REVIEW

Antioxidative and antigenotoxic properties of vegetables and dietary phytochemicals: The value of genomics biomarkers in molecular epidemiology

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There is considerable evidence that consumption of fruits and vegetables may contribute to the prevention of cancer. It is however remarkable that evidence for such a preventive action arising from mechanistic studies is becoming stronger, whereas results of some recent prospective studies are less convincing. This apparent discrepancy may be overcome, or at least understood, by introducing molecular markers in future epidemiological studies, taking modulation of molecular processes as well as genetic variability in human populations into account. Both human and animal studies demonstrated that vegetable intake modulates gene expression in the gastrointestinal tract of many genes involved in biological pathways in favor of cancer risk prevention. Gene sets identified in this type of studies can be further evaluated, linked to the biological effects of phytochemicals and developed into biomarkers for larger human studies. Human dietary intervention studies have demonstrated that, apart from target tissues, also peripheral lymphocytes can be used for biomonitoring of chemopreventive effects. Transcriptomic responses and metabolite profiling may link phenotypic markers of preventive effects to specific molecular processes. The use of genomics techniques appears to be a promising approach to establish mechanistic pathways involved in chemoprevention by phytochemicals, particularly when genetic variability is taken into account.

Keywords:
Antioxidants / Chemoprevention / Genomics biomarkers / Phytochemicals / Vegetables

1 Introduction

Epidemiological studies indicate an interactive association between dietary habits, lifestyle, genetics and the risk of many different chronic diseases, including cancer. 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 types of cancers. In order to establish the strength of the evidence for a cancer preventive effect of fruits and vegetables, the World Cancer Research Fund (WCRF) first reviewed the literature in 1997. At that time, the WCRF panel concluded that the evidence for a protective effect for cancers of the colon/rectum, lung, stomach, esophagus and mouth/pharynx was ''convincing'', whereas the evidence for such an effect in the larynx, pancreas, breast and bladder was considered ''probable'' [1]. More recently, a number of prospective studies failed to support such protective effects, and as a consequence, these initial judgments were adjusted accordingly, both in the IARC handbook of Cancer prevention on Fruits and Vegetables [2] and in the update of the initial WCRF

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Abbreviations: AhR, aryl hydrocarbon receptor; **BPDE,** benzo[a]-pyrenediol-epoxide; **COMT1,** catechol *O*-methyltransferase 1; **CYP,** cytochrome P450; **GSTT1,** glutathione *S*-transferase T1; **NAhRA,** natural aryl hydrocarbon receptor agonist; **TCDD,** 2,3,7,8-tetrachlorodibenzo-p-dioxin; **TEAC,** Trolox equivalent antioxidant capacity

report [3]. Although these null findings may indeed be correct, there are several potential reasons why the effect of vegetable consumption may be attenuated in epidemiological studies. In view of the multi-factorial complexities of dietary exposures, all dietary intake assessment methods applied in epidemiological studies are associated with measurement errors that affect dietary estimates and may obscure disease risk associations [4–6]. Furthermore, genetic variation within study populations may influence dietary intake as well as kinetics and metabolism of nutrients and phytochemicals [7, 8]. However, when reviewing the literature on the effects of bioactive compounds in animal and *in vitro* mechanistic studies, there is ample evidence that specific antioxidants and other phytochemicals present in foods of plant origin protect against genotoxicity and other cancer-initiating or -promoting processes [9, 10]. The majority of potentially anticarcinogenic compounds in the diet is from plant origin [11], and several classes of compounds have been identified and their respective modes of action have been studied for many years [12, 13]. In many of the postulated mechanisms, the modulation of gene expression is found to play 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 generate new hypotheses based on the identification of potentially relevant genes and to characterize the basic molecular pathways of gene regulation by vegetables. This may eventually lead to the development of validated biomarkers for the assessment of dietary effects in humans. Introducing such molecular markers in future epidemiological studies, thereby taking molecular processes as well as genetic variability in human populations into account, may help to eliminate the present controversy between different types of studies and establish the potential of phytochemicals from dietary fruits and vegetables in cancer risk reduction.

In this short review, the potential value of introducing genomics markers in human studies on both risks and benefits of dietary phytochemicals is discussed, with particular emphasis on the chemopreventive effect on colon cancer risk.

2 Colorectal cancer risk prevention by vegetables

The model of colorectal cancer as proposed by Fearon and Vogelstein consists of successive genetic changes, in which a number of genes are involved, including adenopolyposis coli, K-RAS, deleted in colorectal cancer and p53 [14]. During the last decade, additional genetic events and specific molecular pathways have been identified. It became clear that the intact or mutated key molecules of the Vogelstein model interact and form a network of molecular events affecting additional genes [15, 16]. These genetic pathways and the involved genes are obvious molecular targets for the protection against colorectal cancer. Vegetables and fruits contain many different compounds that may influence the risk of cancer development, including dietary fiber, nutritive compounds (*e.g.* selenium, folic acid, vitamin C and E, carotenoids) and non-nutritive phytochemicals that have no nutritional value but may exert specific biological activities (*e.g.* flavonoids, indoles and

Table 1. Main mechanisms of anti-carcinogenicity of bioactive compounds in fruits and vegetables

Mechanism of anti-carcinogenicity	Constituent	Mode of action	Reference
Prevention of carcinogen uptake	Dietary fiber	Adsorption of carcinogens	[19]
Inhibition of enzymatic carcinogen Activation	Flavonoids Isothiocyanates Organosulfur comp. Indoles	Modulation of phase I and II enzymatic Bioactiviation/detoxification pathways	[20–23]
Scavenging reactive metabolites	Flavonoids Isothiocyanates Vitamin C and E Carotenoids	Direct scavenging of reactive oxygen Species and free radicals	[23]
Induction of DNA repair	Flavonoids	Inhibition of DNA topoisomerase II	[24]
Inhibition of cell proliferation	Quercetin Indoles	Blocking of G1-S transition in cell cycle Inhibition of cyclin-dependent kinase 6	[25–27]
Induction of apoptosis	Indoles Isothiocyanates Flavonoids Organosulfur comp.	Induction of caspases, P53, *BAX* Reduction/inhibition of BCL-2 and NF-κB	[25, 28, 29]
Induction of cell differentiation	Flavonoids β-Carotene Vitamin A	Inhibition of abl oncogene tyrosine kinase; Up-regulation of retinoid receptor Expression, induction of TGF-β	[30, 31] [32]

isothiocyanates) [11, 13, 17]. These dietary constituents may interfere with the carcinogenic process through various modes of action at various stages of cancer development. Some of the involved mechanisms may be partially overlapping, complementary or even synergistic [18]. In Table 1, an overview of main mechanisms of anti-carcinogenicity is presented with some examples for each category. In many of the presented mechanisms, the modulation of gene expression is likely to play a role, but the molecular targets at the genome level and the involved genetic pathways are to a large extent unknown. Recently, we reviewed the literature on studies investigating the mechanisms underlying the prevention of colorectal cancer and lung cancer by vegetables or vegetable constituents [12]. It was demonstrated that most of the data on modulation of gene and protein expression in the colon are provided by studies of a few predefined genes and/or proteins, focusing predominantly on those involved in apoptosis, cell cycle, cell proliferation and enzymatic bioactivation/detoxification. A limited number of microarray studies identified additional processes that might be relevant, including cell differentiation [33–35], cell–cell interaction [33, 34], signal transduction [33, 34] and immune-related responses [33]. Updating the number of experimental studies investigating the effect of vegetables and phytochemicals on gene and/or protein expression, as indicated in our previous review, shows that still only 3 out of 12 [33–35] *in vitro* studies and 2 out of 7 animal studies [35, 36] made use of ''omics'' techniques. The animal studies investigating multiple genes and proteins demonstrated that apart from the preselected genes involved in biotransformation, also apoptosis, DNA repair, cell cycle and polyamine metabolism were modulated by vegetables [35, 36]. To the best of our knowledge only one human dietary intervention study with vegetables made use of microarray technologies [37], of which the main results are discussed below.

3 Gene expression modulation in the colon by vegetables and phytochemicals

The first human study that has focused on the effects of a dietary vegetables on the expression of genes in the colon using microarray techniques was published in 2004 [37]. It was investigating the hypothesis that an important contribution of the anticarcinogenenic effects of vegetables in the colorectum is mediated by modulation of the expression of genes involved in biological and genetic pathways that are relevant for chemical carcinogenesis. In this study, both healthy controls and patients with adenomatous polyps (a population regarded to be at increased risk for the development of colorectal cancer) received a diet with either a 50% decreased (= 75 g/day) or a doubled intake (= 300 g/day) of a mixture of vegetables (cauliflower, carrots, peas and onions) during a period of 2 wk. A dedicated microarray, containing 597 genes representing pathways relevant for carcinogenesis, was used to analyze gene expression changes in normal colorectal mucosa, obtained by endoscopic biopsy. Comparison of pre- and post intervention measurements revealed that in total 52 genes were differentially expressed, and according to literature review, 20 of these genes could be linked to the (colon) carcinogenic process. As is shown in Fig. 1, in both patients and controls seven genes were similarly up- or down-regulated, in the high vegetable group for instance the fos proto-oncogene and ornithine decarboxylase. On the other hand, 16 genes were modulated differently in patients as compared with controls. It appeared that in control subjects, genes that are likely to play a role in the relatively early phases of carcinogenesis were modulated (*e.g* the down-regulation of cytochrome P450 (CYP) enzymes involved in the metabolism of xenobiotic agents), whereas effects in patients were found on genes involved in the later stages, such as MDM2. An increased intake of vegetables resulted in the down-regulation of genes promoting cell proliferation and bioactivation of procarcinogens and in up-regulation of genes involved in cell growth arrest. In contrast, a decreased intake of vegetables resulted in down-regulation of genes that inhibit cell growth and up-regulation of genes promoting cellular dedifferentiation and bioactivation of procarcinogens. Table 2 summarizes for the most relevant genes the observed effect of the intervention according to the different study populations and provides an interpretation of the up- or down-regulations in terms of likely to be preventive or risk enhancing, based on their theoretical effect described in the literature. The overall conclusion is that almost all the effects observed after increased vegetable intake may be mechanistically linked to cellular processes that may prevent or reduce colon cancer risk, whereas reduced intake of

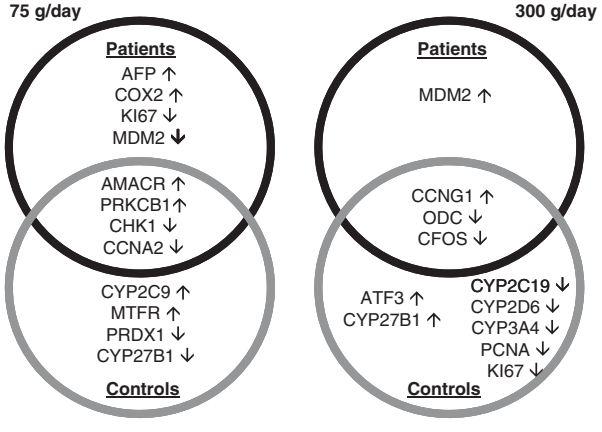

Figure 1. Differentially expressed genes in patients with colorectal adenomas and controls, after a dietary intervention with decreased (75 g/day) or increased (300 g/day) intake of vegetables; arrow up means up-regulation, arrow down means down-regulation.

Table 2. Effect of increased or decreased dietary intake on several genes involved in colon carcinogenesis and the theoretical effect on colorectal cancer risk

Gene	Decreased vegetable intake		Increased vegetable intake		Involved pathway
	Effect on expression	Theoretical effect on CRC	Effect on expression	Theoretical effect on CRC	
AMACR	+	+			Metabolism
ODC1			−	−	
PKCB1	+	+			Cell cycle/growth
CCNA2	−	−			
CCNG1			+	−	
MDM2	−	?	+	?	
CHK1	−	+			
C-FOS			−	−	
COX-2	+	+			
CYP2C9	+	+			Oxidoreductase activity
CYP2C19			−	?	
CYP2D6			−	?	
CYP3A4			−	−	
CYP27B1	−	+	+	−	

+, up-regulation of gene expression or stimulating colorectal cancer risk; -−, down-regulation of gene expression or preventive effect on colorectal cancer risk; ?, not possible to interpret; CRC, colorectal cancer.

vegetables resulted in a greater number of affected genes and is more likely linked to increased cancer risk.

Although this study does not link gene expression modulation directly to cancer as the ultimate disease endpoint, it demonstrates the potential value of gene expression profiling in nutritional epidemiology. If this type of genomics markers is indeed going to be used in epidemiological studies, it should be validated that comparable gene expression changes can also be established in lymphocytes as target tissue that may be available from large cohort studies. Ideally, dietary induced gene expression changes would also reflect interindividual differences as a result of genetic polymorphisms, thereby identifying subpopulations that may particularly benefit from specific dietary preventive factors.

4 Genetic polymorphisms and antioxidative response

In order to establish whether or not lymphocytes can be used in human studies to establish gene expression changes, as well as the influence of genetic polymorphisms, a series of studies has been performed in our department [38–40]. These studies focused on the chemopreventive effect of dietary quercetin, a well studied flavonoid, present in many fruits and vegetables. Quercetin possesses good antioxidant properties and has been shown to protect against the induction of DNA damage [41–44]. A first pilot study demonstrated that it was possible to increase plasma Trolox equivalent antioxidant capacity (TEAC) values and quercetin levels by a 4 wk dietary intervention with a blueberry-apple juice mixture, and that after the intervention, the level of both oxidative damage and DNA adduct levels, induced after *ex vivo* exposure to hydrogen peroxide and benzo[a] pyrene (B[a]P) respectively, were (non-significantly; $n = 8$) reduced [38]. In a second pilot study, the impact was established of genetic polymorphisms in *GSTM1* and glutathione *S*-transferase T1 (*GSTT1*), both encoding for enzymes involved in antioxidative defense, on the induction of oxidative DNA damage and on the effectiveness of quercetin and ascorbic acid to prevent this type of damage in human lymphocytes *in vitro* [39]. We found no differences between base line levels or *ex vivo* induced oxidative DNA damage between variants and wild types. However, the level of protection against hydrogen peroxide induced oxidative DNA damage by a pre-incubation of lymphocytes with quercetin was significantly higher in *GSTT1* wild types than in *GSTT1* variants. Also pre-incubation of lymphocytes with ascorbic acid resulted in a protection against the induction of oxidative DNA damage in *GSTT1* wild types, whereas *GSTT1* variants showed an increase of damage. Based on these promising data, a large scale human dietary intervention study was designed to test the hypothesis that individuals with genetic polymorphisms for genes related to quercetin metabolism, B[a]P metabolism, oxidative stress response and DNA repair differ in their response to the DNA protective effects of increased fruit-borne antioxidant intake [40]. Before and after a 4 wk intervention with a quercetin-rich blueberry-apple juice, freshly isolated lymphocytes of in total 168 participants were challenged *ex vivo* with hydrogen peroxide and B[a]P and analyzed for

oxidative damage and the induction benzo[a]pyrenediol-epoxide (BPDE)-DNA adducts using the comet assay and ^{32}P postlabeling, respectively. All participants were screened for 34 different single nucleotide polymorphisms, of which six were found to influence the outcome of the intervention. Table 3 summarizes the impact of these polymorphisms. Particularly individuals bearing the wild type for *GSTT1* benefit the most of the protection against the induction of oxidative DNA damage, whereas the variant alleles show the highest increase in plasma antioxidant capacity (TEAC). This indicates that increased antioxidant levels in blood plasma is not necessarily a good predictor of protection against oxidative DNA damage. In contrast to the overall preventive effect of the intervention against oxidative DNA damage, *ex vivo* induced BPDE-DNA adduct levels were higher after 4 wk intervention. Again, the effect was linked to genetic polymorphisms, showing relatively low levels of damage in *CYP1B1*∗*5* variants with low enzymatic activity. This may be the consequence of the reduced capacity to metabolize B[a]P and form reactive intermediates, and an additional inhibition of enzyme activity by dietary flavonoids present in the blueberry-apple juice [47, 48]. Furthermore, catechol *O*-methyltransferase 1 (*COMT1*) variants showed a profound increase in adduct formation. This is probably due to low activity inherent to this genotype, resulting in a decreased elimination of reactive B[a]P metabolites [47]. Overall, out of the total research population, 139 *GSTT1* wild types and 30 *CYP1B1*∗*5* variants may benefit most from increased fruit-borne antioxidant intake with respect to reducing risks of DNA damage, while for 43 carriers of the *COMT1* variants, risk of genetic damage may be increased. Ongoing metabolomics and gene expression analysis are expected to provide further insight into the underlying mechanisms of the observed effects. Preliminary data show

Table 3. Impact of six out of 34 analyzed genetic polymorphisms on the outcome of a blueberry apple juice intervention study (based on [40])

Genetic polymorphism	Effect on study outcome	Remarks
NQO1 2* (Phase II detoxification)	Heterozygous subjects showed larger increase in plasma quercetin levels	Wild types have a higher enzyme activity and may have a higher metabolism of quercetin resulting in lower levels of free quercetin in plasma Quercetin may induce NQO1 gene expression, resulting in even more enhanced metabolism of quercetin [45]
CAT 1* (Oxidative stress response)	Heterozygous and homozygous variants show an increased plasma ascorbic acid concentration	This finding suggests that the lack of enzymatic antioxidant activity is compensated by a more efficient uptake of dietary ascorbic c acid
GSTT1 (Phase II detoxification)	The variants had a significantly higher total antioxidant potential (TEAC) after the intervention	GSTT1 deletion is associated with a reduced enzymatic antioxidant defense and could have been expected to cause lower plasma TEAC values; this may be explained by effective compensatory mechanisms
	Wild-type subjects showed higher protection against *ex vivo* induced oxidative DNA damage	The genotype with the optimal antioxidant effect also benefits most of the intervention; this confirms earlier findings [39]; the mechanism behind this protection is unclear
XRCC1 4* (DNA repair)	Included in the same step-wise regression model as GSTT1, and gender, predicting protection against oxidative DNA damage in the COMET assay Variants show reduced DNA-repair capacity	Wild-types show the highest reduction of DNA damage after the intervention, indicating that phytochemicals in blue berry juice further stimulate the most optimally functioning DNA repair system
*CYP1B1*5* (Phase I bioactivation)	Variants have relatively low BPDE-DNA adduct levels as compared with wild-types after the intervention	Variants have lower CYP activity resulting in reduced bioactivation of benzo[a]pyrene Flavonoids like quercetin are known to inhibit CYP activity [46]
COMT1 (Phase II detoxification)	Variants show a profound increase of *ex vivo* induced BPDE-DNA adducts	COMT1 is involved in the elimination of benzo[a]pyrene metabolites [47]; the reduced enzyme activity may result in increase DNA damage

CAT, catalase; XRCC1, X-ray repair cross-complementing group 1; COMT, catechol *O*-methyltransferase.

that also transcriptomic responses to the intervention are different between individuals with different genetic make-up. For instance, *GSTT1* variants show a much stronger response as compared with the wild types; in variants, 1091 genes differentially expressed before and after the intervention (656 up- and 435 down-regulated) *versus* wild types, in which 2579 genes were differentially expressed (1432 up- and 1147 down-regulated) (unpublished results).

Overall, these findings demonstrate that the evaluation of the impact of genetic polymorphisms can provide a useful tool in assessing susceptible subpopulations and groups that benefit from specific dietary modifications or interventions, and that lymphocytes can be used to monitor gene expression responses to dietary factors. Whether or not these gene expression profiles are helpful to understand the molecular mechanisms behind potentially harmful or chemopreventive effects of phytochemicals remains to be established. It is therefore of crucial importance that all identified genomics responses and affected pathways are carefully evaluated in validation studies.

5 Risk–benefit analysis of dietary phytochemicals

It is well documented that phytochemicals from fruits and vegetables are not under all circumstances beneficial, but in fact may also exert toxic effects, particularly when added to the diet as supplements [49]. An elaborate meta analysis by Bjelakovic *et al.* revealed that, of 68 randomized trials of anti-oxidant intake through supplements β-carotene, vitamin A and vitamin E significantly increased all-cause mortality, whereas selenium and vitamin C supplementation had no significant effect on mortality, either positive or negative [50]. Like vitamins, flavonoids are thought to contribute to the protection by fruits and vegetables against cancer and other degenerative diseases, but excessive flavonoid intake should be avoided as anti-oxidant effects can turn into pro-oxidant effects depending on the concentration [51, 52]. This is the result of auto-oxidantion of the flavonoids or by its metabolism resulting in *o*-semiquinone and *o*-quinone structures. It has been shown for instance that the oxidation products of quercetin display various toxic effects due to their ability to arylate protein thiols [52–55]. Excessive flavonoid intake will most likely occur by ingestion of commercially available food supplements of which recommended doses greatly exceed the dose that can be reached by normal or vegetarian diets [56].

With regard to the balance between risk and benefit, another interesting class of phytochemicals is presented by the natural aryl hydrocarbon receptor agonists (NAhRAs), found in cruciferous vegetables and citrus fruits. Because the activation of the aryl hydrocarbon receptor (AhR) is thought to be essential in the toxicity of dioxins, a well-known group of environmental pollutants of which 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most potent one, it is of great importance to investigate whether these naturally occurring agonists may also exert similar toxic effects as dioxins or dioxin-like compounds [57–60]. The importance of investigating such a potential health concern is even more evident in view of the fact that the intake of high doses of purified NAhRAs is being promoted as healthy food supplements. The interest in this type of food supplements is based on the observation that some identified NAhRAs from vegetables and fruits, like indole-3-carbinol in cruciferous vegetables, furocoumarins in citrus fruits and flavonoids such as chrysin, baicalein and cantharidin in vegetables and herbs can show health-promoting effects, especially as tumor suppressors and are thought to be an important cause for the observed beneficial effects of vegetable- and fruit-enriched diets [3, 61–63]. It is remarkable that the proposed mechanism of anti-tumor activity is also based on the AhR activation, and the subsequent induction of phase I and II biotransformation enzymes like *CYP1A1*, *CYP1A2* and *UDPGT1A6* [64, 65]. This seeming contradiction of both toxic and health beneficial effects initiated by the same receptor may be caused by differences downstream of the AhR-activation or alternative mechanisms besides the AhR-activation. Possibly, differences between AhR-mediated toxic effects induced by TCDD and beneficial health effects of NAhRAs may be caused by interactions with enzyme activity at the protein level rather than at the level of gene expression. It was reported that a NAhRA-containing extract of grapefruit juice induced the AhR-related gene expression strongly, but inhibited the enzymatic activity of the gene product *CYP1A1* and *CYP1B1* [66, 67].

In order to establish whether or not activation of the AhR pathway by NAhRAs and dioxin-like substances results in similar cellular responses, gene expression profiles induced in Caco-2 cells were studied using microarray analysis [66]. Cells were exposed to indolo[3,2-b]carbazole and to extracts of citrus pulp and grapefruit juice. Gene expression profiles induced by these NAhRAs were compared with those of the xenobiotic AhR agonists TCDD and B[a]P. More than 20 genes were found more than 1.5 times up- or down-regulated by TCDD, and the expression of most of these genes was modulated in the same direction and to a similar extent by B[a]P and the NAhRAs, and many of these genes may be involved in dioxin-related toxic effects. The same phase I and II biotransformation enzymes like *CYP1A1*, *CYP1B1* and *UDPGT1A6*, and a number of genes involved in cell proliferation were elevated to the same magnitude. Although not extensively investigated, no important difference in gene regulation could be detected between NAhRAs and TCDD.

The TCDD-like gene expression profiles of the NAhRAs suggest that NAhRAs may indeed induce TCDD-like toxicity, and therefore we investigated whether the expression of the most important AhR-responsive genes could also be detected in *ex vivo* exposed human lymphocytes. Blood is a relatively easy obtainable human tissue, and finding geno-

mics biomarkers for exposure or effects in human blood cells would enable toxicological evaluation of NAhRA exposure in human populations. For that purpose, it was investigated whether or not a same kind of gene expression profile as in Caco-2 cells was found in freshly isolated human lymphocytes after *in vitro* exposure to NAhRAs and TCDD [68]. Although the lymphocytes appeared to be less sensitive than the Caco-2 cells, a small number of AhR-specific genes were significantly up-regulated, including *CYP1A1, CYP1B1* and NAD(P)H dehydrogenase, quinone 1 (*NQO1*). As the elevated expression of these three genes was considered to be a promising biomarker for human exposure to AhR-agonists, a pilot study was initiated to verify the up-regulation of these three genes in human blood cells after consumption of NAhRA-rich food items. Screening of foods using the DR CALUX® assay had shown that most NAhRA-activity was found in grapefruit juice and cruciferous vegetables [69], so these were used for a 3-day intervention study. Although the level of NAhRA intake was considerable, no up-regulation of gene expression of *CYP1A1, CYP1B1* or *NQO1* could be detected both 3 and 24 h after the dietary intervention. Two alternative measurements were performed to establish AhR-related activity after the dietary intervention. First, the effect of the intervention on *CYP1A2* induction was determined by measuring caffeine-metabolite ratios in urine samples, and second, the DR CALUX®-activity was measured in plasma samples. No effect on *CYP1A2* induction could be detected, although studies with comparable amounts of cruciferous vegetables showed *CYP1A2* induction by way of altered caffeine metabolism [70, 71], but the DR CALUX®-assay showed a slight increase in activity after consumption of cruciferous vegetables. A newly developed gene reporter assay, the Dioxin Responsive Element-driven Chemical Activated FLUorescent protein eXpression (DRE-CAFLUX) assay confirmed the slight elevation in AhR-activity found in the human plasma samples from the intervention with cruciferous vegetables (unpublished results). Although the AhR-activity in grapefruit juice extract was much higher than in the cruciferous vegetables, the NAhRAs in grapefruit juice extract do not appear to reach the bloodstream in high amounts. Therefore, it can be concluded that gene expression profiling in human lymphocytes is not sensitive enough for the detection for NAhRA-exposure or AhR-related effects in humans.

6 Concluding remarks

The relationship between diet and cancer is extensively studied, and particularly the consumption of vegetables and fruits, containing a wide range of bioactive phytochemicals, has been suggested to reduce cancer risk. A rapidly increasing number of mechanistic studies provide insight into the different modes of action for all different classes of compounds, making a relationship between dietary intake of phytochemicals and beneficial health outcomes biologically plausible. With the introduction of new ''omics'' techniques, new types of intermediate biomarkers can be developed that may not only reflect intake or exposure, but can potentially also provide insight into molecular responses to dietary factors. Results from the relatively small number of studies that have applied such genomics approaches demonstrate that gene expression changes can indeed be established in target tissue after dietary intervention with vegetables, even in a small research population. Also lymphocytes have been used as surrogate tissue for biomarker analysis, showing that individuals with different genetic polymorphisms in a number of relevant genes respond differently to dietary intake of phytochemicals, both with regard to phenotypic markers of genotoxic damage as at the level of modulated gene expression. The link between molecular pathways in which these genes are involved and the altered protection against induced DNA damage remains, however, to be established. It appears an attractive future perspective that the evaluation of the impact of genetic polymorphisms on gene expression profiles may identify susceptible subpopulations and groups that benefit from specific dietary interventions. However, there are still numerous scientific and ethical issues that hamper sound personalized nutritional recommendations. Coming from a ''one diet fits all'' approach to public health, a steadily growing body of literature demonstrates the full complexity of gene-environment interactions and has illustrated that also a ''one gene needs one diet'' translation does not exist. Particularly the selection of genes to be studied and the assessment of the overall interactive effects of different polymorphisms appear of crucial interest. In their recent review, Williams *et al.* [72] identify the challenges for molecular nutrition research in order to establish the contribution of genetics in variable responsiveness to dietary factors and provide directions for future research to come to personalized nutritional recommendations.

It appears to be of particular interest to establish in future studies the potential use of genomics markers in order to discriminate between beneficial and detrimental effects of dietary supplements containing high levels of isolated phytochemicals. Classical exposure biomarkers, such as plasma concentrations of specific compounds may provide information on dietary intake, but cannot indicate toxic responses, whereas specific markers of for instance DNA damage or oxidative stress in general may overlook health effects that are mediated by other molecular mechanisms, such as activation of the AhR. Finally, it has also been demonstrated that transcriptomics analysis has its limitations. As microarray analysis of gene expression responses evaluate the effects on thousands of genes simultaneously; it is inevitable that some differences in gene expression levels will be found. This implies that such approaches may be particularly useful in the identification of potentially relevant genes and pathways and may generate new hypothesis on molecular mechanisms of interest, but also that the findings generated by such techniques require extensive validation studies to ensure that the

indicated responses are indeed reproducible and meaningful. The microarray technologies may not be sensitive enough to detect all relevant gene responses, and modulation of enzyme activity at the protein level may be more relevant than the level of gene expression. This implies that gene expression analyses is most likely to be of additional value in human studies when combined with conventional biomarkers of effect, a fundamental research concept also indicated as phenotypic anchoring [73]. Biomarkers of interest could be all sorts of clinical chemistry markers, histopathology or more specific markers of genotoxic damage, such as DNA strand breaks or oxidative DNA-damage. Once such experiments have provided sufficient proof of principle, further integration of data coming from omics-technologies, especially proteomics and metabolomics analysis, may contribute to the further understanding of phytochemical induced human health effects.

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