

REVIEWS: CURRENT TOPICS

Mechanisms of colorectal and lung cancer prevention by vegetables: a genomic approach

Simone G.J. van Breda, Theo M.C.M. de Kok, Joost H.M. van Delft*

*Department of Health Risk Analysis and Toxicology, Nutrition and Toxicology Research Institute, Maastricht University,
PO Box 616, 6200 MD Maastricht, The Netherlands*

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Abstract

Colorectal cancer (CRC) and lung cancer (LC) occur at high incidence, and both can be effectively prevented by dietary vegetable consumption. This makes these two types of cancer highly suitable for elucidating the underlying molecular mechanisms of cancer chemoprevention. Numerous studies have shown that vegetables exert their beneficial effects through various different mechanisms, but effects on the genome level remain mostly unclear. This review evaluates current knowledge on the mechanisms of CRC and LC prevention by vegetables, thereby focusing on the modulation of gene and protein expressions. The majority of the effects found in the colon are changes in the expression of genes and proteins involved in apoptosis, cell cycle, cell proliferation and intracellular defense, in favor of reduced CRC risk. Furthermore, vegetables and vegetable components changed the expression of many more genes and proteins involved in other pathways for which biologic meaning is less clear. The number of studies investigating gene and protein expression changes in the lungs is limited to only a few *in vitro* and animal studies. Data from these studies show that mostly genes involved in biotransformation, apoptosis and cell cycle regulation are affected. In both colon and lungs, genomewide analyses of gene and protein expression changes by new genomics and proteomics technologies, as well as the investigation of whole vegetables, are few in number. Further studies applying these ‘omics’ approaches are needed to provide more insights on affected genetic/biologic pathways and, thus, in molecular mechanisms by which different chemopreventive compounds can protect against carcinogenesis. Particularly studies with combinations of phytochemicals and whole vegetables are needed to establish gene expression changes in the colon, but especially in the lungs.

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1. Introduction

Food preparation and dietary habits are very relevant exogenous factors affecting cancer risk. The most consistent finding on diet as a determinant of cancer risk prevention is the association between consumption of vegetables and fruits and reduced risk of several cancers. Convincing epidemiological evidence for this preventive action exists for cancer of the gastrointestinal tract and cancer of the respiratory tract [1]. The colon, rectum and lungs are of particular interest in these studies. In terms of

incidence, lung cancer (LC) is the most common cancer worldwide, closely followed by cancer of the colorectum as the third most frequent [2]. Diets containing considerable amounts of a variety of vegetables (>400 g/day) may reduce these types of cancer by 45% and 40%, respectively (range, 30–50%) [3].

The high incidence of colorectal cancer (CRC) and LC, together with epidemiological evidence for effective chemopreventive action, makes these two types of cancer highly suitable for elucidating the underlying molecular mechanisms of cancer prevention by vegetables. These two organ systems differ in respect to the way in which bioactive components of vegetables reach the tissues. In contrast to the lungs, the colon and the rectum are both sites of direct contact (i.e., as vegetables pass through the colon and the

* Corresponding author. Tel.: +31 43 3881092; fax: +31 43 3884146.
E-mail address: j.vandelft@grat.unimaas.nl (J.H.M. van Delft).

rectum, they come into close contact with the intestinal lining). After digestion and absorption in the gastrointestinal tract, substances will first pass the liver, where they may undergo extensive metabolism, before they reach the systemic circulation. As a result, bioactive components originating from dietary vegetables can only reach the lung after this first-pass metabolism.

Numerous studies in different experimental systems have shown that vegetables exert their beneficial effects through various different mechanisms. In many of these postulated mechanisms, the modulation of gene expression plays a role. Since only a limited number of genes have been investigated, mostly in pseudotarget cells such as lymphocytes instead of the ultimate target organ, molecular targets at the genome level are mostly unknown. Nowadays, high-throughput technologies such as microarrays and 2-D gel electrophoresis can be used to investigate the effect of a specific diet on the expression of thousands of genes and proteins in a single experiment. These technologies can help us to identify and characterize the basic molecular pathways of gene regulation by vegetables.

This review provides an overview of present knowledge on molecular mechanisms by which vegetables exert their anticarcinogenic effects by focusing on effects at the genome level on the target organs colorectum and lungs. First, a summary of the target organs colorectum and lungs in terms of cancer epidemiology and cancer etiology and of genetic pathways involved in cancer development will be given. Next, a general overview of mechanisms by which vegetables can protect against cancer, followed by a detailed analysis of protective mechanisms involved in CRC and LC at the genome level, will be given.

2. Epidemiology and etiology

2.1. CRC

Worldwide, CRC is the third most common type of cancer and a major cause of death from cancer. In the year 2000, approximately 943,000 new cases were diagnosed and 491,000 people died of CRC. The peak incidence of the disease is on the seventh decade of life, and it is fairly equally distributed between men and women. Incidence rates vary around the world; the developed world (Europe, Japan, Australia, New Zealand and North America) accounts for almost 65% of the total global incidence. Central and South America, Asia and Africa are areas of low risk, but the incidence is now increasing in these regions [2]. Migrant and temporal trend studies show that rates in migrants from low-risk to high-risk countries tend to increase to the rates of host countries within one or two generations, or sometimes even as early as within the migrating generation itself. This indicates that environmental factors play an important role in the etiology of CRC [4,5].

Increased CRC risk is associated with both dietary factors (intake of excess fat, sugar and alcohol; low amount of

vegetables and fiber) and lifestyle factors (low physical activity, high body mass and smoking) typical of Western societies [2,6–8]. Based on epidemiological studies, Doll and Peto [9] assessed that 90% of CRC mortality was attributed to dietary factors. This estimation was adjusted to 70% (range, 50–80%) by Willett [3] because part of the reduction was explained by physical activity rather than by diet. But it remains clear that CRC can largely be prevented by dietary changes. Especially diets containing considerable amounts of a variety of vegetables (>400 g/day) may reduce the relative risk of CRC by 40% (range, 30–50%) [10].

Apart from the effects of exogenous factors, CRC risk may be influenced by hereditary factors or a combination of genetic and environmental factors. Patients with familial risk (those who have two or more first-degree or second-degree relatives, or both, with CRC) make up approximately 20% of all patients with CRC, whereas about 5% of the total annual burden of CRC is caused by autosomal dominant genetic factors. This last category can be divided into two major forms of hereditary CRC: familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC). FAP (incidence, <0.5%) is an autosomal dominant syndrome associated with mutations of the adenomatous polyposis coli (*APC*) gene, which results in the development of hundreds of polyps by the third decade of life. CRC inevitably occurs unless the colon is prophylactically removed. HNPCC is more common than FAP (incidence, 4–6%) and is a much more heterogeneous syndrome with a 70–90% lifetime risk of developing CRC. It is caused by a germline mutation in any of the mismatch repair (MMR) genes human mutL homolog 1 (*hMLH1*), human mutS homolog 2 (*hMSH2*), human mutS homolog 3 (*hMSH3*), human mutS homolog 6 (*hMSH6*), human postmeiotic segregation 1 (*hPMS1*) or human postmeiotic segregation 2 (*hPMS2*) [11–13]. The experience derived from these two hereditary syndromes has been helpful in advancing our understanding of the molecular basis of CRC.

2.2. LC

LC is the most common malignant disease worldwide and a major cause of death from cancer. It was a rare disease until the beginning of the 20th century. Since then, the occurrence of LC has increased rapidly, and it now accounts for approximately 902,000 new cases each year among men and 337,000 new cases each year among women. An estimated 1,100,000 people died of LC in the year 2000 [14]. In both men and women, the incidence is low before the age of 40 years and increases up to at least 70 years. The difference in incidence between men and women correlates with the difference in the overall pattern of tobacco smoking, which is the predominant risk factor. However, for the same lifetime exposure to tobacco, risks are almost similar in men and women. In men, >80% of LC cases are caused by smoking; in women, the attributable risk is less: about 70% in Northern Europe and 45% worldwide

[14,15]. The geographical and temporal patterns of LC incidence are predominantly determined by the use of tobacco. The highest incidence rates have been recorded in North America, Europe, Japan, Australia, New Zealand and China, and the highest lowest rates have been recorded in South America, Southern Asia and Africa [16].

Next to smoking, several other exposures are known to increase LC risk, including environmental tobacco smoke (passive smoking); occupational exposures to asbestos, arsenic, chloromethyl ethers, chromium VI and nickel; residential and occupational exposures to radon; and exposures to carcinogenic air pollutants [15–17].

Like CRC, LC risk is influenced by dietary [15,18] and genetic factors [19–21]. In 1981, Doll and Peto [9] assessed that 20% of LC mortality was attributed to dietary factors. Willett [3] came to the same conclusion after revising epidemiological studies and taking into account the studies published since. LC can be prevented by dietary changes. Diets high in vegetables and fruits convincingly decrease risk; diets high in selenium, vitamin C and vitamin E possibly decrease risk; and diets high in carotenoids probably decrease risk; whereas diets high in total fat and alcohol possibly increase risk [15,18]. Especially diets containing considerable amounts of a variety of vegetables (>400 g/day) may reduce the relative risk of LC by 45% [10]. So for LC, it seems that the absence of protective dietary factors — rather than the presence of promoting dietary factors — contributes more to the risk.

Evidence from studies on familial aggregation of LC suggests that genetic factors are also involved in human lung tumor development. A positive familial history of LC has been identified as a risk factor [22]. More specifically, segregation analysis of LC proband families indicates that Mendelian codominant inheritance of a rare major autosomal gene is involved [23]. In addition to the gene at this locus, several other genetic factors have been linked to LC susceptibility. Genetic variants or polymorphisms that are hypothesized to affect LC risk have been identified. In particular, genetic differences in the genes cytochrome P4502D6 (*CYP2D6*), cytochrome P4501A1 (*CYP1A1*) and glutathione S-transferase μ (*GSTM1*), which are responsible for the metabolism of tobacco carcinogens, have been implicated in susceptibility to LC [24,25]. Finally, LC also appears at an increased rate in several genetic syndromes, including Li–Fraumeni syndrome, hereditary retinoblastoma (RB), familial breast cancer and Bloom syndrome [20].

3. Genetic pathways involved in carcinogenesis

3.1. CRC: genetic pathways involved in the Vogelstein model

For about a decade, the model proposed by Fearon and Jones [26] and Fearon and Vogelstein [27] has been the paradigm of genetic alterations involved in the development of CRC. In this model, CRC develops as a result of the

progressive accumulation of genetic and epigenetic changes that lead to the transformation of normal colonic epithelium to colon adenocarcinoma, with adenomatous polyp as an intermediate step in this sequence. The loss of genomic stability is an important step in this process as it contributes to the occurrence of mutations.

Sporadic CRC arises mainly through two distinct pathways. In the first pathway, chromosome instability, the initial inactivation of the *APC* tumor-suppressor gene (chromosome 5q), is followed by the accumulation of alterations in additional oncogenes (*K-RAS*, v-ki-ras Kirsten rat sarcoma viral oncogene, on chromosome 12p) and tumor-suppressor genes (chromosome 18q: *DCC*, deleted in CRC; chromosome 17p: *TP53*, p53 gene). The second pathway, associated with microsatellite instability (MSI), occurs in 15–20% of sporadic CRC cases. Alterations have been found to cluster in genes encoding enzymes involved in the repair of DNA mismatches (in particular, *hMLH1* and *hMSH2*). These alterations provide growth advantage and lead to clonal expansion of distorted cells [26–30].

Since the development of the Vogelstein model, much information on the function of key genes in this model, as well as on their interactions, has become available. Additional genetic events and specific molecular pathways that are perturbed by each of the mutations have been identified (Fig. 1). Additionally, the importance of epigenetic mechanisms, such as DNA methylation, that regulate gene expression is becoming increasingly clear [29–32]. The genetic pathways that are most intensively investigated are summarized below.

3.1.1. Wingless/Wnt signaling pathway

One of the central tumor-promoting effects of *APC* mutations is to lead to the overactivation of the Wingless/Wnt signaling pathway, with subsequent expression of genes that favor cell growth. *APC* mutations disrupt the association of *APC* with β -catenin, resulting in excessive amounts of β -catenin and overactivation of the Wnt signaling pathway. Consequently, genes that promote tumor formation are transcribed. Translocation of cytoplasmic β -catenin to the nucleus results in its interaction with other transcription factors such as T-cell factor (TCF)/lymphoid-enhancing factor. TCF-4 is the predominant TCF family member expressed in the colonic epithelium. Relevant targets up-regulated by TCF-4 identified to date include cyclin D1 (*CCND1*), c-myc-binding protein (*MYCBP*), matrilysin (*MMP*), c-jun protein (*c-Jun*), fos-related antigen 1 (*FRA-1*), urokinase-type plasminogen activator receptor (*PLAUR*) and peroxisome proliferator activator receptor δ (*PPAR δ*). Other genes that are regulated by the Wnt signaling pathway and are up-regulated in colon cancer include the WISP genes *WISP-1* and *WISP-3*. Consistent with the concept that overexpression of β -catenin is a central tumor-promoting effect of *APC* mutation, oncogenic mutations in the β -catenin gene (*CTNNB1*) have been observed in some CRC cases [29–32].

COLORECTAL CANCER: GENETIC PATHWAYS INVOLVED IN THE VOGELSTEIN MODEL

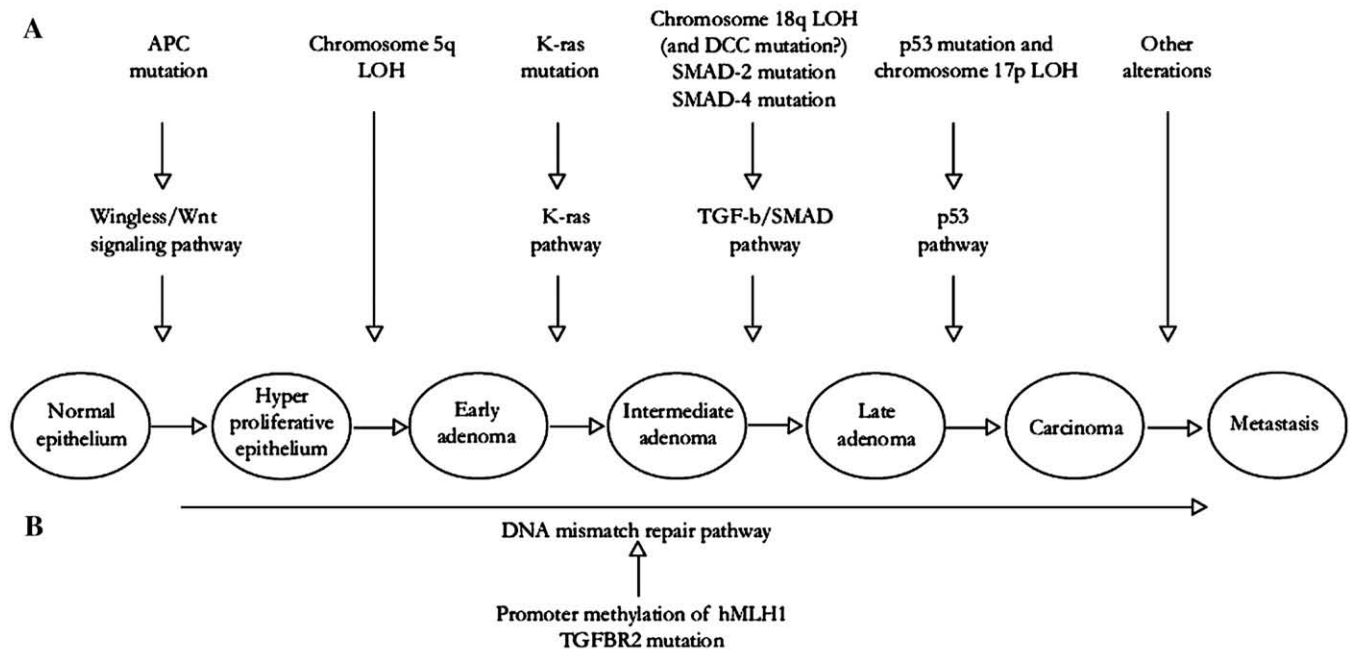


Fig. 1. Overall scheme of key genetic events (genetic alterations and involved pathways) in colorectal tumorigenesis [29–32]. Key genetic events can be grouped into two broad categories: (A) 80–85% of tumors exhibit chromosomal instability; (B) the remaining 15–20% of tumors are characterized by MSI.

3.1.2. *K-RAS* pathway

K-RAS mutations appear to occur after *APC* mutations are formed and are associated with advanced adenomatous lesions. *K-RAS* pathways transduce signals from extracellular growth factors to regulate progression through the cell cycle and proliferation. *K-RAS* mutations are associated with up-regulation of DNA methyltransferase (*DNMT*), *CCND1* and gastrin (*GAST*) [29–32].

3.1.3. Transforming growth factor β /SMAD pathway

Transforming growth factor β (TGF- β) is a multifunctional cytokine that can induce growth inhibition, apoptosis and differentiation in intestinal epithelial cells. The downstream transcriptional targets of TGF- β signaling pathways are the so-called SMAD proteins, which have been shown to regulate genes encoding plasminogen activator inhibitor 1 (*SERPINE1*); the cyclin-dependent kinase inhibitors cyclin-dependent kinase inhibitor 2B (*p15*, *CDKN2B*), cyclin-dependent kinase inhibitor 1A (*p21*, *CDKN1A*) and cyclin-dependent kinase inhibitor 1B (*p27*, *CDKN1B*); *CCND1*; *MYCBP*; and TGF- β itself. Mutational inactivation of *SMAD2* and *SMAD4* has been observed in a high percentage of pancreatic cancer and in 5–10% of colon cancers. Next to *DCC*, these two tumor-suppressor genes are the targets of 18q loss of heterozygosity (LOH) [29–32].

3.1.4. *TP53* pathway

TP53 mutations appear to be late events in the colon adenoma–carcinoma sequence that may mediate the transi-

tion from adenoma to carcinoma. It is expressed at very low levels in cells until it is activated by genotoxic stress via incompletely understood mechanisms. Its activation results in the transcription of genes that directly regulate cell cycle progression and apoptosis. These genes include *CDKN1A*, growth arrest and DNA-damage-inducible (*GADD*; *GADD45*) genes, transformed 3T3 cell double minute-2 p53-binding protein (*MDM2*), *14-3-3- σ* , BCL-2-associated X protein (*BAX*), cyclin G (*CCNG1*) and others. Expression of many of these genes effectively halts DNA replication, induces DNA repair and inhibits angiogenesis [29,30].

3.1.5. DNA MMR pathway

Somatic inactivation of the MMR system additionally gives rise to approximately 15% of sporadic colon cancers. In either instance, the resulting colon cancers display the phenotype of MSI. The predominant mechanism for inactivating MMR was found to be the epigenetic silencing of the *hMLH1* promoter owing to aberrant promoter methylation. The most frequently targeted gene for mutation in this pathway is the TGF- β receptor Type II tumor-suppressor gene (*TGFBR2*). Other less frequently targeted genes include the insulin-like growth factor 2 receptor (*IGF2R*), *BAX* and caspase-5 (*CASP5*), genes encoding enzymes that regulate apoptosis; E2F transcription factor 4 (*E2F4*) and transcription factor 4 (*TCF-4*), transcription factors; mutS homolog 3 (*MSH3*) and mutS homolog 6 (*MSH6*), DNA MMR genes; *RIZ*, the RB protein-interacting zinc finger gene; and caudal type homeobox transcription factor 2 (*CDX2*), an intestinal homeobox

factor. Importantly, MSI and subsequent target gene mutations appear to occur throughout the adenoma-to-carcinoma progression [11,28–30].

3.2. LC: genetic alterations and involved pathways

During the past two decades, considerable effort has been exerted for the identification of genes that drive LC development. Histological apparent LC is due to the sequential accumulation of specific genetic and morphological changes to the normal epithelial cells of the lungs. The major histological types of LC are (1) non-small cell lung cancers (NSCLCs), which represent about 80% of LC and are divided into squamous cell carcinoma, adenocarcinoma, large cell carcinoma and mixed types; and (2) small cell lung cancers (SCLCs), which represent about 20% of all LC cases. Some genetic alterations are specific to one of the two major LC types, and there are those that are common in both [19,33–35]. Until now, no single genetic event or a combination of genetic events has been identified in 100% of LC tissues.

Table 1 gives an overview of the main genetic alterations in LC and the relative abundance in NSCLC and SCLC, respectively [19]. Particularly, these comprise mutations or deletions of tumor-suppressor genes (resulting in inactivation) and proto-oncogenes (resulting in activation) and epigenetic inactivation of these genes via DNA methylation (hypermethylation of gene promoter regions). The role of these genes and their involved pathways are described shortly.

Changes in several tumor-suppressor genes, including *APC* and *TP53* (see Section 3.1 for involved genetic pathways), are related to the development of LC. Distinct from CRC, *APC* is not mutated in LC but is inactivated through hypermethylation of the promoter region [19,34,35].

The p16–cyclin D1–cyclin-dependent kinase 4 (CDK4)–RB pathway is disturbed in both SCLC and NSCLC. This pathway is central to controlling the G₁–S-phase transition of the cell cycle. One of four gene products that comprise this pathway is mutated or functionally altered in many human cancers; the two tumor-suppressor gene products affected in LC are cyclin-dependent kinase inhibitor 2A (*p16*, *CDKN2A*) in NSCLC and RB in SCLC. *CCND1* overexpression can coexist with these changes. RB binds to the E2F transcription factor and, therefore, E2F cannot activate genes needed to initiate S phase. Moreover, this complex also represses the transcription of other target genes. Phosphorylation of RB by cyclin D1/CDK4–6 releases E2F, which initiates the S phase. This pathway can be turned on by mutations inactivating RB, mutations inactivating p16, overexpression of *CCND1* or overexpression of *CDK4*. Other tumor-suppressor genes affected in LC are, for example, the fragile histidine triad (*FHIT*) gene, *RAS* association (RalGDS/AF-6) domain family 1 (tumor-suppressor RASSF1 isoform A; *RASSF1A*) and cadherin 13 (H-cadherin; *CDH13*). These genes undergo

Table 1
Major genetic alterations in LC [19]

	SCLC (%)	NSCLC (%)
Tumor-suppressor genes		
p53 abnormalities		
Mutation with 17p13 LOH	75–100	~50
Abnormal p53 expression	40–70	40–60
p16–cyclin D1–CDK4–RB pathway lesions		
p16 mutation or DNA methylation with 9p21 LOH	<0.1	10–40
Absent p16 expression	0–10	30–70
Absent RB expression with 13q14 LOH	~90	15–30
<i>APC</i> (5q21 LOH) DNA methylation	26	46
Chromosome 3p LOH (several sites)	100	90
RAR-β 3p24 (DNA methylation)	76	40
<i>RASSF1A</i> 3p21.3 (DNA methylation)	90	30–40
<i>FHIT</i> 3p14.2 (DNA methylation and deletion)	64	~50
<i>CDH13</i> (DNA methylation)	20	45
Proto-oncogenes and growth stimulation		
Putative autocrine loops		
	GRP/GRP receptor	<i>HGF/MET</i>
	SCF/KIT	<i>NDF/ERBB</i>
<i>RAS</i> mutation	<1	15–20
MYC amplification	15–30	5–10
Other changes		
BCL-2 expression	75–95	10–35
Telomerase activity	~100	80–85
MSI	~35	~22
Promoter hypermethylation	Marker development	Marker development

RAR-β: retinoic acid receptor β; NDF: new differentiation factor; ERBB1: epidermal growth factor receptor; ERBB2: Her2/neu; BCL-2: breakdown cluster lymphoma antiapoptotic proto-oncogene.

promoter hypermethylation, causing loss of expression. Other tumor-suppressor genes present on chromosome 3p are tumor-suppressor candidate 2 (*TUSC2*), sema domain secreted 3B (*SEMA3B*), cytochrome *b*-561 domain containing 2 (*CYB561D2*) and tumor-suppressor candidate 4 (*TUSC4*), which are affected by LOH [19,25,33–35].

Next to changes in tumor-suppressor genes, proto-oncogenes are affected in such a way that cell growth is stimulated. Furthermore, autocrine and paracrine growth factor stimulatory loops exist in LC. Several, but not all, components of these stimulatory pathways are proto-oncogene products. An important positive signaling pathway is gastrin-releasing peptide (GRP) and its receptor family. This growth-stimulatory loop has a role in lung development and repair, but it also becomes ‘reactivated’ in LC, particularly in SCLC. Another important autocrine loop is the stem cell factor/tyrosine kinase receptor oncogene *KIT* (*SCF/KIT*), which is also active in SCLC. An autocrine loop specific for NSCLC is the hepatocyte growth factor/metastasis proto-oncogene (*HGF/MET*). The *HGF* stimulates the mitogenesis or motogenesis of human bronchial epithelial, alveolar Type II. Furthermore, NSCLC demonstrates abnormalities of the neuregulin receptors v-erb-b2 erythroblastic leukemia viral oncogene homolog 1 (ERBB1) and v-erb-b2 erythroblastic leukemia viral oncogene

homolog 2 (ERBB2), which are part of a family of transmembrane receptor tyrosine kinases. On ligand binding, ERBB receptors homodimerize or heterodimerize, thereby inducing intrinsic kinase activities that initiate intracellular signal transduction cascades, which regulate epithelial proliferation and differentiation. Overexpression of ERBB correlates with increased tumorigenicity of human bronchial epithelial cells and the metastatic potential of NSCLC [19,34].

K-RAS is the most frequently activated *RAS* gene in LC, resulting in inappropriate signaling for continued cell division (see involved pathways in Section 3.1) [19,21,34,35]. *RAS* signaling ultimately activates nuclear proto-oncogenes such as *MYC*, which, on heterodimerization, transcriptionally activates downstream genes that drive cells to grow. Activation occurs by gene amplification or transcriptional dysregulation, both leading to protein overexpression [19,21,34,35].

Other changes relevant to LC development are increased B-cell CLL/lymphoma 2 (*BCL-2*) expression, telomerase activity, MSI and promoter hypermethylation. The product of the *BCL-2* antiapoptotic gene is the key player in apoptosis, which is highly expressed in SCLC, making it less sensitive to apoptosis [19,34].

4. Cancer prevention by vegetables: overview of mechanisms

4.1. General overview

Numerous constituents found in vegetables, including micronutrients (nutritive compounds; e.g., carotenoids, vitamins C and E, folic acid and selenium), dietary fiber and phytochemicals (non-nutritive bioactive compounds with no known nutritional value; e.g., flavonoids, indoles, isothiocyanates and glucosinolates) and interactions among these constituents might contribute to the ability of these foods to reduce cancer risk. Evidence for the cancer-preventing potencies of these agents mainly comes from animal and in vitro studies, which have shown that their capacity to prevent cancer either comes from direct action (such as radical scavenging) or is induced via complex interactions with the body's metabolic and molecular processes [36–39]. It is thought that anticarcinogenic agents need to act in concert with other compounds to prevent cancer ('teamwork'), rather than a single substance being responsible for the protective effect ('magic bullet') [40].

Because cancer is a multistep process occurring over an extended period of time, there is a number of possible stages at which the process could be halted, inhibited, delayed or even reversed [41]. The multiple phases involved in the pathogenesis of cancer offer the basis of most classifications of anticarcinogenic mechanisms of agents present in vegetables [42–45]. Some mechanisms are reiterated several times in different phases of the process, while others are strictly interconnected or partially overlapping. Furthermore,

agents that are possibly responsible have both complementary and overlapping mechanisms of action. Table 2 gives an overview of a selection of proposed anticarcinogenic mechanisms, together with examples of protective agents, their specific mode of action and their vegetable source. Here, the mechanisms are divided into two categories: blocking mechanisms (inhibition of mutation and cancer initiation) and suppressing mechanisms (suppression of promotion, progression, invasion and metastasis).¹ In most of the presented mechanisms, modulation of gene expression may play a role; however, molecular targets at the genome level and involved genetic pathways are mostly unknown.

4.2. Modulation of gene expression by vegetables

Several studies have been conducted to get more insights into mechanisms underlying the prevention of CRC and LC risk by vegetables or vegetable components. Tables 3 and 4 give an overview of in vitro, animal and human studies in which the effect of vegetables or vegetable components on CRC and LC risk, respectively, is investigated by focusing on gene or protein expression changes. In these tables, a distinction has been made between studies investigating gene and/or protein expression changes by conventional methods (such as Northern and Western blot analyses), in which the number of genes and/or proteins investigated at once is limited, and studies using technologies such as microarrays and 2-D gel electrophoresis, in which hundreds to thousands of genes and/or proteins are examined in a single experiment.

Most of the information on gene and protein expression changes in the colon comes from studies on a few predefined genes and/or proteins. Many of these studies focus on gene and protein expression changes related to apoptosis [47–54], cell cycle [47,48,50,52,53], cell proliferation [47,52,54] and/or intracellular defense/biotransformation [49,55–58]. In addition to these processes, microarray studies identify expression changes in genes and/or proteins that are involved in other processes such as cell differentiation [59–61], immune system [59], cell–cell interaction [59,60] and signal transduction [59,60].

Although evidence from epidemiological studies is quite convincing that vegetables and/or vegetable components decrease the risk of LC, only a relatively small number of experimental studies have been conducted in lung tissues or lung cells in relation to the exposure of vegetables or vegetable components. Only a few studies have been carried out: three in vitro studies [62–64] and four animal studies [57,65–67]. Two of the in vivo animal studies used microarrays for gene expression analyses [66,67].

Most in vitro studies on the effect of vegetables on CRC risk use colon carcinoma cell lines as model system and one or more specific vegetable constituents as test compounds.

¹ The classification was taken from Wattenberg [46], supplemented with information from De Flora [43] and Kelloff [44].

Table 2
Anticarcinogenic mechanisms of agents present in vegetables

Process of anticarcinogenesis	Protective agent	Mode of action	Vegetable source	Reference(s)
Blocking mechanisms				
Prevention of carcinogen uptake	Dietary fiber	Adsorption of carcinogens	All vegetables	[113]
Inhibition of nonenzymatic endogenous carcinogen formation	Vitamin C	Scavenging of nitrite to prevent the formation of nitrosamines	All vegetables	[114]
Inhibition of enzymatic carcinogen formation/activation: modulation of biotransformation enzymes	Flavonoids	Inhibition of the conversion of nitrate to nitrite	Allium vegetables	[114]
	Isothiocyanates, indoles	Inhibition of activation by Phase I enzymes; induction of Phase I detoxification and Phase II conjugation pathways	Cruciferous vegetables	[115–117]
	Flavonoids	Inhibition of activation by Phase I enzymes	Allium vegetables	[118,119]
	Organosulfur compounds	Inhibition of Phase I enzymes and induction of Phase II enzymes	Allium vegetables	[120]
Blocking, quenching or scavenging of reactive metabolites	Flavonoids	Direct scavenging of reactive oxygen species and free radicals; inhibition of xanthine oxidase activity	Allium vegetables	[118,119]
	Isothiocyanates	Scavenging of free radicals	Cruciferous vegetables	[121,122]
	Vitamin C	Scavenging of free radicals; quenching of singlet oxygen	All vegetables	[114,123]
	Carotenoids	Quenching of singlet oxygen; protection of DNA nucleophilic sites	Orange and green leafy vegetables	[124–126]
	Vitamin E	Intracellular antioxidant: protection of cell membranes from lipid peroxidation by trapping peroxy radicals	Green vegetables	[127]
Induction of DNA repair and synthesis	Protease inhibitors	Inducing DNA repair by enhancing poly(ADP-ribosyl)transferase	Legumes	[128]
	Flavonoids	Inhibition of DNA topoisomerase II	Allium vegetables	[119]
Suppressing mechanisms				
Inhibition of cell proliferation and replication (cell cycle arrest)	Quercetin	Blocking of G ₁ –S-phase transition of the cell cycle	Allium vegetables	[48,86,129]
	Protease inhibitors	Repression of the <i>NF-κB</i> signaling pathway	Legumes	[130]
	β-Carotene	Inhibition of polyamine metabolism by down-regulating ornithine decarboxylase	Orange and green leafy vegetables	[50,131,132]
	Dietary fiber	Inhibition of secondary bile acids	All vegetables	[113]
Induction of cell apoptosis	Indoles	Inhibition of cyclin-dependent kinase 6	Cruciferous vegetables	[133]
	Flavone	Down-regulation of <i>CCNB</i> and <i>CCNE</i> ; induction of p21	Allium vegetables	[47]
	Isothiocyanates, indoles	Induction of caspases	Cruciferous vegetables	[48,49]
	Flavonoids	Induction of p53 and <i>BAX</i> ; reduction of BCL-2	Allium vegetables	[47]
Induction of cell differentiation	Organosulfur compounds	Inhibition of <i>NF-κB</i> and BCL-X _L ; induction of proapoptotic <i>BAX</i>	Allium vegetables	[120]
	β-Carotene, vitamin A	Up-regulation of retinoid receptor expression; induction of TGF-β	Orange and green leafy vegetables	[134]
Protection of intercellular communication between normal cells	Flavonoids	Inhibition of <i>abl</i> oncogene tyrosine kinase	Allium vegetables	[135,136]
	Carotenoids	Up-regulation of <i>connexin 43</i> gene expression	Orange and green leafy vegetables	[137,138]
Modulation of immune system	Protease inhibitors	Protection of extracellular matrix	Legumes	[139]
	Flavonoids	Inhibition of COX and lipoxygenase activities	Allium vegetables	[118]
Inhibition of impairment of the immune system	Vitamin E	Induction of antibody production, natural killer cell activity and macrophage phagocytosis	Green vegetables	[140]
Stimulation of an efficient and effective immune system	Vitamin C	Production of interferon	All vegetables	[141]
	Vitamin A	Induction of antibody response; proliferation of T-lymphocytes; production of interferon	Orange and green leafy vegetables	[142]
	Carotenoids	Inhibition of NF-κB activity	Orange and green leafy vegetables	[143]
Beneficial effect on hormone status	Apigenin, kaempferol	Inhibition of estrone formation	Allium vegetables	[119]
	Indoles	Increase in estrogen conjugation	Cruciferous vegetables	[144]
Inhibition of tumor-induced neovascularization (angiogenesis)	Isoflavones	Binding to estrogen receptors	Legumes	[145]
	Flavonoids	Inhibition of tyrosine kinases; inhibition of vascular endothelial growth factor and TGF-β expression	Allium vegetables	[118,146]
Inhibition of the metastasis: activation of antimetastatic genes	Indoles	Induction of E-cadherin, α-catenin, β-catenin, γ-catenin and BRCA1	Cruciferous vegetables	[147]

These studies demonstrate that specific vegetable components such as flavonoids, indoles, isothiocyanates and β -carotene are able to induce cell cycle arrest and apoptosis and to reduce cell proliferation, and that this coincides with the specific modulation of genes and/or proteins, such as cyclins and caspases [47–53,59,60,68–70]. In vitro studies using lung cells have mainly focused on gene expression effects at the level of cell cycle and cell growth, induced by the specific vegetable compounds β -carotene, flavone and quercetin [62–64].

A limited number of animal studies have focused on the effect of vegetables or vegetable components on gene and/or protein expression changes in the colon [54–57,61,71,72]. In the majority of these studies, conventional methods are used to measure gene and/or protein expression changes, with specific focus on the modulation of biotransformation genes and enzymes in the colon [54–57,71]. In only two studies were multiple genes and proteins investigated [61,72]. These studies identify changes in gene and protein expressions involved in pathways other than biotransformation, such as apoptosis, DNA repair, polyamine metabolism and cell cycle. In addition, animal studies focus on the effect of vegetables or vegetable components on outcomes other than protein and gene expressions in the colon, such as development of intestinal neoplasia [73–76] and aberrant crypt foci [77]. Only four animal studies in which the effect of vegetables on gene and/or protein expression changes in the lungs has been investigated [57,65–67] have been carried out, two of which used microarrays for gene expression analyses [66,67]. Three of four animal lung studies reported changes in the expression of biotransformation genes and proteins [57,65,67].

In three human studies, the effect of vegetables or vegetable components on the modulation of gene or protein expression is investigated in the colon [56,58,78]. More research has been done in the field of human dietary intervention trials, in which the effect of nutritional interventions on the recurrence of precancerous polyps has been investigated. The results of these trials are, however, disappointing [79–81]. In most of the studies, no reduction in the number of adenomas was found after an intervention with vegetables or components. At the moment, no studies have been conducted to investigate gene and/or protein expression changes in the lungs caused by vegetable consumption in humans. Dietary intervention studies in which the effect of β -carotene, retinol and vitamin E intake on LC occurrence was investigated have been performed [17,18,82–84]. The results of these trials proved, however, the opposite of what was expected. In contrast to reduction of LC risk, smoking subjects taking these supplements experienced a higher risk of LC.

In summary, the number of in vitro, animal and human studies in which the effect of vegetables on gene expression and/or protein expression changes at the target level is examined is limited. Most studies use surrogate tissues, and only a few genes and/or proteins are investigated. The

majority of studies in both colon and lungs identify changes in the expression of genes involved in apoptosis [47–54,59,64,67], cell cycle [47,48,50,52,53,63,64], cell proliferation [47,52,54,62,63,67] and/or intracellular defense and biotransformation [49,55–58,65,67,70,72,78]. The effects of vegetables and/or vegetable components in the colon and lungs on the expression changes of genes and/or proteins involved in these four pathways are now discussed in more detail.

4.2.1. Apoptosis

The number of cells in multicellular organisms is tightly regulated by controlling the rate of cell division and cell death. If cells are no longer needed, they commit suicide by activating an intracellular death program called apoptosis. The cell that undergoes this process dies neatly, without damaging surrounding cells. Stimulation of apoptosis by vegetables and/or vegetable components provides a protective mechanism against cancer by eliminating genetically damaged cells before they can transform to tumor cells. Apoptosis is mediated via an intrinsic pathway in which activation takes place inside the cells' mitochondria or via an extrinsic pathway in which activation occurs from outside the cell by means of cell surface receptors [APO1/CD95 and the tumor necrosis factor (TNF) receptor family]. In the intrinsic pathway, cytochrome *c* is released from the mitochondria into the cytosol, where it binds and activates an adaptor protein called apoptotic-protease-activating factor 1 (APAF1). This protein binds and aggregates procaspase-9 molecules, which leads to the cleavage of these molecules and the triggering of the caspase cascade [85]. The BCL-2 family is an important family of proteins that are the main intracellular regulators of the cell death program. The proteins of this family help to regulate apoptosis. Some members of this family are antiapoptotic, such as BCL-2 itself or BCL-X_L: they inhibit apoptosis, at least partly by blocking the release of cytochrome *c* from the mitochondria. In contrast, the proapoptotic members of the family, such as BAX and BCL-2 antagonist/killer (BAK), promote procaspase activation and cell death [85]. In the extrinsic pathway, activation of the cell surface receptor by an extracellular ligand causes an intracellular region (the death effector domain) to form a cytosolic complex with procaspase-8, followed by the release of caspase-8, which initiates the downstream activation of effector caspase-3 [86], caspase-4, [87], caspase-6 and caspase-7 [86], resulting in programmed cell death [86].

The results of colon studies regarding the effect of vegetables and/or vegetable components on gene and/or protein expression changes involved in apoptosis show changes in genes and proteins involved in both extrinsic and intrinsic pathways [47,48,51,70,72]. These changes involve, among others, the modulation of the expression of genes and proteins of the BCL-2 family [47,48,50,70]. Flavone, a dietary flavonoid, inhibits antiapoptotic BCL-X_L and induces proapoptotic BAK in HT-29 colon cells. This is

accompanied by the inhibition of cyclooxygenase-2 (*COX-2*) and nuclear factor of κ light polypeptide gene enhancer (*NF- κ B*), promoting apoptosis even further [47]. In a successive study using the same model and treatment of flavone, gene and protein expression changes are measured by means of microarrays and 2-D gel polyacrylamide gel electrophoresis, identifying, among others, additional proapoptotic and antiapoptotic genes and proteins, such as the heat shock protein HSTCP1 and plectin (*PLECT*) [59].

Sulforaphane, an isothiocyanate, is able to induce the proapoptotic protein BAX in HT-29 colon cells, which is correlated with cytochrome *c* release from the mitochondria and the presence of a proteolytic fragment of the caspase-3 substrate poly(ADP-ribose)polymerase (PARP), suggesting the activation of caspase-dependent apoptosis [48]. Isothiocyanates are not naturally present in vegetables, but they are generated from secondary glucosinolates during the crushing or chewing of cruciferous vegetable. β -Carotene, which is present in orange and yellow vegetables, is able to decrease the expression of antiapoptotic proteins BCL-2 and BCL-X_L in a dose-dependent manner in COLO 320 HSR colon cells, leading to the induction of apoptosis [50]. Finally, quercetin, a dietary flavonoid present in, for instance, onions, is able to modulate several apoptosis-related genes in CO115 colon cells — induction of proapoptotic *BAX* and reduction of antiapoptotic *BCL-2LII*, among others [70]. In addition to these two members of the BCL-2 family, quercetin up-regulates the expression of the proapoptotic genes BCL-2/adenovirus E1B 19-kDa-interacting protein 3-like (*BNIP3L*), tumor protein p53-inducible nuclear protein 1 (*TP53INP1*), tumor protein p53-inducible protein 3 (*TP53I3*), TNF receptor superfamily member 10D (*TNFRSF10D*), *CASP8*, TNF receptor superfamily member 6 (*TNFRSF6*), *APAF1*, myxovirus (influenza virus) resistance 1 (*MX1*) and programmed cell death 4 (*PDCD4*), and it down-regulates the expression of the antiapoptotic genes helicase lymphoid-specific HELLS (*HELLS*) and baculoviral IAP repeat containing 5 (*BIRC5*) [70]. Three of four reported studies on changes involved in the BCL-2 family of proteins investigate *TP53* gene expression, and, remarkably, no change is detected, although the expression of its targets changes considerably [47,48,70]. The effect of quercetin on gene expression changes was also investigated in Caco-2 cells by van Erk et al. [60]. Only six genes [i.e., *APAF1*; *CASP1*; secreted frizzled-related protein 1 (*SFRP*); tumor protein p53-binding protein 2 (*TP53BP2*); serine or cysteine proteinase inhibitor, clade B member 3 (*SERPINB3*); and serine or cysteine proteinase, clade B member 9 (*SERPINB9*)] involved in apoptosis are modulated, and modulation is directed towards both apoptosis induction and reduction. In addition to gene and protein expression changes involved in apoptosis in the colon, quercetin modulated apoptotic-related proteins in NCI-H209 lung cells. After quercetin treatment of these cells, concurrent decrease in mitochondrial membrane potential, release of cytochrome *c*, up-regulation of *BAX*,

down-regulation of BCL-2 and activation of *CASP3*, followed by cleavage of PARP, are observed, leading to apoptosis [64].

In a study of Hu et al. on the induction of apoptosis in HT-29 colon cells by phenethyl isothiocyanate (PEITC), it was demonstrated that three mitogen-activated protein kinase (MAPK) signaling pathways are induced: c-Jun N-terminal kinase (JNK), extracellular-signal-regulated protein kinase (ERK) and p38 kinase, which leads to cytochrome *c* release. In the HT-29 cell line, the activation of JNK appears to be critical for the initiation of the apoptotic process. Caspase-9 and caspase-3 are then subsequently activated, followed by nucleus condensation and DNA fragmentation, which are hallmarks of apoptosis [51]. Another isothiocyanate, isothiocyanate sulforaphane, is also able to induce ERK, JNK and p38 in HT-29 colon cells [53]; however, no apoptosis was measured in this study. The effect of PEITC is investigated in other cell lines (i.e., HCT-116 and HCT15 colon cells) [69]. Several *GADD* genes are up-regulated, among which are *GADD45*, *GADD34* and *GADD153*. Up-regulation of these genes is accompanied by morphological changes such as condensed and fragmented chromatin, which are also characteristic of apoptosis. It is, however, unclear how these proteins induce apoptosis [69].

Instead of investigating a single vegetable compound, another study investigates the effect of different doses of a mixture of four vegetables and of individual vegetables present in the mixture (i.e., cauliflower, carrots, peas and onions) on the colon and lungs of female C57BL/6 mice [67,72]. In the colon mucosa of mice receiving the highest vegetable dose diet, the *TNFRSF6*, *CASP4*, *CASP7*, *CASP3*, cathepsin B (*CTSB*), thymosin β 10 (*TMSB10*) and signal transducer and activator of transcription 1 (*STAT1*) genes are up-regulated, theoretically leading to increased apoptosis [72]. *TNFRSF6* is part of the TNF receptor family, facilitating the extrinsic apoptotic pathway. *CASP4*, *CASP7* and *CASP3* encode for proteins that are part of the caspase cascade leading to apoptosis. *CTSB* can trigger the mitochondrial pathway of apoptosis. This protease is released from lysosomes into the cytosol during intracellular cytotoxic signaling cascades, thereby increasing cytochrome *c* release from the mitochondria [88]. Cytochrome *c* forms a protein complex with *APAF1* that recruits procaspase-9, resulting in its activation. *CASP9* activates *CASP3*, initiating a caspase cascade that leads to cell death [86,89]. *CTSB* can also trigger apoptosis in a caspase-independent pathway, whereby *CTSB* functions as the dominant execution protease [90]. This mechanism could represent a backup program in tumor cells because here the standard apoptosis pathway is frequently impaired and the expression of *CTSB* is increased. *TMSB10*, a small actin-binding protein, has dual functions: programmed cell death [91] and tumor invasion [92]. The mechanism by which *STAT1* induces apoptosis is not clear, but may involve indirect up-regulation of *TNFRSF6* and caspase activation [93]. In a successive study by Breikers

Table 3

Selection of in vitro, animal and human colon studies involved in the modulation of gene or protein expression by vegetables or vegetable components

Model system	Vegetable or vegetable component tested	Main results [(1) gene and/or protein expression changes; (2) additional measurements]	Involved mechanism(s) expression changes	Reference(s)
(1A) In vitro studies using single gene and/or protein analyses				
HT-29 cells	Flavone (150 μ M)	(1) Inhibition of <i>COX-2</i> , <i>NF-κB</i> , <i>CCNE</i> , <i>CCNB</i> and antiapoptotic <i>BCL-X_L</i> ; induction of <i>p21</i> and proapoptotic <i>BAK</i> ; no change in <i>TP53</i> gene expression (2) Reduced cell proliferation; induction of cell differentiation and apoptosis; cell cycle arrest in post-G ₁ phase	Cell cycle, apoptosis Cell proliferation	[47]
HT-29 cells	Sulforaphane (15 μ M) (isothiocyanate)	(1) No change in p53; induction of proapoptotic <i>BAK</i> ; induction of <i>CCNA</i> and B1 protein expression (2) Induction of apoptosis and cell cycle arrest	Apoptosis Cell cycle	[48]
COLO 205 cells, COLO 320 cells, COLO 201 cells, LS-174 cells, WiDr cells, primary colorectal tumors	Quercetin (10 μ M)	(1) Inhibition of <i>p21-RAS</i> , <i>K-RAS</i> , <i>N-RAS</i> and <i>H-RAS</i> gene and protein expressions	Cell growth	[68]
LS-174 cells, Caco-2 cells	Four different indoles; three different isothiocyanates (IC ₅₀ concentration)	(1) Induction of <i>CYP1A1</i> gene and protein expressions by indoles; induction of AKR1C1 protein and gene expressions by isothiocyanates; GCS _h protein expression by isothiocyanates; induction of NQO1 protein and gene expressions by both indoles and isothiocyanates (2) Less DNA damage after preincubation; induction of apoptosis	Apoptosis Intracellular defense	[49]
COLO 320 cells, HSR cells, LS-174 cells, HT-29 cells, WiDr cells	β -Carotene (0–50 μ M)	(1) Inhibition of the expression of the proteins <i>CCNA</i> , <i>BCL-2</i> and <i>BCL-X_L</i> ; no changes in <i>p21</i> , <i>p27</i> and <i>BAX</i> (2) Inhibition of cell growth; induction of cell cycle arrest; induction of apoptosis	Cell cycle Apoptosis	[50]
HT-29 cells	PEITC (5–50 μ M)	(1) Induction of the expression of the proteins JNK, c-Jun, N-terminal kinase, ERK, p38 kinase (2) Induction of apoptosis; activation of <i>CASP3</i> and <i>CASP9</i> ; release of cytochrome <i>c</i>	Apoptosis	[51]
HCT-116 cells, DLD-1 cells	NI3C and I3C, at several different concentrations and time points	(1) Increase in <i>p21</i> and <i>CCNE1</i> and decrease in <i>CDK4</i> and <i>CDC2</i> gene expressions by NI3C; increase in p21 and decrease in CDK2 protein expressions by NI3C and I3C; p27 protein expression increased by NI3C; <i>CCNB</i> level decreased by I3C and NI3C (2) Reduced cell growth; NI3C more potent than I3C; accumulation of cells in G ₂ –M phases and delay in G ₁ –S phases by NI3C and in G ₀ –G ₁ phases by I3C; induction of apoptosis by I3C	Cell cycle, cell proliferation, apoptosis	[52]
HT-29 cells	Isothiocyanate sulforaphane (6.25, 12.5, 25, 50 and 100 μ M for 12 and 24 h)	(1) Inhibition of <i>CCND1</i> , <i>CCNA</i> and <i>MYCBP</i> protein and gene expressions; induction of p21 gene and protein expressions; activation of MAPK pathways, including ERK, JNK and p38 protein expressions; no change in <i>p27</i> gene expression (2) Reduction in cell growth; induction of cell cycle arrest in G ₁ phase	Cell growth, cell cycle, apoptosis	[53]
(1B) In vitro studies using microarrays and/or 2-D gel protein analyses				
HCT-116 cells, HCT-15 cells	Phenethylisothiocyanate (0 or 100 μ M for 0 or 48 h)	(1) Increased <i>GADD</i> gene expression (2) Induction of apoptosis; induction of DNA strand breaks	Apoptosis	[69]
HT-29 cells	Flavone (150 μ M)	(1) 488 mRNAs were modulated at least twofold compared with control; 25 proteins differed at least twofold compared with control — 20 of them could be identified by MALDI-TOFMS, including, for example, annexin II, proteins of the citric acid cycle, catalase, protein kinase C- β 488 mRNAs were at least twofold changes compared to control; NADH-dehydrogenase, annexin II, apolipoprotein, protein disulfide isomerase, HSTCP1, succinate dehydrogenase, 3 α -hydroxysteroid dehydrogenase and catalase were regulated on both protein and mRNA levels in the same direction (2) Induction of caspase-3 activity and nuclear fragmentation	Cell cycle, cell growth, cell differentiation, cytoskeleton, cell–cell interaction, immune system, transcription, signaling, metabolism, apoptosis	[59]

Table 3 (continued)

Model system	Vegetable or vegetable component tested	Main results [(1) gene and/or protein expression changes; (2) additional measurements]	Involved mechanism(s) expression changes	Reference(s)
Caco-2 cells	Quercetin (5 and 50 μ M) for 48 h	(1) 150–200 genes were differentially expressed by 5 and 50 μ M quercetin; genes were grouped in categories (2) Reduction of cell growth; induction of cell cycle arrest in S phase	Cell cycle, differentiation, cell–cell interaction, apoptosis, transcription, signal transduction, energy metabolism	[60]
CO115 cells	Quercetin (10 or 100 μ M for 24 or 48 h)	(1) Genes involved in cell cycle control (e.g., <i>CDKN2B</i> , <i>CDKN1C</i> , <i>CDKN1A</i>), apoptosis (e.g., <i>PDCD2</i> , <i>BAX</i> , <i>TNFRSF6</i>) and xenobiotic metabolism (e.g., <i>NQO1</i> , <i>AKR1C1</i> , <i>EPHX1</i>) were significantly modulated (2) Inhibition of cell growth	Cell growth, apoptosis, cell cycle, biotransformation of xenobiotics	[70]
(2A) Animal studies using single gene and/or protein analyses				
Wistar rats	10% broccoli diet for 1 week	(1) Induction of CYP1A1 mRNA and protein; reduction of <i>CYP11B</i> mRNA; induction of <i>CYP11B</i> protein	Intracellular defense	[55]
ICR(Ha) mice	Lyophilized broccoli (1 g/kg, po); sacrifice at 1 and 2 days posttreatment	(1) Induction of GST- μ and GST- π activity and protein	Intracellular defense	[56]
Wistar rats	Organosulfur compounds: diallyl sulfide, diallyl disulfide, dipropyl sulfide, or dipropyl disulfide (1 mmol/kg/day) by gavage (4 days)	(1) Induction of UGT and MEH protein expression by diallyl disulfide; induction of QR and GST activity by diallyl disulfide and dialyl sulfide	Intracellular defense	[57]
C57BL/6j-Min/+ mice	Diets for 9 weeks: caffeic acid phenethyl ester (CAPE; 0.03% or 0.15%), curcumin (0.1%), quercetin (2%) or rutin (2%)	In CAPE-fed and curcumin-fed mice: (1) Inhibition of β -catenin protein expression in enterocytes (2) Increased enterocyte apoptosis; proliferation of enterocytes back to normal levels; decreased tumor formation	Apoptosis, cell proliferation, Wnt signaling pathway	[54]
C57BL/6 mice	20% soybean diet for 14 weeks or 250 μ g of genistein, po (single dose); sacrifice at 24 h	(1) Induction of CYP27B1 and inhibition of CYP24 gene and protein expressions after both the soybean diet and the genistein gavage	Synthesis of 1,25-dihydroxyvitamin D ₃	[71]
(2B) Animal studies using microarrays and/or 2-D gel protein analyses				
C57BL/6 mice	8 different diets for 2 weeks: (1) control (no vegetables) diet; a diet containing (2) 10%, (3) 20% or (4) 40% of the vegetable mixture; a diet containing individual vegetables present in the mixture: (5) 7% cauliflower, (6) 7.3% carrots, (7) 22.6% peas or (8) 3.1% onions (lyophilized)	(1) 39 genes were differentially expressed between the control group and one of the groups receiving the vegetable mixture, including genes involved in biotransformation (e.g., <i>SULT1A1</i> , <i>GSTA2</i>), apoptosis (e.g., <i>TNFRSF6</i> , <i>CASP3</i> , <i>CASP4</i> , <i>CASP7</i>), polyamine metabolism (<i>OAT</i>) and DNA repair (<i>RAD51</i>); 18 genes were differentially expressed between the control group and one of the groups receiving individual vegetables, including genes involved in polyamine metabolism (<i>SAT</i> , <i>ODC1</i>), tumor invasion (e.g., <i>MYBBP1a</i>), homeostasis (e.g., <i>HSPD1</i>), cell cycle (<i>CDKN1A</i>) and DNA metabolism (<i>TOP2A</i>)	Apoptosis, biotransformation of xenobiotics, DNA repair, polyamine metabolism, tumor invasion, homeostasis, cell cycle, DNA metabolism	[72]
C57BL/6 mice	4 different diets for 2 weeks: (1) control (no vegetables) diet; a diet containing (2) 10%, (3) 20% or (4) 40% of the vegetable mixture (lyophilized)	(1) 30 proteins were differentially expressed in one or more vegetable diets compared to control; six proteins could be identified by MALDI-TOFMS: myosin regulatory light chain, carbonic anhydrase I, high-mobility group protein 1, pancreatitis-associated protein 3, <i>GAPDH</i> , ATP synthase oligomycin sensitivity conferral protein	Transcription, differentiation, apoptosis homeostasis	[61]
(3A) Human studies using single gene and/or protein analyses				
Human subjects	300 g/day brussels sprouts	(1) Induction of GST- μ and GST- π protein expression levels in the rectum	Intracellular defense	[58]
Human subjects at increased risk	Broccoli-supplemented diet (tablets; 6 g/day) or cruciferous-free diet for 14 days	(1) No difference in GST- μ and GST- π protein activity	–	[56]
(3B) Human studies using microarrays and/or 2-D gel protein analyses				
Adenoma patients and healthy controls	Low (i.e., 75 g/day) or high (i.e., 300 g/day) diet of four different vegetables (i.e., cauliflower, carrots, peas and onions) for 2 weeks	(1) 58 different genes were differentially expressed after the intervention, of which 17 responded similarly between patients and controls; 20 genes were known to be involved in (colon) carcinogenesis according to literature review, including, for example, <i>PKCB1</i> , <i>CHK1</i> , <i>CCNG1</i> , <i>C-FOS</i> , <i>ODC1</i> , <i>C-MYC</i> , <i>COX-2</i> , <i>CYP2C9</i> , <i>CYP2C19</i> , <i>CYP2D6</i> , <i>CYP3A4</i> , <i>CYP27B1</i>	Cell cycle, polyamine metabolism, biotransformation of xenobiotics, cell proliferation	[78]

MALDI-TOFMS: matrix-assisted laser desorption initiation time-of-flight mass spectrometry.

Table 4

Selection of in vitro, animal and human lung studies involved in the modulation of gene or protein expression by vegetables or vegetable components

Model system	Vegetable or vegetable component tested	Main results [(1) gene and/or protein expression changes; (2) additional measurements]	Involved mechanism(s) expression changes	Reference(s)
(1A) In vitro studies using single gene and/or protein analyses				
C10, E9 and 82-132 murine lung epithelial cells	β -Carotene (1–10 μ M for 1–5 days)	(1) No effect on connexin 43 protein expression in C10, E9 and 82-132 cells; increased connexin 43 protein expression in C3H10T1/2 murine fibroblasts	Cell growth, intercellular communication	[62]
C3H10T1/2 murine fibroblasts		(2) No effect on growth and gap junctional intercellular communication in C10, E9 and 82-132 cells; growth reduction and enhancement of GJIC in C3H10T1/2 murine fibroblasts		
A549 human lung adenocarcinoma cells	Flavone (30, 60 and 100 μ M for 24, 48 or 72 h)	(1) Induction of p21 mRNA and protein; inhibition of RB protein phosphorylation	Cell growth, cell cycle	[63]
NCI-H209 lung carcinoma cells	Quercetin	(1) Increased expression of CCNB, CDC25c-ser-216-p and WEE1 proteins; up-regulation of <i>BAX</i> ; down-regulation of <i>BCL-2</i> ; activation of <i>CASP3</i>	Cell cycle, apoptosis	[64]
		(2) Cell cycle arrest in G ₂ -M and sub-G ₀ -G ₁ phases; induction of apoptosis and reduction of cell proliferation; release of cytochrome c; cleavage of PARP	Cell proliferation	
(2A) Animal studies using single gene and/or protein analyses				
Wistar rats	Organosulfur compounds: dialyl sulfide, dialyl disulfide, dipropyl sulfide, or dipropyl disulfide (1 mmol/kg) by gavage daily (4 days)	(1) Induction of UGT, MEH, GST activity and QR by dialyl disulfide	Biotransformation of xenobiotics	[57]
Sprague–Dawley rats	Glucoraphanin (120 or 240 mg/kg) by gavage in a single dose or daily for 4 days	(1) Induction of UGT and GST; induction of CYP1A1, CYP1A2, CYP3A1, CYP3A2, CYP2B1, CYP2B2 and CYP2C11 enzymes (2) Induction of reactive oxygen species	Biotransformation of xenobiotics	[65]
(2B) Animal studies using microarrays and/or 2-D gel protein analyses				
C57BL/6 mice	8 different diets for 2 weeks: (1) control (no vegetables) diet; a diet containing (2) 10%, (3) 20% or (4) 40% of the vegetable mixture; a diet containing individual vegetables present in the mixture: (5) 7% cauliflower, (6) 7.3% carrots, (7) 22.6% peas or (8) 3.1% onions (lyophilized)	(1) 18 genes were differentially expressed between the control group and one of the groups receiving the vegetable mixture, including genes involved in cell growth (<i>IGFBP3</i>), apoptosis (<i>IGFBP3</i> , <i>GAPDH</i> , <i>TGM2</i>), metabolism (<i>TOP2A</i> , <i>GLUL</i>) and immune response (<i>FCER1G</i> , <i>CTSS</i>); 11 genes were differentially expressed between the control group and one of the groups receiving individual vegetables, including <i>GLUL</i> , <i>CTSS</i> , <i>SLC6A4</i> , <i>HPGD</i> , <i>SULT1A1</i> , <i>SELENBP1</i> , <i>HBA-A1</i> , <i>GSR</i> , <i>LDLR</i> , <i>BNIP1</i> , <i>G1P2</i>	Apoptosis, cell growth, DNA metabolism, immune response, biotransformation	[67]
Sprague–Dawley rats	Oltipraz (OPZ) 400 mg/kg; PEITC 500 mg/kg; 5,6-benzoflavone (5,6-BF) 500 mg/kg; I3C 2500 mg/kg diet; <i>N</i> -acetyl-L-cysteine (NAC) 1000 mg/kg bw; PEITC+I3C; OPZ+NAC; in combination with environmental cigarette smoke, for 28 days	(1) The expression of 4858 genes was measured to assess the safety of the different compounds; no individual gene differences were reported (2) Inhibition of DNA adducts induced by environmental cigarette smoke (5,6-BF, I3C, PEITC and NAC)	–	[66]

Until today, no human studies have been reported (Medline; accessed February 2007).

et al., the effect induced by different doses of vegetables on protein changes is examined. Only a few proteins are identified, among which is glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) [61]. This protein displays wide functional diversity, including apoptosis, although the

mechanism is not clear [94]. In addition to investigations into the colon of these mice, gene expression changes in the lungs were examined [67]. In the lungs of mice receiving the highest vegetable dose, three apoptosis-related genes were modulated: insulin-like growth factor binding protein 3

(*IGFBP3*), *GAPDH* and transglutaminase 2 C polypeptide (*TGM2*). The protein encoded by *IGFBP3* belongs to one of six members of the IGFBP family and regulates the activity of insulin-like growth factor 1 (IGF-1), thereby inhibiting the mitogenic and antiapoptotic actions of IGF-1. *IGFBP3* also inhibits cell growth and induces apoptosis independently of IGF-1 by inhibiting the p13K/Akt/PKB and MAPK signaling pathways [95]. In mice, IGFBP-3 inhibited the growth of lung tumors and, in humans, a higher IGFBP-3 level in serum was associated with a lower LC risk. In vitro and in vivo, the expression of *IGFBP3* was induced by dietary factors such as lycopene, retinoic acid, flavonoids and green tea [96]. However, in the lungs of mice receiving a vegetable mixture, *IGFBP3* was down-regulated. Possible constituents other than lycopene, retinoic acid and flavonoids were present in the vegetables, which had a down-regulating effect on the expression of this gene. Next to *IGFBP3*, two other apoptosis genes were modulated in this study: *GAPDH* and *TGM2*. The mechanism by which *GAPDH* modulates apoptosis in the lungs is also not clear. Increased *TGM2* expression is associated with apoptosis, probably by the induction of massive Ca^{2+} -mediated intracellular cross-linking [97]. Furthermore, it has been found that *TGM2* was up-regulated in bronchial epithelial cells by retinoic acid, which has a role in cancer treatment and prevention by inducing growth suppression [98]. *GAPDH* was up-regulated and *TGM2* was down-regulated in the highest vegetable group in the current study. The effect of modulation by the vegetable mixture on *IGFBP3* and *TGM2* is expected to be reduction of apoptosis, which is not in line with the proposed hypothesis of cancer risk prevention by vegetables. In addition to the investigation of the vegetable mixture on gene expression changes in the colon and lungs of these mice, the effects of individual vegetables present in the mixture were investigated. Cauliflower was able to increase the expression of BCL-2/adenovirus E1B 19-kDa interacting protein 1 (*BNIP1*). The protein product of this gene interacts with members of the BCL-2 family, which consists of proapoptotic (such as *BAX*) and antiapoptotic (such as BCL-2) proteins. It is suggested that *BNIP1* displays proapoptotic properties by interacting with BCL-2 and BCL-2L1 [99,100]. It was shown that *BNIP1* was down-regulated in mouse lung tumor tissues, possibly leading to reduced apoptosis of tumor cells [101]. However, the precise function of *BNIP1* is unclear [99,100]. Up-regulation of *BNIP1* by cauliflower could lead to increased apoptosis, which is generally regarded as a protective mechanism against cancer, by removing genetically damaged lung cells before they can undergo clonal expansion.

In conclusion, in the colon, vegetables and vegetable components are able to modulate genes and proteins involved in both the extrinsic and the intrinsic pathways of apoptosis in such a way that mostly an increase in apoptosis is observed or expected. In the lungs, however, the number of investigations is limited, and effects on apoptosis are not unanimous. No clear conclusions can be

drawn from these studies. Furthermore, in addition to effects on genes and/or proteins within the intrinsic and extrinsic pathways, the expressions of other proapoptotic and antiapoptotic genes and proteins are changed in the colon and lungs, of which the precise mechanism of action remains unclear.

4.2.2. Cell cycle and cell proliferation

Apoptosis is often accompanied by arrest of the cell cycle, thereby reducing proliferation signals in the cell. The cell grows continuously in the interphase, which consists of three phases: DNA replication is confined to S phase, G_1 is the gap between M phase and S phase, while G_2 is the gap between S phase and M phase. In M phase, first, the nucleus and then the cytoplasm divide. Cell cycle progression in G phases can be regulated by various intracellular and extracellular signals. If environmental conditions are unfavorable, cells delay progress through G phase, in which they can stay for different time periods before resuming proliferation. The different processes that take place in the cell cycle are highly controlled by the cell cycle control system. This system can arrest the cell cycle at specific checkpoints (e.g., if the cell cycle event has not been completed yet as well by signals from the environment). Key components of the cell cycle control system are formed by CDKs and cyclins. The complex of cyclin with CDK triggers cell-specific events [85]. Several studies have investigated the effect of vegetables and/or vegetable components on the expression of cell-cycle-related genes and/or proteins [47,48,50,52–54,59,60,63,64,68,70,72,78], and most of them have also measured cell cycle arrest [47,48,50,52–54,60,63,64,70].

Colon cells incubated with flavone [47], sulforaphane [48], β -carotene [50] and *N*-methoxyindole-3-carbinol (NI3C) [52] accumulate in the G_2 –M phases of the cell cycle, and this is accompanied by expression changes of genes and/or proteins involved in these phases. Both flavone [47] and NI3C [52] are able to induce *p21* expression, which encodes for a Cdk inhibitor. It inhibits the cell division cycle 2 (*CDC2*)–cyclin B (*CCNB*) complex, thereby inhibiting the transition of G_2 phase into M phase. *CCNB* is down-regulated by both flavone [47] and NI3C [52], and *CDC2* is down-regulated by NI3C, thereby hampering transition even more. The cell cycle arrest in G_2 –M phases induced by sulforaphane is accompanied by increased levels of cyclin A (*CCNA*) and cyclin B1 (*CCNB1*), which are proteins that regulate *CDC2* kinase activity in this phase [48]. In contrast, *CCNA* is inhibited by β -carotene in colon cells, but is nevertheless accompanied by G_2 –M-phase cell cycle arrest. No change in *p21* and *p27* protein expression is observed [50]. Down-regulation of *CCNA* is also observed in the colon of human subjects receiving a 50% decreased vegetable diet (i.e., 75 g/day) for 2 weeks. The diet consisted of a mixture of four different vegetables (i.e., cauliflower, carrots, peas and onions) [78]. Induction of *p21* gene expression is observed in mice receiving a diet containing

cauliflower, carrots or peas for 2 weeks. No cell cycle assay is used in this study [72]. Quercetin is also able to modulate several genes involved in the G₂–M phase: down-regulation of the mitosis-promoting genes polo-like kinase 1 (*PLK1*), CDC20 cell division 20 homologue (*CDC20*), transcription factor 19 (*TCF-19*), FYVE Rho GEF and PH domain containing 2 (*FGD2*) and budding uninhibited by benzimidazole 1B (*BUB1B*), which is accompanied by a reduction in the cell growth of CO115 colon cells [70].

Besides the induction of G₂–M-phase cell cycle arrest, vegetables and/or vegetable components are able to stop progression in other phases. Flavone blocks the S-phase entry of HT-29 colon cells, which is accompanied by a decrease in cyclin E (*CCNE*) expression. This cyclin is needed to activate cyclin-dependent kinase 2 (CDK2), which promotes S-phase entry [47]. Delayed G₁–S-phase transition is observed by NI3C treatment. This is accompanied by increased *p21*, *p27* and *CCNE* expressions and reduced expression of *CDK4*. Both p21 and p27 inhibit the CDK2/CCNE complex, and p21 inhibits the activity of CDK4 leading to reduced activation of the RB protein, which regulates the transcription of S-phase genes, including *CDC2* [52]. In A549 lung cells, flavone induced cell cycle arrest in G₁ and reduction in cell growth, which is accompanied by increased p21 gene and protein expressions and decreased phosphorylation of the RB protein, which could explain the observed arrest. These effects occur independently of p53 [63], which is normally responsible for the transcription of *p21*. These results indicate that the increase in *p21* gene expression can be regulated through other means.

In HT-29 colon cells, sulforaphane blocks the cell cycle in G₁ phase, which goes along with the down-regulation of cell-proliferation-related *MYCBP* gene and protein expressions; the down-regulation of positive cell cycle regulator (such as *CCND1*, *CCNA* and *CCNE*) gene and protein expressions; and the up-regulation of negative cell cycle regulator (such as p21) gene and protein expressions [53]. In addition, indole-3-carbinol (I3C) is able to arrest colon carcinoma cells in G₀–G₁ phases, in combination with decreased CCNB1 expression and increased p21 expression [52].

In addition to the modulation of G₂–M-phase gene expressions, quercetin modulates genes involved in G₁–S-phase transition. *p15*, *p21*, cyclin-dependent kinase inhibitor 1C (*p57*, *CDKN1C*) and sestrin 2 (*SESN2*) are up-regulated; at the same time, cell-cycle-promoting genes, such as *CDK4*, are down-regulated in CO115 colon cells 24 h upon quercetin treatment independently of *TP53* gene expression. Late-response genes (i.e., 48 h upon quercetin treatment), which are modulated and involved in G₁–S phases, included the cell-proliferating suppressing TNF (ligand) superfamily, member 15 (*TNFSF15*) gene (up-regulation) and the cell-cycle-promoting genes *E2F1*, *CDK2*, calcium/calmodulin-dependent protein kinase (CAM kinase) II δ (*CAMK2D*) and cell division cycle 25 homolog A (*CDC25A*) (down-regulation) [70]. In other colon cells (i.e., Caco-2 cells), quercetin treatment for 48 h increases the number of cells in

S phase at the expense of the number of cells in G₁ phase. This coincides with the down-regulation of the expression of *CDK4* and *CCND1* genes in a dose-dependent manner [60]. In NCI-H209 lung carcinoma cells, quercetin treatment arrests cells in G₂–M and G₀–G₁ phases, leading to a decrease in cell growth. This coincides with an increased expression of CCNB, CDC25c-ser-216-p and Wee1 homolog (WEE1) proteins, indicating G₂–M-phase arrest.

In conclusion, vegetables and/or vegetable components are able to arrest the cell cycle of different colon cells at different phases or to delay transition from one phase to another, and this coincides with specific gene and/or protein expression changes involved in these phases, in particular, CDKs, cyclins and CDK inhibitors. Furthermore, cell cycle arrest is often accompanied by a reduction in cell growth. Although the number of studies using lung cells is limited, the results of these studies show that vegetable components can arrest lung cells at different stages of the cell cycle, which goes together with a reduction in cell growth.

4.2.3. Intracellular defense and biotransformation

The human body can defend itself against the initiation of cancer by intercepting and detoxifying potentially DNA-damaging xenobiotic or endogenous substances by Phase I and Phase II biotransformation enzymes. The activity of the involved biotransformation enzymes can be modulated by vegetables and/or vegetable components. The enzymes of the Phase I biotransformation system introduce a polar group into the compound, whereas Phase II biotransformation enzymes conjugate an endogenous hydrophilic substance. The modified compound is then more water soluble, and the body can excrete it more easily [102].

The majority of studies on gene and/or protein expression changes in the colon, which focus on the Phase I and II biotransformation systems, have investigated the effects of cruciferous vegetables and components [49,55,56,58,72,78]. Indoles are able to induce CYP1A1 protein and gene expressions, part of the Phase I biotransformation system, in LS74 and Caco-2 cells. In addition, the Phase II biotransformation enzyme NAD(P)H dehydrogenase quinone 1 (*NQO1*) is up-regulated, accompanied by increased mRNA expression. Both indoles and isothiocyanates induce aldo-keto reductase family 1, member C1 (AKR1C1) mRNA and protein expressions, which are involved in Phase II biotransformation. Only isothiocyanates are able to induce the Phase II biotransformation enzyme glycine cleavage system protein H (GCS_h). Pretreatment of colon cells with indoles and isothiocyanates protects cells against DNA damage induced by benzo(a)pyrene and hydrogen peroxide. This can be explained by the induction of the biotransformation enzymes by indoles and isothiocyanates, thereby eliminating DNA-damaging compounds before they can become harmful [49].

In vivo, broccoli induces CYP1A1 mRNA and protein and CYP1B1 protein in the colon of Wistar rats [55], and

GST- μ and GST- π proteins in the colon of ICR(Ha) mice. However, no GST- μ and GST- π protein changes are observed in the colon of healthy volunteers after a 10% broccoli diet for 1 week [56]. In contrast, in the rectum of healthy volunteers consuming 300 g/day brussels sprouts for 7 days, indeed an induction of GST- μ and GST- π proteins is detected [58]. GST enzymes are involved in Phase II biotransformation and catalyze a wide variety of electrophiles with glutathione, leading to inactivation of many carcinogens [103].

In addition to studies on cruciferous vegetables, the effects of a limited number of other vegetables on biotransformation genes and/or enzymes are investigated. The expression of *GSTA2* is induced in the colon of C57BL/6 mice, which received a diet containing 40% of a mixture of four vegetables (i.e., cauliflower, carrots, peas and onions). *GSTA2* enzyme is able to detoxify heterocyclic aromatic amines (HCAs) [104,105], which are implicated in the pathogenesis of colorectal and other cancers [106,107], next to a number of other electrophilic carcinogenic metabolites such as benzo(*a*)pyrene-7,8-dihydrodiol-9,10-epoxide and aflatoxin B1-8,9-oxide [108]. In addition to this major activity, GSTs from the α class, such as *GSTA2*, are known to catalyze glutathione peroxidase reactions and are therefore an essential component of the cellular antioxidant defense mechanism [109]. In addition to *GSTA2*, sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1 (*SULT1A1*) is down-regulated in the colon of these mice. Sulfotransferase enzymes catalyze the sulfate conjugation of many hormones, neurotransmitters, drugs and xenobiotic compounds, and play an important role in the detoxification of these compounds. However, they also play an important role in the metabolism and bioactivation of many dietary and environmental mutagens, including HCAs, which are implicated in the pathogenesis of CRC. In contrast to the down-regulation of this gene in the colon, this gene was up-regulated in the lungs of mice receiving a carrot diet. The role of this gene in the lungs is, however, probably different from its role in the colon. It was shown that a higher activity of the *SULT1A1* enzyme in the lungs was associated with a decreased LC risk [110].

In a human dietary intervention study with healthy controls and adenoma patients, several Phase I biotransformation genes are modulated in the colon of healthy subjects after an altering vegetable diet for 2 weeks [78]. The vegetable diet consisted of the same four vegetables as in the previously mentioned animal study [72]. In the group receiving a low dose of vegetables (i.e., 75 g/day), *CYP2C9* is up-regulated, and in the group receiving a high dose of vegetables (i.e., 300 g/day), *CYP2C19*, *CYP2D6* and *CYP3A4* are down-regulated. *CYP2C9* [24,111] and *CYP3A4* are responsible for the metabolism of a number of drugs and the (colon) carcinogens polycyclic aromatic hydrocarbons and HCAs [112]. In addition, *CYP2C19* and *CYP2D6* metabolize a number of drugs, but have not been thoroughly studied in relation to cancer susceptibility [24].

The effect of different naturally occurring organosulfur compounds, which are present in, for instance, garlic and onions, on Phase II biotransformation enzymes in the colon of Wistar rats has been investigated by Guyonnet et al. [57]. Several Phase II biotransformation enzymes are induced, among which are UDP-glucuronyl transferase (UGT) and microsomal epoxide hydrolase (MEH) induced by dialyl disulfide, and quinone reductase (QR) and GST activity induced by dialyl disulfide and dialyl sulfide. In the lungs of these rats, a higher activity of the Phase II enzymes UGT, MEH, GST and QR has been observed [57]. Induction of UGT and GST activity is also observed in the lungs of Sprague–Dawley rats receiving the precursor of sulforaphane [65]. However, in addition to these Phase II biotransformation enzymes, several cytochrome *P450* enzymes, which are known to activate several xenobiotics into more reactive compounds, are induced. Furthermore, it is shown that this precursor of sulforaphane (i.e., glucoraphanin) is able to generate reactive oxygen species in the lungs. These results suggest that, rather than behaving as a chemopreventive agent, glucoraphanin can actually exert toxicological effects by inducing carcinogen-bioactivating enzymes and generating oxidative stress [65]. In CO115 colon cells, the flavonoid quercetin, which is also present in vegetables such as onions, modulates both Phase I and II biotransformation genes, which are also involved in antioxidant defense [70]. Early-response genes (i.e., 24 h upon quercetin treatment) include the Phase II biotransformation genes *ACK1C1*, *NQO*, epoxide hydrolase 1 (*EPHX1*), lysyl oxidase-like 3 (*LOXL3*), thioredoxin reductase (*TXNR1*) and γ -glutamyl-cysteine synthetase (*GCLM*), which are all up-regulated. Forty-eight hours upon quercetin treatment, two cytochrome *P450* genes are up-regulated (i.e., *CYP4FII* and *CYP3A5*, both involved in detoxification).

In summary, the colon, which is exposed to several vegetables and/or vegetable components, expresses higher levels of detoxification genes and/or enzymes, indicating an increased level of protection against xenobiotics. In particular, cruciferous vegetables and/or components have been investigated, and both Phase I and Phase II biotransformation genes and/or enzymes have been modulated, among which cytochrome *P450*s and GSTs are most abundant. The number of studies using lung cells or tissues in which effects on biotransformation genes and/or proteins are observed is low, and results predict both an increase and a decrease in the risk of LC.

5. Conclusions

Here current knowledge on the effects of vegetables and vegetable components on gene and/or protein expression changes in the colon and lungs is reviewed. Predominantly affected pathways include apoptosis, cell cycle, cell proliferation and intracellular defense. Gene and/or protein changes coincide with actual changes in the involved

processes. The number of studies in the colon that use high-throughput methods is still limited, although these provide a wealth of information on multiple expression changes and involved pathways. Additionally, these studies result in a higher number of gene expression changes for which biologic relevance is not yet clear. Furthermore, single vegetable components in high doses, instead of whole vegetables, are mostly investigated. But the majority of gene and protein expression changes are in favor of a reduced CRC risk. For the lungs, limited information on gene and protein expression changes induced by vegetables and vegetable components is available today. Few studies show both protective and promoting effects on LC risk. Also with respect to studies performed on the lungs, high-throughput analyses of gene and protein expression changes by new “omics” technologies, as well as the investigation of whole vegetables, are still rare. Eventually, these types of studies should give more insight into the genetic pathways affected and, thus, into the molecular mechanisms of cancer prevention by vegetables — in particular whole vegetables — in the colon and lungs.

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