body weights were significantly reduced by apple pectin intervention, although the animals consumed the same amount of food/day as the control group (not adjusted for differences in available energy). Since treatment with citrus pectin similarly reduced body weight increase, but not tumor numbers, the authors argued that reduction of bw alone was not sufficient to explain the strong cancer preventive effect. Rather, a transient effect on fecal bacterial enzyme activities was discussed [127].

The C57BL/6-Apc^{Min} (Apc^{Min}) mouse strain commonly used in chemoprevention studies is a genetically engineered model that develops multiple intestinal neoplasia. Ten weeks of intervention with both cloudy apple juice and a polyphenol-enriched apple juice extract (1 : 1 mix of AE02 and AE03 at 0.2 % in drinking water) in male Apc^{Min} mice significantly lowered the number of adenomas in the small intestine by 38 % and 40 %. Extract treatment also improved hematocrit values, which are reduced in Apc^{Min} mice as a sign of intestinal bleeding. Also, spleen weights, which were about 5-fold increased in Apc^{Min} compared to wild-type mice, were significantly reduced by extract intervention [128], [129]. In a recent study by Mandir et al., the influence of dehydrated apple pomace was investigated in the Apc^{Min} mouse model. Apple pomace is a waste product of apple juice production and a good source of highly fermentable non-starch polysaccharides (23.9 g/100 g). At a dose of 20 % in the diet, apple pomace significantly *enhanced* the number of small intestinal polyps and polyp burden in female Apc^{Min} mice. Also, colonic adenoma size and burden was significantly enhanced. This was surprising, but is consistent with some earlier reports on fermentable resistant carbohydrates. The results were explained by increased formation of short-chain fatty acids (SCFA), which may

stimulate intestinal cell growth [130]. However, other studies have demonstrated a protective effect, e.g., with rye bran, indicating that the role of dietary fiber in colon cancer prevention is still an area of controversial debate ([96], [131] and references cited therein).

In Vivo Effects of Apple-Derived Dietary Fiber in Animal Models

Several short-term dietary intervention studies in rodents have been performed to investigate the influence of apple-derived dietary fiber and cell wall components on intestinal fermentation products, fecal steroids and serum lipids. This was of interest since, e.g., the ratio of the secondary bile acids lithocholic acid to deoxycholic acid, formed by bacterial metabolism from primary bile acids synthesized in the liver, is regarded as a risk index for colorectal cancer [132]. Shimizu et al. examined the effect of a commercially available apple pulp powder low in fiber (32.8%) and rich in carbohydrates (59.8%, dextrin) on fecal steroid profiles in rats [133]. The apple powder was added at a concentration of 15.2% to the diet and fed for three weeks. The intervention significantly reduced serum triacylglycerol and serum and liver phospholipid levels in comparison with a cellulose control group. Excretion of total and several major bile acids was significantly increased, whereas the ratio between secondary bile acids and total bile acids was significantly reduced by the apple pulp powder [133].

So-called "enzymatic liquefaction" of apple pomace with cellulases and pectinases to produce pomace liquefaction juices (B-juices) may increase the extraction of valuable apple components including dietary fiber and polyphenols [134]. B-juices are rich in colloids composed of galacturonic acid (49–64 mol%), arabinose (14–23 mol%) and galactose (6–15 mol%), with minor amounts of rhamnose, xylose, and glucose [135]. Sembries et al. compared the influence of colloids from B-juices and an `alcohol-insoluble substance' (AIS) on SCFA profiles and intestinal microbiota in rats. Similar to the results with apple pectin [127] (see above), animals fed for 6 weeks with 5% B-juice colloids gained about 25% less weight than control rats, although food consumption was not significantly different. Both interventions significantly lowered luminal pH values and increased the weight of cecal contents. Apple colloids enhanced total SCFA, acetate, and propionate concentrations in cecum and distal colon, whereas AIS also increased butyrate levels. This was attributed to cellulose present only in AIS, but not apple colloids. AIS intervention enhanced the numbers of cecal microbiota of the *Eubacterium rectale* cluster. In contrast, apple colloids increased fecal concentrations of *Bacteriodaceae* [136]. Similar to the results of Shimizu et al. [133], apple colloids and AIS also increased excretion of primary bile acids in feces up to 30% and 88%, whereas concentrations of secondary bile acids were reduced [137]. Analogous effects were obtained when diluted B-juice was applied to rats directly as a drink, suggesting that administration of extraction juices enriched in phytochemicals and dietary fiber resulted in beneficial nutritional effects [138]. These are promising results, but long-term cancer preventive effects of B-juice colloids need to be investigated.

The effect of apple components on cecal fermentations and lipid metabolism was also tested by Aprikian et al. [139]. Rats were fed with diets supplemented with 5% apple pectin, 10% high-polyphenol freeze-dried apple, or both, for three weeks. Cecal short chain fatty acids were significantly enhanced by all apple diets in comparison with the controls (190 μmol/cecum = 84 mM) with highest concentrations of 560 μmol/cecum (= 122 mM) in the combination group. Overall, the authors concluded that the effect of apple pectin and the polyphenol-rich fraction on large intestine fermentations and lipid metabolism was enhanced when both fractions were fed in combination, suggesting interactions between fiber and polyphenols of apple [139].

Human Short-Term Intervention Studies: Modulation of Antioxidant Status and Markers of Oxidative Stress

In vitro, apple polyphenols have been identified as potent radical-scavengers and antioxidants (see above). But are these mechanisms effectively translated to the *in vivo* situation? Although animal studies have indicated cancer preventive efficacy of apple products, extrapolation of the results to the human situation is difficult. In humans, exposure to low doses of endogenous or exogenous carcinogens and tumor promoters may occur constantly and life-long. In addition, genetic polymorphisms, variations in DNA methylation and epigenomic events may influence the response of humans to carcinogens and protective agents [31]. Consequently, proof of cancer preventive efficacy in humans requires very large and long-lasting controlled clinical trials.

Short-term human intervention studies can provide an indication of potential health-promoting or cancer preventive activity based on the modulation of biomarkers. Several recent studies have focused on the modulation of antioxidant status and markers of oxidative stress by consumption of apple and apple juice. In a study by Ko et al., improved antioxidant capacity *vs*. hydroxyl radicals was detected in serum of 10 healthy male volunteers 30 min after consumption of 150 mL apple juice. Apple juice was compared with a variety of fruit juices. Orange juice provided the best antioxidant effect, whereas pear juice was inactive [140]. Similarly, one serving of 1 L of apple juice caused a significant increase of serum DPPH radical scavenging activity in 12 healthy subjects 1 h after juice ingestion [141]. In a study conducted by Maffei et al., 6 healthy, non-smoking male volunteers consumed a homogenate obtained from 600 g unpeeled apples. Results indicated a significant inhibition of H_2O_2-induced micronuclei frequency in lymphocytes collected at 3 h after apple consumption, compared with samples at 0 h. Values gradually returned to baseline starting from 6 to 24 h [142]. In line with these observations, Briviba et al. reported that consumption of 1 kg organically or conventionally grown apples once by 10 healthy male volunteers did not change antioxidant capacity in plasma, endogenous DNA strand breaks, and protection from H_2O_2-induced DNA damage in lymphocytes when measured 24 h after consumption. However, lymphocyte DNA had lower levels of so called Endo-III sensitive sites (specific for oxidized pyrimidines) and was protected from hydroxyl radicals. The type of apple production had no influence on polyphenol levels and any biological effect measured in this study [143]. Mayer et al. used a high-throughput fluorescence screening method to measure antioxidative capacity in human serum [144]. Two fluorophores were developed as hydrophilic and hydrophobic oxidation markers for the aqueous and lipid phase of serum. Forty-seven healthy human volunteers consumed 1 kg of apples daily

for four days, providing 2.7 g total phenolics/kg fresh apples. Apple consumption increased the anti-oxdidant capacity in the aqueous phase, but not in the lipophilic phase 3 h after the first apple consumption. The effect was only transient and did not increase with longer apple intake for four days [144]. Consumption of a blueberry/apple juice mixture (1 L daily, providing an extra 18 mg quercetin) for four weeks significantly increased the total antioxidant capacity in plasma of 8 female volunteers. Also, quercetin plasma levels increased significantly from 1.5 ng/mL plasma (5.0 nM) before intervention to 3.1 ng/mL (10.6 nM) at the end of the study [59]. This study was followed by a larger scale study with 168 subjects, who consumed a blueberry/apple juice mixture (1 L daily, providing an extra 97 mg quercetin) for four weeks. In this follow-up study, analysis of effects of 34 genetic polymorphisms on lymphocytic DNA damage was included. Plasma concentrations of quercetin and ascorbic acid, and antioxidant capacity were significantly increased. Lymphocytic DNA was protected against *ex vivo* H_2O_2-induced oxidative DNA damage, whereas levels of *ex vivo* induced B(a)P-diol epoxide-DNA adducts were 28% *increased* upon intervention. Six genetic polymorphisms significantly influenced the outcome of the intervention [NQO1*2 → quercetin levels; Cat1 → vitamin C levels; GSTT1 deletion → plasma antioxidant capacity and levels of induced oxidative damage in lymphocytes; Cyp1B1*5 and COMT1 → B(a)P-diole epoxide-DNA adduct levels] [60].

Overall, these studies suggest that apple or apple juice consumption results in a brief, transient increase in plasma antioxidant capacity 0.5 to 3 h after consumption. Eventually, longer continuous exposure for 2 – 4 weeks is required to obtain a sustained increase. Based on reports of Lotito and Frei, these results have to be interpreted with caution. In a series of *in vitro*, *ex vivo* and *in vivo* studies they demonstrated that the increase in human plasma antioxidant capacity after apple consumption is not caused by apple-derived antioxidants, but most likely due to a metabolic effect of fructose, which is provided by apple products in large quantities, on urate, an important endogenous antioxidant in plasma [145], [146], [147].

Apple Consumption and Cancer Incidence in Humans: Epidemiological Evidence

Observations of the eating behavior of the general public in retro- or prospective epidemiological studies allow to draw conclusions on relations between consumption of specific food items and cancer risk [148]. Best evidence comes from large scale prospective cohort studies, which select a collective of healthy people and regularly interview for dietary habits and cancer incidence over a long period of time. These studies permit epidemiologists to calculate a relative risk (RR), that is the ratio of the probability of cancer occurring in the exposed (here: apple-eating) group versus the non-exposed group (here: no or very low apple consumption). The collective can be divided in a number of subgroups with increasing exposure (mostly 3, tertiles; 4, quartiles; 5 quintiles), and the RR is calculated for each subgroup. This allows evaluation of dose-response relationships. RR values can be > 1.0 within a certain confidence interval (CI), indicating an increased risk, or < 1.0, indicating protection. Only studies with a CI not including 1.0 are considered as statistically significant.

Case-control studies compare a group of patients who have a disease with a group of patients (or healthy controls) who do not. Case-control studies acquire data on food consumption etc. retrospectively and are more prone to errors. The results are expressed as an odds ratio (OR), defined as the ratio of the odds of an event (here: apple-eating) occurring in the group of cancer cases, to the odds of it occurring in the control group. OR = 1.0 would indicate that apple consumption is equal in both groups, whereas an OR < 1.0 would demonstrate that the control group is more likely to eat apples than the group of cases. This observation would suggest (but not prove) that regular apple consumption may be linked to a reduced risk of getting cancer.

Epidemiological evidence accumulated over the past years points to the cancer preventive potential of apples especially for lung and colorectal cancer. The results of several cohort and case-control studies are summarized in **Table 4**. In the Nurses' Health Study (NHS), a large prospective cohort study conducted in the USA, a significant reduced risk for lung cancer was observed among the women, while no effect was seen among men in the Health Professionals' Follow-up Study [149]. The Zutphen Elderly Study aimed at identifying risk factors for chronic diseases in elderly men in The Netherlands. Uptake of apples was non-significantly related with reduced lung cancer risk, whereas tea consumption as the major source (87%) of catechins had no protective effect [150]. Also, in a large Finnish cohort study, the risk for lung cancer was significantly reduced by 60% in men who ate most apples (>47 g/d) compared to those who did not eat apples at all [151]. Finally, statistically significant inverse associations between lung cancer risk and onions and apples as the main food source of the flavonoid quercetin were found in a case-control study conducted in Hawaii. No significant differences were observed when apple consumption was related to the most common lung cancer cell types, *i.e.* squamous cell carcinoma and adenocarcinoma [152].

In addition to the consistent association with lung cancer prevention, recent publications also indicate preventive effects of apple consumption on colorectal carcinogenesis. In the NHS, those 20% of women who consumed the most apples had a significantly reduced risk of developing colorectal adenomas in comparison to the 20% with the lowest intake [153]. In a case-control study conducted in Uruguay, apple consumption was associated with a significant, dose-dependent reduction in colorectal cancer risk in men and women [154]. In a South-Korean case-control study, fruit consumption (apples combined with banana, pear and watermelon) lowered the risk for colon cancer in men, but not in women [155]. A meta-analysis of multicenter case control studies conducted in Italy revealed that consumption of ≥1 apple/day in comparison with ≤ 1 apple/day significantly reduced the odds ratio (OR) for colorectal cancer as well as for cancers of the oral cavity (OR 0.79, 95% CI 0.62 – 1.0), larynx (OR 0.58, 95% CI 0.44 – 0.76), breast (OR 0.82, 95% CI 0.73 – 0.92) and ovary (OR 0.85, 95% CI 0.72 – 1.0) [156]. A recent case-control study from Scotland did not find a statistically significant association between apple consumption and colon cancer risk [157]. High apple consumption (> 94 g/day) was associated with a reduced renal cancer risk. The reduction was particularly strong for the 10% of people who ate the most apples and for non-smokers, whereas no effect was seen in smokers [158].

Summary and Conclusions

Apples are a rich source of phytochemicals (polyphenols, triterpenoids) and dietary fiber, which have been associated with can-

Table 4 Epidemiological cohort and case-control studies on apple consumption and cancer risk

Type of cancer	Type of study	Study population	No. of cancer cases	Years of follow-up	Effect of apple consumption	Ref.
Lung cancer						
Nurses' Health Study (NHS)	cohort	77283 women	519	12 y	For increases of one serving of apples and pears, RR = 0.63; 95 % CI = 0.43 – 0.91	[149]
Health Professionals' Follow-up Study (HPFS)	cohort	47778 men	274	10 y	no effect	[149]
Zuthphen Elderly Study	cohort	728 men	42	10 y	Catechins from apples account for 8 % of total catechin intake. RR for 7.5 mg increase in catechin from apples = 0.67, 95 % CI = 0.38 – 1.17	[150]
Finnish study	cohort	5218 men	169	max. 30 y	RR for highest (> 47 g/d) vs. lowest (0 g/d) quartile: 0.4 (95 % CI = 0.22 – 0.74)	[151]
Hawaii study	case-control	582 cases + 582 controls			OR for highest (> 49.7 g/d) vs. lowest (< 2.3 g/d) quartile: 0.6, 95 % CI = 0.4 – 1.0 (*P* for trend 0.03)	[152]
Colorectal cancer						
Nurses' Health Study (NHS)	cohort	34467 women	1 720	18 y	OR for adenoma prevalence comparing highest vs. lowest quintile: 0.83, 95 % CI = 0.7 – 0.98 (*P* for trend 0.05)	[153]
Uruguay	case-control	160 cases + 287 hospital controls			OR for highest vs. lowest tertile: 0.4, 95 % CI = 0.25 – 0.66 (*P* for trend <0.001)	[154]
South-Korea	case-control	162 cases + 2 576 hospital controls			OR for highest vs. lowest quartile (combined with banana, pear and watermelon): 0.36, 95 % CI = 0.16 – 0.84 (only in men)	[155]
Italy (meta-analysis)	case-control	1953 cases + 4154 hospital controls			OR for ≥ 1 apple/d vs. <1 apple/d: 0.8, 95 % CI = 0.71 – 0.9	[156]
Scotland	case-control	1456 cases + 1456 population-based controls			OR for highest vs. lowest quartiles: 0.94, 95 % CI = 0.62 – 1.5 (*P* for trend 0.9)	[157]
Renal cancer						
Sweden	case-control	379 cases + 353 population-based controls			OR for highest (> 94 g/d) vs. lowest (< 15 g/d) quartile: 0.65, 95 % CI = 0.43 – 0.98 (*P* for trend 0.02). Top 10 % of consumption: OR = 0.36, 95 % CI = 0.14 – 0.93	[158]
		Non-smokers only: 175 cases + 191 controls			OR for highest (> 94 g/d) vs. lowest (< 15 g/d) quartile: 0.5, 95 % CI = 0.28 – 0.88 (*P* for trend 0.01). n. s. for smokers	

Data were corrected for potential confounding factors, such as age, gender, smoking habits, alcohol consumption, medical treatments, and vitamin supplementation as indicated in the original references. RR, relative risk; OR, odds ratio; 95 % CI, confidence interval. Only results with a 95 % CI not including 1.0 are considered as statistically significant. n. s. not significant.

cer preventive mechanisms in *in vitro* studies. These include anti-mutagenic effects, enhanced detoxification through modulation of xenobiotic metabolism, antioxidant effects (demonstrated *in vitro* and *in vivo*), anti-inflammatory activity by inhibition of Cox activity and NF-κB, inhibition of signaling pathways, including the EGF/EGFR-mediated activation of the MAP kinase pathway, PKC and polyamine metabolism, and GSK3β involved in Wnt-signaling, cell growth inhibitory mechanisms, and induction of programmed cell death by activation of both the death receptor and the mitochondrial pathway. Recent studies have identified chymotrypsin-like activity of the 26S proteasome, tumor suppressor protein DEP-1, epigenetic mechanisms, and modulation of immune functions as novel targets of apple products.

Bioavailability studies have indicated that low molecular weight polyphenols of apples may be absorbed after hydrolysis and further conjugated. Overall, plasma levels appear to be low. Transient anti-oxidant activity 0.5 to 3 h after apple and apple juice consumption has been observed in human short-term intervention studies. Application for 2 – 4 weeks may be required for sustained antioxidant effects. Some studies indicate that these antioxidant activities are more likely due to metabolic effects of fructose than to polyphenolic antioxidants [147]. OPC, which are the most abundant polyphenols in apples, are poorly absor-

bed, pass the stomach and will reach the colon, where they may exert local antioxidant, anti-proliferative, or immune modulating effects.

Apple extracts and apple pectin are fermented by the colonic microflora and provide SCFA, which inhibit histone deacetylases and are generally assumed to possess antiproliferative potential. Some studies indicate that combination of polyphenols and apple-derived dietary fiber may lead to enhanced biological effects. Unexpectedly, in a recent study with $APC^{Min/+}$ mice, intervention with dehydrated apple pomace, which is regarded as a good source of both dietary fiber and cell wall-bound flavonols, at a relatively high dose of 20% in the diet increased the incidence of small intestinal adenoma significantly. This was discussed in relation to a stimulating effect of SCFA on cell growth. Overall, the role of dietary fiber in colon cancer prevention is still an area of controversial discussion.

Although extrapolation from animal studies to the human situation is difficult, doses equivalent to 800 mL of cloudy apple juice [128] or 6 apples [122], respectively, have been shown to reduce colon and mammary cancer in carcinogenesis models. There is consistent evidence from additional animal studies (with one exception as outlined above) and epidemiological observations that regular consumption of one or more apples per day may contribute to the prevention of certain types of cancer.

This review emphasizes the importance of apples and apple products for cancer prevention. Apples and apple juice are an integral part of the human diet and are consumed by a majority of the population, including children. Almost all studies summarized here suggest that apples, natural cloudy apple juice, and apple extracts should be further investigated as part of a prevention strategy for cancer, especially of hereditary and sporadic colorectal cancer and lung cancer.

Acknowledgement

I would like to thank the German Federal Ministry of Education and Research (BMBF) for financial support of our studies with apple juice in the framework of the Nutrition Net (www.nutrition-net.org). The Nutrition Net is a network of German research groups headed by H. Becker, P. Schreier, F. Will and H. Dietrich, E. Richling, D. Marko, D. Schrenk, C. Janzowski, B.L. Pool-Zobel, F. Böhmer, S. Wölfl, S. Barth, A. Bub, K. Briviba, B. Watzl, and G. Rechkemmer, aimed at investigating the role of food ingredients on development of intestinal diseases and potential of their prevention by nutrition, especially by apple products.

References

1 *Sporn MB, Newton DL.* Chemoprevention of cancer with retinoids. Fed Proc 1979; 38: 2528 – 34
2 *Kelloff GJ, Lippman SM, Dannenberg AJ, Sigman CC, Pearce HL, Reid BJ et al.* Progress in chemoprevention drug development: the promise of molecular biomarkers for prevention of intraepithelial neoplasia and cancer – a plan to move forward. Clin Cancer Res 2006; 12: 3661 – 97
3 *Ellinger W.* ZMP-Marktbilanz Obst 2007, Deutschland, Europäische Union, Weltmarkt. Bonn: ZMP Zentrale Markt- und Preisberichtstelle GmbH; 2007
4 Wirtschaftsvereinigung Alkoholfreie Getränke e.V. Pro-Kopf-Verbrauch alkoholfreier Getränke in Deutschland 2006. Germany: Wirtschaftsvereinigung Alkoholfreie Getränke; 2008
5 *Boyer J, Liu RH.* Apple phytochemicals and their health benefits. Nutr J 2004; 3: 5
6 *Souci SW, Fachmann W, Kraut H, revised by Kirchhoff E.* Food composition and nutrition tables, based on the 6th edition. Stuttgart: medpharm GmbH Scientific Publishers; 2005
7 *Thielen C, Will F, Zacharlas J, Dietrich H, Jacob H.* Polyphenols in apples: Distribution of polyphenols in apple tissue and comparison of fruit and juice. Dtsch Lebensmitt Rundsch 2004; 100: 389 – 98
8 *Lata B, Tomala K.* Apple peel as a contributor to whole fruit quantity of potentially healthful bioactive compounds. Cultivar and year implication. J Agric Food Chem 2007; 55: 10795 – 802
9 *Lata B, Przeradzka M, Binkowska M.* Great differences in antioxidant properties exist between 56 apple cultivars and vegetation seasons. J Agric Food Chem 2005; 53: 8970 – 8
10 *Van Buren J.* Fruit phenolics. In: Hulme AC, editor. The biochemistry of fruit and their products. New York: Academic Press; 1970: 269 – 304
11 *Guyot S, Marnet N, Sanoner P, Drilleau JF.* Variability of the polyphenolic composition of cider apple (*Malus domestica*) fruits and juices. J Agric Food Chem 2003; 51: 6240 – 7
12 *Kahle K, Kraus M, Richling E.* Polyphenol profiles of apple juices. Mol Nutr Food Res 2005; 49: 797 – 806
13 *Vrhovsek U, Rigo A, Tonon D, Mattivi F.* Quantitation of polyphenols in different apple varieties. J Agric Food Chem 2004; 52: 6532 – 8
14 *Keller P, Strekker P, Arnold G, Schieber A, Carle R.* Bestimmung phenolischer Verbindungen in Tafel- und Mostäpfeln mittels HPLC. Fluessiges Obst 2001; 68: 480 – 3
15 *Schmitz-Eiberger M, Weber V, Treutter D, Baab G, Lorenz J.* Bioactive components in fruits from different apple varieties. J Appl Bot 2003; 77: 167 – 71
16 *Mangas JJ, Suarez B, Picinelli A, Moreno J, Blanco D.* Differentiation by phenolic profile of apple juices prepared according to two membrane techniques. J Agric Food Chem 1997; 45: 4777 – 84
17 *Tsao R, Yang R, Young JC, Zhu H.* Polyphenolic profiles in eight apple cultivars using high-performance liquid chromatography (HPLC). J Agric Food Chem 2003; 51: 6347 – 53
18 *Pearson DA, Tan CH, German JB, Davis PA, Gershwin ME.* Apple juice inhibits human low density lipoprotein oxidation. Life Sci 1999; 64: 1913 – 20
19 *Sanoner P, Guyot S, Marnet N, Molle D, Drilleau JP.* Polyphenol profiles of French cider apple varieties (*Malus domestica* sp.). J Agric Food Chem 1999; 47: 4847 – 53
20 *Alonso-Salces RM, Barranco A, Abad B, Berrueta LA, Gallo B, Vicente F.* Polyphenolic profiles of Basque cider apple cultivars and their technological properties. J Agric Food Chem 2004; 52: 2938 – 52
21 *Guyot S, Marnet N, Laraba D, Sanoner P, Drilleau JF.* Reversed-phase HPLC following thiolysis for quantitative estimation and characterization of the four main classes of phenolic compounds in different tissue zones of a French cider apple variety (*Malus domestica* var. Kermerrien). J Agric Food Chem 1998; 46: 1698 – 705
22 *Guyot S, Marnet N, Drilleau J.* Thiolysis-HPLC characterization of apple procyanidins covering a large range of polymerization states. J Agric Food Chem 2001; 49: 14 – 20
23 *Shibusawa Y, Yanagida A, Isozaki M, Shindo H, Ito Y.* Separation of apple procyanidins into different degrees of polymerization by high-speed counter-current chromatography. J Chromatogr A 2001; 915: 253 – 7
24 *Yanagida A, Kanda T, Shoji T, Ohnishi-Kameyama M, Nagata T.* Fractionation of apple procyanidins by size-exclusion chromatography. J Chromatogr A 1999; 855: 181 – 90
25 *Yanagida A, Kanda T, Takahashi T, Kamimura A, Hamazono T, Honda S.* Fractionation of apple procyanidins according to their degree of polymerization by normal-phase high-performance liquid chromatography. J Chromatogr A 2000; 890: 251 – 9
26 *Shoji T, Masumoto S, Moriichi N, Kanda T, Ohtake Y.* Apple (*Malus pumila*) procyanidins fractionated according to the degree of polymerization using normal-phase chromatography and characterized by HPLC-ESI/MS and MALDI-TOF/MS. J Chromatogr A 2006; 1102: 206 – 13
27 *Aron PM, Kennedy JA.* Flavan-3-ols: nature, occurrence and biological activity. Mol Nutr Food Res 2008; 52: 79 – 104
28 *Huemmer W, Dietrich H, Will F, Schreier P, Richling E.* Content and mean polymerization degree of procyanidins in extracts obtained from clear and cloudy apple juices. Biotechnol J 2008; 3: 234 – 43
29 *Frighetto RTS, Welendorf RM, Nigro EN, Frighetto N, Siani AC.* Isolation of ursolic acid from apple peels by high speed counter-current chromatography. Food Chem 2008; 106: 767 – 71
30 *He X, Liu RH.* Triterpenoids isolated from apple peels have potent antiproliferative activity and may be partially responsible for apple's anticancer activity. J Agric Food Chem 2007; 55: 4366 – 70
31 *Milner JA.* Diet and cancer: facts and controversies. Nutr Cancer 2006; 56: 216 – 24

32 *Scalbert A, Williamson G.* Dietary intake and bioavailability of polyphenols. J Nutr 2000; 130: 2073S – 85S
33 *Yang CS, Landau JM, Huang MT, Newmark HL.* Inhibition of carcinogenesis by dietary polyphenolic compounds. Annu Rev Nutr 2001; 21: 381 – 406
34 *Manach C, Williamson G, Morand C, Scalbert A, Remesy C.* Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr 2005; 81: 230S – 42S
35 *Crespy V, Morand C, Besson C, Manach C, Demigne C, Remesy C.* Comparison of the intestinal absorption of quercetin, phloretin and their glucosides in rats. J Nutr 2001; 131: 2109 – 14
36 *Crespy V, Aprikian O, Morand C, Besson C, Manach C, Demigne C et al.* Bioavailability of phloretin and phloridzin in rats. J Nutr 2001; 131: 3227 – 30
37 *Kahle K, Kraus M, Scheppach W, Richling E.* Colonic availability of apple polyphenols – a study in ileostomy subjects. Mol Nutr Food Res 2005; 49: 1143 – 50
38 *Kahle K, Huemmer W, Kempf M, Scheppach W, Erk T, Richling E.* Polyphenols are intensively metabolized in the human gastrointestinal tract after apple juice consumption. J Agric Food Chem 2007; 55: 10 605 – 14
39 *Olthof MR, Hollman PCH, Katan MB.* Chlorogenic acid and caffeic acid are absorbed in humans. J Nutr 2001; 131: 66 – 71
40 *Olthof MR, Hollman PCH, Buijsman MNCP, van Amelsvoort JMM, Katan MB.* Chlorogenic acid, quercetin-3-rutinoside and black tea phenols are extensively metabolized in humans. J Nutr 2003; 133: 1806 – 14
41 *Gonthier M-P, Verny M-A, Besson C, Remesy C, Scalbert A.* Chlorogenic acid bioavailability largely depends on its metabolism by the gut microflora in rats. J Nutr 2003; 133: 1853 – 9
42 *Walle T, Otake Y, Walle UK, Wilson FA.* Quercetin glucosides are completely hydrolyzed in ileostomy patients before absorption. J Nutr 2000; 130: 2658 – 61
43 *Walle T, Walle UK, Halushka PV.* Carbon dioxide is the major metabolite of quercetin in humans. J Nutr 2001; 131: 2648 – 52
44 *Gu L, Kelm MA, Hammerstone JF, Beecher G, Holden J, Haytowitz D et al.* Concentrations of proanthocyanidins in common foods and estimations of normal consumption. J Nutr 2004; 134: 613 – 7
45 *Prior RL, Gu L.* Occurrence and biological significance of proanthocyanidins in the American diet. Phytochemistry 2005; 66: 2264 – 80
46 *Spencer JP, Schroeter H, Rechner AR, Rice-Evans C.* Bioavailability of flavan-3-ols and procyanidins: gastrointestinal tract influences and their relevance to bioactive forms *in vivo.* Antioxid Redox Signal 2001; 3: 1023 – 39
47 *Rios LY, Bennett RN, Lazarus SA, Remesy C, Scalbert A, Williamson G.* Cocoa procyanidins are stable during gastric transit in humans. Am J Clin Nutr 2002; 76: 1106 – 10
48 *Gerhauser C, Klimo K, Kahle K, Garreta A, Steinle R, Scheppach W et al.* Cancer chemopreventive potential of apple juice – Results of a short-term human intervention study with ileostomy patients. EJC Suppl 2008; 6: 50
49 *Deprez S, Brezillon C, Rabot S, Philippe C, Mila I, Lapierre C et al.* Polymeric proanthocyanidins are catabolized by human colonic microflora into low-molecular-weight phenolic acids. J Nutr 2000; 130: 2733 – 8
50 *De Flora S, Ferguson LR.* Overview of mechanisms of cancer chemopreventive agents. Mutat Res 2005; 591: 8 – 15
51 *Ferguson LR.* Antimutagens as cancer chemopreventive agents in the diet. Mutat Res 1994; 307: 395 – 410
52 *Hensel A, Meier K.* Pectins and xyloglucans exhibit antimutagenic activities against nitroaromatic compounds. Planta Med 1999; 65: 395 – 9
53 *Ferguson LR, Zhu S, Kestell P.* Contrasting effects of non-starch polysaccharide and resistant starch-based diets on the disposition and excretion of the food carcinogen, 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), in a rat model. Food Chem Toxicol 2003; 41: 785 – 92
54 *Kestell P, Zhu S, Ferguson LR.* Mechanisms by which resistant starches and non-starch polysaccharide sources affect the metabolism and disposition of the food carcinogen, 2-amino-3-methylimidazo[4,5-*f*]quinoline. J Chromatogr B Analyt Technol Biomed Life Sci 2004; 802: 201 – 10
55 *Kohle C, Bock KW.* Coordinate regulation of Phase I and II xenobiotic metabolisms by the Ah receptor and Nrf2. Biochem Pharmacol 2007; 73: 1853 – 62
56 *Pohl C, Will F, Dietrich H, Schrenk D.* Cytochrome P450 1A1 expression and activity in Caco-2 cells: modulation by apple juice extract and certain apple polyphenols. J Agric Food Chem 2006; 54: 10 262 – 8
57 *Gerhauser C, Klimo K, Heiss E, Neumann I, Gamal-Eldeen A, Knauft J et al.* Mechanism-based *in vitro* screening of potential cancer chemopreventive agents. Mutat Res 2003; 523 – 524: 163 – 72
58 *Zessner H, Pan L, Will F, Klimo K, Knauft J, Niewohner R et al.* Fractionation of polyphenol-enriched apple juice extracts to identify constituents with cancer chemopreventive potential. Mol Nutr Food Res 2008; 52 1: S28 – 44
59 *Wilms LC, Hollman PC, Boots AW, Kleinjans JC.* Protection by quercetin and quercetin-rich fruit juice against induction of oxidative DNA damage and formation of BPDE-DNA adducts in human lymphocytes. Mutat Res 2005; 582: 155 – 62
60 *Wilms LC, Boots AW, de Boer VC, Maas LM, Pachen DM, Gottschalk RW et al.* Impact of multiple genetic polymorphisms on effects of a 4-week blueberry juice intervention on *ex vivo* induced lymphocytic DNA damage in human volunteers. Carcinogenesis 2007; 28: 1800 – 6
61 *Veeriah S, Kautenburger T, Habermann N, Sauer J, Dietrich H, Will F et al.* Apple flavonoids inhibit growth of HT29 human colon cancer cells and modulate expression of genes involved in the biotransformation of xenobiotics. Mol Carcinogen 2006; 45: 164 – 74
62 *Veeriah S, Miene C, Habermann N, Hofmann T, Klenow S, Sauer J et al.* Apple polyphenols modulate expression of selected genes related to toxicological defence and stress response in human colon adenoma cells. Int J Cancer 2008; 122: 2647 – 55
63 *Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J.* Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007; 39: 44 – 84
64 *Eberhardt MV, Lee CY, Liu RH.* Antioxidant activity of fresh apples. Nature 2000; 405: 903 – 4
65 *Burda S, Oleszek W.* Antioxidant and antiradical activities of flavonoids. J Agric Food Chem 2001; 49: 2774 – 9
66 *Wolfe KL, Liu RH.* Apple peels as a value-added food ingredient. J Agric Food Chem 2003; 51: 1676 – 83
67 *Lee KW, Kim YJ, Kim DO, Lee HJ, Lee CY.* Major phenolics in apple and their contribution to the total antioxidant capacity. J Agric Food Chem 2003; 51: 6516 – 20
68 *Chinnici F, Bendini A, Gaiani A, Riponi C.* Radical scavenging activities of peels and pulps from cv. Golden Delicious apples as related to their phenolic composition. J Agric Food Chem 2004; 52: 4684 – 9
69 *McGhie TK, Hunt M, Barnett LE.* Cultivar and growing region determine the antioxidant polyphenolic concentration and composition of apples grown in New Zealand. J Agric Food Chem 2005; 53: 3065 – 70
70 *van der Sluis AA, Dekker M, Verkerk R, Jongen WM.* An improved, rapid *in vitro* method to measure antioxidant activity. Application on selected flavonoids and apple juice. J Agric Food Chem 2000; 48: 4116 – 22
71 *Vanzani P, Rossetto M, Rigo A, Vrhovsek U, Mattivi F, D'Amato E et al.* Major phytochemicals in apple cultivars: contribution to peroxyl radical trapping efficiency. J Agric Food Chem 2005; 53: 3377 – 82
72 *Schaefer S, Baum M, Eisenbrand G, Dietrich H, Will F, Janzowski C.* Polyphenolic apple juice extracts and their major constituents reduce oxidative damage in human colon cell lines. Mol Nutr Food Res 2006; 50: 24 – 33
73 *McCann MJ, Gill CI, G OB, Rao JR, McRoberts WC, Hughes P et al.* Anticancer properties of phenolics from apple waste on colon carcinogenesis *in vitro.* Food Chem Toxicol 2007; 45: 1224 – 30
74 *D'Angelo S, Cimmino A, Raimo M, Salvatore A, Zappia V, Galletti P.* Effect of reddening-ripening on the antioxidant activity of polyphenol extracts from cv. `Annurca' apple fruits. J Agric Food Chem 2007; 55: 9977 – 85
75 *Shi D, Jiang BH.* Antioxidant properties of apple juice and its protection against Cr(VI)-induced cellular injury. J Environ Pathol Toxicol Oncol 2002; 21: 233 – 42
76 *Trosko JE, Tai MH.* Adult stem cell theory of the multi-stage, multi-mechanism theory of carcinogenesis: role of inflammation on the promotion of initiated stem cells. Contrib Microbiol 2006; 13: 45 – 65
77 *Lee KW, Lee SJ, Kang NJ, Lee CY, Lee HJ.* Effects of phenolics in Empire apples on hydrogen peroxide-induced inhibition of gap-junctional intercellular communication. Biofactors 2004; 21: 361 – 5
78 *Ulrich CM, Bigler J, Potter JD.* Non-steroidal anti-inflammatory drugs for cancer prevention: promise, perils and pharmacogenetics. Nat Rev Cancer 2006; 6: 130 – 40
79 *Ahn KS, Sethi G, Aggarwal BB.* Nuclear factor-kappa B: from clone to clinic. Curr Mol Med 2007; 7: 619 – 37

80 *Davis PA, Polagruto JA, Valacchi G, Phung A, Soucek K, Keen CL et al.* Effect of apple extracts on NF-kappaB activation in human umbilical vein endothelial cells. Exp Biol Med 2006; 231: 594 – 8
81 *Yoon H, Liu RH.* Effect of selected phytochemicals and apple extracts on NF-kappaB activation in human breast cancer MCF-7 cells. J Agric Food Chem 2007; 55: 3167 – 73
82 *Surh YJ.* Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer 2003; 3: 768 – 80
83 *Aggarwal BB, Shishodia S.* Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol 2006; 71: 1397 – 421
84 *Ramos S.* Cancer chemoprevention and chemotherapy: dietary polyphenols and signalling pathways. Mol Nutr Food Res 2008; 52: 507 – 26
85 *Kern M, Tjaden Z, Ngiewih Y, Puppel N, Will F, Dietrich H et al.* Inhibitors of the epidermal growth factor receptor in apple juice extract. Mol Nutr Food Res 2005; 49: 317 – 28
86 *Fridrich D, Kern M, Pahlke G, Volz N, Will F, Dietrich H et al.* Apple polyphenols diminish the phosphorylation of the epidermal growth factor receptor in HT29 colon carcinoma cells. Mol Nutr Food Res 2007; 51: 594 – 601
87 *Gosse F, Guyot S, Roussi S, Lobstein A, Fischer B, Seiler N et al.* Chemopreventive properties of apple procyanidins on human colon cancer-derived metastatic SW620 cells and in a rat model of colon carcinogenesis. Carcinogenesis 2005; 26: 1291 – 5
88 *Kern M, Pahlke G, Balavenkatraman KK, Bohmer FD, Marko D.* Apple polyphenols affect protein kinase C activity and the onset of apoptosis in human colon carcinoma cells. J Agric Food Chem 2007; 55: 4999 – 5006
89 *Kern M, Pahlke G, Ngiewih Y, Marko D.* Modulation of key elements of the Wnt pathway by apple polyphenols. J Agric Food Chem 2006; 54: 7041 – 6
90 *Yang GY, Liao J, Kim K, Yurkow EJ, Yang CS.* Inhibition of growth and induction of apoptosis in human cancer cell lines by tea polyphenols. Carcinogenesis 1998; 19: 611 – 6
91 *Long LH, Clement MV, Halliwell B.* Artifacts in cell culture: rapid generation of hydrogen peroxide on addition of (–)-epigallocatechin, (–)-epigallocatechin gallate, (+)-catechin, and quercetin to commonly used cell culture media. Biochem Biophys Res Commun 2000; 273: 50 – 3
92 *Lapidot T, Walker MD, Kanner J.* Can apple antioxidants inhibit tumor cell proliferation? Generation of H_2O_2 during interaction of phenolic compounds with cell culture media. J Agric Food Chem 2002; 50: 3156 – 60
93 *Liu RH, Sun J.* Antiproliferative activity of apples is not due to phenolic-induced hydrogen peroxide formation. J Agric Food Chem 2003; 51: 1718 – 23
94 *Veeriah S, Hofmann T, Glei M, Dietrich H, Will F, Schreier P et al.* Apple polyphenols and products formed in the gut differently inhibit survival of human cell lines derived from colon adenoma (LT97) and carcinoma (HT29). J Agric Food Chem 2007; 55: 2892 – 900
95 *Waldecker M, Kautenburger T, Daumann H, Veeriah S, Will F, Dietrich H et al.* Histone-deacetylase inhibition and butyrate formation: Fecal slurry incubations with apple pectin and apple juice extracts. Nutrition 2008; 24: 366 – 74
96 *Sengupta S, Muir JG, Gibson PR.* Does butyrate protect from colorectal cancer? J Gastroenterol Hepatol 2006; 21: 209 – 18
97 *Davie JR.* Inhibition of histone deacetylase activity by butyrate. J Nutr 2003; 133: 2485S – 93S
98 *Meier P, Finch A, Evan G.* Apoptosis in development. Nature 2000; 407: 796 – 801
99 *Vermeulen K, Van Bockstaele DR, Berneman ZN.* Apoptosis: mechanisms and relevance in cancer. Ann Hematol 2005; 84: 627 – 39
100 *Kisselev AF, Goldberg AL.* Proteasome inhibitors: from research tools to drug candidates. Chem Biol 2001; 8: 739 – 58
101 *Chen D, Milacic V, Chen MS, Wan SB, Lam WH, Huo C et al.* Tea polyphenols, their biological effects and potential molecular targets. Histol Histopathol 2008; 23: 487 – 96
102 *Chen MS, Chen D, Dou QP.* Inhibition of proteasome activity by various fruits and vegetables is associated with cancer cell death. In Vivo 2004; 18: 73 – 80
103 *Park SY, Kim EJ, Shin HK, Kwon DY, Kim MS, Surh YJ et al.* Induction of apoptosis in HT-29 colon cancer cells by phloretin. J Med Food 2007; 10: 581 – 6
104 *Gosse F, Roussi S, Guyot S, Schoenfelder A, Mann A, Bergerat JP et al.* Potentiation of apple procyanidin-triggered apoptosis by the polyamine oxidase inactivator MDL 72 527 in human colon cancer-derived metastatic cells. Int J Oncol 2006; 29: 423 – 8
105 *Maldonado-Celis ME, Roussi S, Foltzer-Jourdainne C, Gosse F, Lobstein A, Habold C et al.* Modulation by polyamines of apoptotic pathways triggered by procyanidins in human metastatic SW620 cells. Cell Mol Life Sci 2008; 65: 1425 – 34
106 *Miura T, Chiba M, Kasai K, Nozaka H, Nakamura T, Shoji T et al.* Apple procyanidins induce tumor cell apoptosis through mitochondrial pathway activation of caspase-3. Carcinogenesis 2008; 29: 585 – 93
107 *Hibasami H, Shohji T, Shibuya I, Higo K, Kanda T.* Induction of apoptosis by three types of procyanidin isolated from apple (Rosaceae Malus pumila) in human stomach cancer KATO III cells. Int J Mol Med 2004; 13: 795 – 9
108 *Ovesna Z, Vachalkova A, Horvathova K, Tothova D.* Pentacyclic triterpenoic acids: new chemoprotective compounds. Minirev Neoplasma 2004; 51: 327 – 33
109 *Balavenkatraman KK, Jandt E, Friedrich K, Kautenburger T, Pool-Zobel BL, Ostman A et al.* DEP-1 protein tyrosine phosphatase inhibits proliferation and migration of colon carcinoma cells and is upregulated by protective nutrients. Oncogene 2006; 25: 6319 – 24
110 *Jaenisch R, Bird A.* Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003; 33 Suppl: 245 – 54
111 *Mulero-Navarro S, Esteller M.* Epigenetic biomarkers for human cancer: The time is now. Crit Rev Oncol Hematol 2008; doi:10.1016/j.critrevonc.2008.03.001
112 *Fang M, Chen D, Yang CS.* Dietary polyphenols may affect DNA methylation. J Nutr 2007; 137: 223S – 8S
113 *Fini L, Selgrad M, Fogliano V, Graziani G, Romano M, Hotchkiss E et al.* Annurca apple polyphenols have potent demethylating activity and can reactivate silenced tumor suppressor genes in colorectal cancer cells. J Nutr 2007; 137: 2622 – 8
114 *Percival SS, Bukowski JF, Milner J.* Bioactive food components that enhance $\gamma\delta$T cell function may play a role in cancer prevention. J Nutr 2008; 138: 1 – 4
115 *Holderness J, Jackiw L, Kimmel E, Kerns H, Radke M, Hedges JF et al.* Select plant tannins induce IL-2Ralpha up-regulation and augment cell division in gammadelta T cells. J Immunol 2007; 179: 6468 – 78
116 *Akiyama H, Sato Y, Watanabe T, Nagaoka MH, Yoshioka Y, Shoji T et al.* Dietary unripe apple polyphenol inhibits the development of food allergies in murine models. FEBS Lett 2005; 579: 4485 – 91
117 *Graff JC, Jutila MA.* Differential regulation of CD11b on $\gamma\delta$T cells and monocytes in response to unripe apple polyphenols. J Leukoc Biol 2007; 82: 603 – 7
118 *Ohnishi-Kameyama M, Yanagida A, Kanda T, Nagata T.* Identification of catechin oligomers from apple (*Malus pumila* cv. Fuji) in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and fast-atom bombardment mass spectrometry. Rapid Commun Mass Spectrom 1997; 11: 31 – 6
119 *Akazome Y.* Characteristics and physiological functions of polyphenols from apples. Biofactors 2004; 22: 311 – 4
120 *Shoji T, Akazome Y, Kanda T, Ikeda M.* The toxicology and safety of apple polyphenol extract. Food Chem Toxicol 2004; 42: 959 – 67
121 *Ding M, Lu Y, Bowman L, Huang C, Leonard S, Wang L et al.* Inhibition of AP-1 and neoplastic transformation by fresh apple peel extract. J Biol Chem 2004; 279: 10 670 – 6
122 *Liu RH, Liu J, Chen B.* Apples prevent mammary tumors in rats. J Agric Food Chem 2005; 53: 2341 – 3
123 *Miura D, Miura Y, Yagasaki K.* Effect of apple polyphenol extract on hepatoma proliferation and invasion in culture and on tumor growth, metastasis, and abnormal lipoprotein profiles in hepatoma-bearing rats. Biosci Biotechnol Biochem 2007; 71: 2743 – 50
124 *Barth SW, Fahndrich C, Bub A, Dietrich H, Watzl B, Will F et al.* Cloudy apple juice decreases DNA damage, hyperproliferation and aberrant crypt foci development in the distal colon of DMH-initiated rats. Carcinogenesis 2005; 26: 1414 – 21
125 *Oszmianski J, Wolniak M, Wojdylo A, Wawer I.* Comparative study of polyphenolic content and antiradical activity of cloudy and clear apple juices. J Sci Food Agric 2007; 87: 573 – 9
126 *Barth SW, Faehndrich C, Bub A, Watzl B, Will F, Dietrich H et al.* Cloudy apple juice is more effective than apple polyphenols and an apple juice derived cloud fraction in a rat model of colon carcinogenesis. J Agric Food Chem 2007; 55: 1181 – 7

127 *Ohkami H, Tazawa K, Yamashita I, Shimizu T, Murai K, Kobashi K et al.* Effects of apple pectin on fecal bacterial enzymes in azoxymethane-induced rat colon carcinogenesis. Jpn J Cancer Res 1995; 86: 523 – 9

128 *Pan L, Zessner H, Will F, Klimo K, Frank N, Dietrich H et al.* et al. Natural cloudy apple juice and a polyphenol-enriched apple juice extract prevent intestinal adenoma formation in the App (Min/+) model for colon cancer prevention. Cancer Epidemiol Biomarkers Prev 2005; 14: 2715s

129 *Gerhauser C.* Chemoprävention von Krebs. Forum Deutsche Krebsgesellschaft 2007; Sonderheft; 2007: Fokus Prävention: 5 – 8

130 *Goodlad RA.* Fiber can make your gut grow. Nutrition 2007; 23: 434 – 5

131 *Mandir N, Englyst H, Goodlad RA.* Resistant carbohydrates stimulate cell proliferation and crypt fission in wild-type mice and in the Apc mouse model of intestinal cancer, association with enhanced polyp development. Br J Nutr; 2008: 1 – 11

132 *Owen RW, Thompson MH, Hill MJ, Wilpart M, Mainguet P, Roberfroid M.* The importance of the ratio of lithocholic to deoxycholic acid in large bowel carcinogenesis. Nutr Cancer 1987; 9: 67 – 71

133 *Shimizu J, Yamada N, Nakamura K, Takita T, Innami S.* Effects of different types of dietary fiber preparations isolated from bamboo shoots, edible burdock, apple and corn on fecal steroid profiles of rats. J Nutr Sci Vitaminol (Tokyo) 1996; 42: 527 – 39

134 *Will F, Bauckhage K, Dietrich H.* Apple pomace liquefaction with pectinases and cellulases: analytical data of the corresponding juices. Eur Food Res Technol 2000; 211: 291 – 7

135 *Mehrlander K, Dietrich H, Sembries S, Dongowski G, Will F.* Structural characterization of oligosaccharides and polysaccharides from apple juices produced by enzymatic pomace liquefaction. J Agric Food Chem 2002; 50: 1230 – 6

136 *Sembries S, Dongowski G, Jacobasch G, Mehrlander K, Will F, Dietrich H.* Effects of dietary fibre-rich juice colloids from apple pomace extraction juices on intestinal fermentation products and microbiota in rats. Br J Nutr 2003; 90: 607 – 15

137 *Sembries S, Dongowski G, Mehrlander K, Will F, Dietrich H.* Dietary fiber-rich colloids from apple pomace extraction juices do not affect food intake and blood serum lipid levels, but enhance fecal excretion of steroids in rats. J Nutr Biochem 2004; 15: 296 – 302

138 *Sembries, Dongowski G, Mehrlander K, Will F, Dietrich H.* Physiological effects of extraction juices from apple, grape, and red beet pomaces in rats. J Agric Food Chem 2006; 54: 10 269 – 80

139 *Aprikian O, Duclos V, Guyot S, Besson C, Manach C, Bernalier A et al.* Apple pectin and a polyphenol-rich apple concentrate are more effective together than separately on cecal fermentations and plasma lipids in rats. J Nutr 2003; 133: 1860 – 5

140 *Ko SH, Choi SW, Ye SK, Cho BL, Kim HS, Chung MH.* Comparison of the antioxidant activities of nine different fruits in human plasma. J Med Food 2005; 8: 41 – 6

141 *Chrzczanowicz J, Gawron A, Zwolinska A, de Graft-Johnson J, Krajewski W, Krol M et al.* Simple method for determining human serum 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity – possible application in clinical studies on dietary antioxidants. Clin Chem Lab Med 2008; 46: 342 – 9

142 *Maffei F, Tarozzi A, Carbone F, Marchesi A, Hrelia S, Angeloni C et al.* Relevance of apple consumption for protection against oxidative damage induced by hydrogen peroxide in human lymphocytes. Br J Nutr 2007; 97: 921 – 7

143 *Briviba K, Stracke BA, Rufer CE, Watzl B, Weibel FP, Bub A.* Effect of consumption of organically and conventionally produced apples on antioxidant activity and DNA damage in humans. J Agric Food Chem 2007; 55: 7716 – 21

144 *Mayer B, Schumacher M, Brandstatter H, Wagner FS, Hermetter A.* High-throughput fluorescence screening of antioxidative capacity in human serum. Anal Biochem 2001; 297: 144 – 53

145 *Lotito SB, Frei B.* Relevance of apple polyphenols as antioxidants in human plasma: contrasting *in vitro* and *in vivo* effects. Free Radic Biol Med 2004; 36: 201 – 11

146 *Lotito SB, Frei B.* The increase in human plasma antioxidant capacity after apple consumption is due to the metabolic effect of fructose on urate, not apple-derived antioxidant flavonoids. Free Radic Biol Med 2004; 37: 251 – 8

147 *Lotito SB, Frei B.* Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? Free Radic Biol Med 2006; 41: 1727 – 46

148 *Hoffmann RG, Lim HJ.* Observational study design. Methods Mol Biol 2007; 404: 19 – 31

149 *Feskanich D, Ziegler RG, Michaud DS, Giovannucci EL, Speizer FE, Willett WC et al.* Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. J Natl Cancer Inst 2000; 92: 1812 – 23

150 *Arts IC, Hollman PC, Bueno De Mesquita HB, Feskens EJ, Kromhout D.* Dietary catechins and epithelial cancer incidence: the Zutphen elderly study. Int J Cancer 2001; 92: 298 – 302

151 *Knekt P, Kumpulainen J, Jarvinen R, Rissanen H, Heliovaara M, Reunanen A et al.* Flavonoid intake and risk of chronic diseases. Am J Clin Nutr 2002; 76: 560 – 8

152 *Le Marchand L, Murphy SP, Hankin JH, Wilkens LR, Kolonel LN.* Intake of flavonoids and lung cancer. J Natl Cancer Inst 2000; 92: 154 – 60

153 *Michels KB, Giovannucci E, Chan AT, Singhania R, Fuchs CS, Willett WC.* Fruit and vegetable consumption and colorectal adenomas in the Nurses' Health Study. Cancer Res 2006; 66: 3942 – 53

154 *Deneo-Pellegrini H, De Stefani E, Ronco A.* Vegetables, fruits, and risk of colorectal cancer: a case-control study from Uruguay. Nutr Cancer 1996; 25: 297 – 304

155 *Lee SY, Choi KY, Kim MK, Kim KM, Lee JH, Meng KH et al.* [The relationship between intake of vegetables and fruits and colorectal adenoma-carcinoma sequence]. Korean J Gastroenterol 2005; 45: 23 – 33

156 *Gallus S, Talamini R, Giacosa A, Montella M, Ramazzotti V, Franceschi S et al.* Does an apple a day keep the oncologist away? Ann Oncol 2005; 16: 1841 – 4

157 *Theodoratou E, Kyle J, Cetnarskyj R, Farrington SM, Tenesa A, Barnetson R et al.* Dietary flavonoids and the risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2007; 16: 684 – 93

158 *Lindblad P, Wolk A, Bergstrom R, Adami HO.* Diet and risk of renal cell cancer: a population-based case-control study. Cancer Epidemiol Biomarkers Prev 1997; 6: 215 – 23