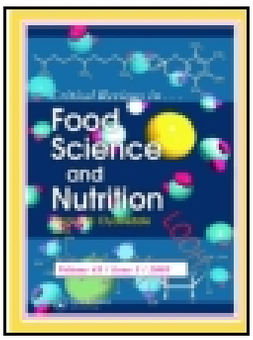

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

Cruciferous vegetables and colorectal cancer prevention through microRNA regulation: A review

Jeong Hoon Pan, Breann Abernathy, Young Jun Kim, Jin Hyup Lee, Jun Ho Kim, Eui Cheol Shin & Jae Kyeom Kim

To cite this article: Jeong Hoon Pan, Breann Abernathy, Young Jun Kim, Jin Hyup Lee, Jun Ho Kim, Eui Cheol Shin & Jae Kyeom Kim (2017): Cruciferous vegetables and colorectal cancer prevention through microRNA regulation: A review, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2017.1300134

To link to this article: https://doi.org/10.1080/10408398.2017.1300134

Accepted author version posted online: 10 Apr 2017.
Published online: 12 Jun 2017.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2017.1300134

Cruciferous vegetables and colorectal cancer prevention through microRNA regulation: A review

Jeong Hoon Pan[a], Breann Abernathy[b], Young Jun Kim[c], Jin Hyup Lee[c], Jun Ho Kim[c], Eui Cheol Shin[d], and Jae Kyeom Kim[a]

[a]School of Human Environmental Sciences, University of Arkansas, Fayetteville, Arkansas, USA; [b]Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota, USA; [c]Department of Food and Biotechnology, Korea University, Sejong, Republic of Korea; [d]Department of Food Science, Gyeongnam National University of Science and Technology, Jinju, Republic of Korea

ABSTRACT

Diets rich in fruits and vegetables may lower colorectal cancer risk. In particular, a number of *in vitro* and *in vivo* studies demonstrated that cruciferous vegetables and their active compounds elicit chemopreventive potency through multiple mechanisms. However, it is relatively unexplored whether these vegetables modulate the risk of cancer development through epigenetic mechanisms including noncoding RNAs. Therefore, the objective of the present review is to report and discuss existing evidence with regards to modulation of microRNAs (miRNAs), one variety of noncoding RNAs, by cruciferous vegetables and their chemo-preventive effects against colorectal cancers. As results, it seems clear, considering accumulating evidence regarding their interactions with cancer related genes and relevant signaling pathways, that miRNA modulation via cruciferous vegetables is an attractive target for the prevention of colorectal cancer. In addition, miRNAs have been characterized as diagnostic and prognostic biomarkers and utilized in cancer therapeutics. Thus, it is very possible that natural agents (not limited to those in cruciferous vegetables) enhance cancer therapeutic efficacy and elicit chemopreventive effects through modulating key miRNAs.

KEYWORDS

Glucosinolates; colorectal cancer; cruciferous vegetables; microRNA

Introduction

Colorectal cancer is the third most common type of cancer in the world. Globally, it is estimated that around 600,000 deaths occur per year, making it the fourth leading cause of cancer related death worldwide (Vargas and Thompson, 2012). The incidences and mortality rates of colorectal cancer differ dramatically due to various factors such as sex, race, ethnicity, and age (Vargas and Thompson, 2012). Of note, the rates of this cancer also increase with the degree of industrialization and urbanization, leading many scientists to investigate the significance of diet as a modifying factor in cancer etiology (Marshall, 2009).

Carcinogenesis is a long, multistage process, thus there are multiple chances to modulate this sequence. It is accepted that diet may act as either a pro- or antitumor risk modifier in cancer development. For instance, while pyrolysis products from cooked foods and myoglobin in red meat can act as an initiator (Felton and Knize, 1991) and promoter (Pierre et al., 2003) respectively, certain phytochemicals and nutrients may inhibit colorectal carcinogenesis at different stages (Coates et al., 2007; Majumdar et al., 2004). According to the recent report of the World Cancer Research Fund and the American Institute for Cancer Research, there is convincing evidence that certain food items (e.g., garlic) protect against colorectal cancer whereas eating red meat, substantial consumption of alcoholic drinks, and factors that lead to greater total body fat and abdominal fat are causes of this cancer, confirming the notion that diet might be an important risk modifier in colorectal cancer development (2007).

Cruciferae is a family of vegetables named after their crucifer shape flowers, which includes vegetables such as cabbage, broccoli, and Brussels sprouts. As with many other vegetables, cruciferous vegetables are high in minerals, nutrients, and phytochemicals (e.g., selenium, folate, and fiber). Particularly, what makes these vegetables unique is the sulfur containing compounds, called glucosinolates which are responsible for their pungent and spicy taste (Higdon et al., 2007). It has been heavily investigated that the chemopreventive potential of cruciferous vegetables is likely due to glucosinolates and their secondary metabolites [e.g., isothiocynates (ITCs)]. So far, more than 120 different glucosinolates have been identified from various plants and the profiles of these compounds vary depending on cultivars and growing conditions. Depending upon the molecular structure, they can be classified as aliphatic, aromatic, ω-methylthioalkyl, and heterocyclic glucosinolates (Fahey et al., 2001; Holst and Williamson, 2004).

Cruciferous vegetables, their bioactives, and colorectal cancers

As previously mentioned, increasing fruit and vegetable intake may provide cancer prevention benefits. Specifically,

CONTACT Jae Kyeom Kim jkk003@uark.edu 1 University of Arkansas, School of Human Environmental Science, Fayetteville, AR 72701, USA.
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/bfsn.

many epidemiological studies and meta-analyses have shown the inverse association between cruciferous vegetable intake and the risk of many cancers (Lam et al., 2010; Liu and Lv, 2013; Tang et al., 2010). However, assessing epidemiological evidence for the association between cancer risk and fruit and vegetable intake requires more specifics in terms of vegetable types, responsible active compounds, and underlying mechanisms for their chemopreventive benefits considering the number of different phytochemicals in vegetables, as well as the anatomical characteristics of colorectal subsites (Sanz-Pamplona et al., 2011). In that regard, it is worth noting that a meta-analysis recently evaluated the association between specific cruciferous vegetables and colorectal cancer incidences (Wu et al., 2013); the authors pooled 35 observational studies and found that high cruciferous vegetable intake was associated with a reduced colorectal cancer risk (relative risk of 0.82; 95% confidence interval: 0.75–0.90). More specifically, cabbage intake represented a statistically significant inverse association with a colorectal cancer risk; the relative risk of colorectal cancer was 0.76 in the high cabbage intake group compared to the lowest category ($P = 0.014$; 95% confidence interval: 0.60–0.97). Similarly, but with a different magnitude, higher intake of broccoli showed lower relative risk of colorectal cancer (relative risk of 0.82; 95% confidence interval: 0.65–1.02; $P = 0.05$) which is biologically plausible as demonstrated in previous *in vitro* and *in vivo* studies (Navarro et al., 2009; Robbins et al., 2010; Zhu et al., 2000; Bonnesen et al., 2001; Hwang and Lee, 2010).

The colorectal cancer preventive effects of cruciferous vegetables (and their bioactives) likely involve multiple molecular mechanisms (as summarized in the Table 1); the major mechanisms include (1) modulation of the biotransformation enzymes (e.g., Phase I and Phase II enzymes) that may facilitate elimination or detoxification of xenobiotics from the body (Kim et al., 2016; Walters et al., 2004); (2) induction of apoptosis by secondary metabolites of glucosinolates [e.g., sulforaphane (Byun et al., 2016); benzyl isothiocyanate (BITC; Antony et al., 2012); allyl isothiocyanate (AITC; Lau et al., 2010); 3,3′-diindolylmethane (DIM; Kim et al., 2007)]; (3) controlling cancer cell growth via cell cycle arrest (Choi et al., 2009; Parnaud et al., 2004) or antiproliferation (Chung et al., 2015; Leem et al., 2015); (4) anti-oxidative capacity (Ferrarini et al., 2012; Miller-Cebert, 2016); (5) antitumorigenesis by inhibiting angiogenesis (Davaatseren et al., 2014), cancer cell migration (Lai et al., 2010a; Lai et al., 2010b), invasion (Lai et al., 2010a; Lai et al., 2010b), and metastasis (Lai et al., 2014); and (6) epigenetic regulation [e.g., histone deacetylase modulation; (Ho et al., 2009; Rajendran et al., 2015)]. Of note, despite the fact that a number of studies demonstrated the beneficial roles of cruciferous vegetables in colorectal cancer models, there is limited information whether these vegetables modulate the risk of cancer development through epigenetic mechanisms including noncoding RNAs (ncRNAs). Therefore, the objective of the present review is to report and discuss existing evidence with regards to modulation of microRNAs (miRNAs), one variety of noncoding RNAs, by cruciferous vegetables and their chemo-preventive effects against colorectal cancers.

MiRNA and current understanding

Typically, small ncRNAs originate from the processing of long ncRNA precursors. The classification of small ncRNAs includes short interfering RNAs, miRNAs, PIWI-interacting RNAs, repeat-associated small interfering RNAs, heterochromatin small RNAs, NAT-derived siRNAs, trans-acting siRNAs, 21U-RNA, and tiny ncRNAs. Of those, miRNAs, which are approximately 24 nucleotide-long noncoding endogenous RNAs, are the most abundant class in animals (Farazi et al., 2008). Despite their small size, miRNAs have been known to play a pivotal role in diverse regulatory pathways, including differentiation (Fukuda et al., 2005), proliferation (Pogue et al., 2010), apoptosis (Cheng et al., 2005), and metabolism (Wilfred et al., 2007). Approximately one third of all human genes appear to be modulated by miRNAs, a notion that supports their significance (Lewis et al., 2005). The expression of some miRNAs differs from others both qualitatively (i.e., certain miRNAs are specific to tissue and cell types) and quantitatively (i.e., some miRNAs are expressed more in specific tissue and cells than those of others) (Bravo-Egana et al., 2008; Poy et al., 2004; Yang et al., 2007).

A single miRNA can target more than one mRNA (or even more than thousands of mRNAs); this is because miRNAs bind with specific sequences of mRNA not specific to a certain gene. Thus, different mRNAs can have similar binding sequences for

Table 1. Chemo-preventive mechanisms of cruciferous vegetables and their bioactive components against colorectal cancer.

Mechanisms		Involved genes, proteins or signaling pathways	References
Regulation of xenobiotics metabolism	Phase I enzymes	CYP1A1, CYP1A2	(Kim et al., 2016; Vang et al., 2001)
	Phase II enzymes	UGT1A1, GST	(Kassie et al., 2003; Walters et al., 2004)
Inhibition of cell proliferation	Induction of apoptosis	p53, PARP, Caspase	(Antony et al., 2012; Byun et al., 2016; Kim et al., 2007; Lau et al., 2010)
	Cell cycle arrest	G2/M phase	(Choi et al., 2009; Parnaud et al., 2004)
	Antiproliferation	SKP2, Wnt/β-catenin	(Chung et al., 2015; Leem et al., 2015)
Inhibition of tumorigenesis	Inhibition of angiogenesis	VEGF	(Davaatseren et al., 2014)
	Inhibition of migration, invasion, and metastasis	MMP, MAPK, NF-κB	(Lai et al., 2010a; Lai et al., 2010b; Lai et al., 2014)
Epigenetic regulation	Modulation of HDAC	HDAC, p16	(Ho et al., 2009; Rajendran et al., 2015)
Anti-oxidative capacity	Modulation of anti-oxidative enzumes	SOD, CAT	(Ferrarini et al., 2012; Miller-Cebert, 2016)

Abbreviations: CAT, catalase; CYP, Cytochrome P450; GST, Glutathione *S*-Transferase; HDAC, Histone deacetylase; MAPK, Mitogen-activated protein kinase; MMP, Matrix metallopeptidase; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2, Nuclear factor-erythroid 2 p45-related factor 2; PARP, Poly (ADP-ribose) polymerase; SOD, superoxide dismutase; SKP2, S-phase kinase-associated protein 2; UGT, UDP-glucuronosyltransferase; VEGF, Vascular endothelial growth factor.

the same miRNAs and, moreover, one mRNA may possess many binding sequences (Lewis et al., 2005; Miranda et al., 2006). A 5′-proximal seed region of miRNA (i.e., nucleotide position 2–8) binds with 3′ untranslated region (3′-UTR) sequences in target mRNA. Depending on the degree of binding complementarity and binding numbers, these target mRNAs could be modulated differently (Place et al., 2008; Vella et al., 2004). However, recent studies have indicated that miRNA binding may occur with the 5′-UTR or coding region of mRNA as well (Forman et al., 2008; Lytle et al., 2007).

The current understanding regarding miRNA biogenesis and its maturation came from the observation that about 70 nucleotide precursor miRNAs (pre-miRNAs) are generated from long transcripts in the nucleus (i.e., primary miRNAs; pri-miRNAs) and are then processed into mature miRNAs in the cytoplasm (Lee et al., 2002). Most transcriptions of miRNAs are initiated by RNA polymerase II, resulting in pri-miRNAs that are normally several kilobases long and have a local hairpin structure (Lee et al., 2004). Pri-miRNAs are then subsequently cleaved by the action of endonucleases [i.e., Drosha-DGCR8 (DiGeorge syndrome critical region 8) complex] in order to form pre-miRNAs (Han et al., 2006). Following cleavage, these pre-miRNAs are exported by exportin-5 (i.e., nuclear pore complexes embedded in the nuclear membrane) to the cytoplasm and further processed to become mature, double-strand miRNAs by Dicer, another cytoplasmic RNase III (Hutvagner et al., 2001). Double-strand miRNAs are incorporated into an RNA-induced silencing complex (RISC) to be unwound and "activated." Once associated with RISC, miRNAs are capable of carrying out the process of silencing the target mRNA (Gregory et al., 2005). Interestingly, Okamura et al. proposed an alternative pathway for miRNA, which bypasses the Drosha cleavage process. MiRNAs can be derived from short intronic hairpins called "mirtrons" that are spliced and debranched to yield "pre-miRNA-like hairpins"; then, this pre-miRNA undergoes the same pathway as others (Okamura et al., 2007).

There are multiple possible control points during the biogenesis of miRNAs that may regulate expression. Although the specific mechanisms that regulate miRNA expression are not fully understood, several examples regarding possible regulatory factors on mRNA expression are summarized in Table 2. Even though it seems evident that miRNAs may play an important role in the modulation of gene expression, the mechanistic aspects are not fully understood. Firstly, it was proposed that miRNAs may inhibit initiation by interfering with the recognition of M7G-cap (Pillai et al., 2005). Another study proposed that the mechanism of the mRNA repression is mainly because miRNAs promote the drop-off of ribosomes (Petersen et al., 2006). In addition, let-7a, a miRNA, inhibited actively translating polyribosomes in HeLa cells (Nottrott et al., 2006). Lastly, it was reported that miRNAs induced deadenylation, possibly resulting in mRNA decay in P-body, which is enriched in the mRNA decay pathway's components (Wu et al., 2006).

Table 2. Possible regulatory factors regarding miRNAs with various mechanisms.

Possible regulatory factors	Suggested mechanisms	References
Drosha binding proteins: DEAD-box RNA helicase subunits Nuclear factor 90 and nuclear factor 45 proteins	Either positively or negatively regulate Drosha mediated process	(Fukuda et al., 2007; Sakamoto et al., 2009)
Drosha/DGCR8 expression	Altered expression levels of Drosha and DGCR8 result in miRNA level	(Muralidhar et al., 2007; Stark et al., 2008)
Dicer binding proteins: TRBP PACT	TRBP and PACT may play a role in the assembly of RISC	(Chendrimada et al., 2005; Lee et al., 2006)
Epigenetic control: MDB1	MiRNAs could be directly repressed by MDB1	(Liu et al.)
Exportin-5 expression	Expression of exportin-5 could modulate the activity and level of miRNAs	(Yi et al., 2005)
Genetic mutations: SNPs	SNPs within miRNAs may be associated with the alteration of processing efficiency	(Iwai and Naraba, 2005)

Abbreviations: Drosha/DGCR8, DiGeorge syndrome critical region; MDB1, Methyl-CpG binding protein 1; PACT, Protein kinase R-activating protein; RISC, RNA-induced silencing complex; SNP, Single Nucleotide Polymorphism; TRBP, TAR RNA binding protein.

MiRNA, colorectal cancer, and modulation of cancer risk via diet

Global dysregulation of miRNA is considered a hallmark of carcinogenesis, but the exact roles of miRNAs in cellular transformation and cancer etiology need to be further elucidated. In human colorectal cancer tissues, for instance, the expression of several miRNAs is either significantly higher (e.g., miR-31, miR-183, and miR-92) or lower (e.g., miR-143 and miR-145) than those in normal tissues (Motoyama et al., 2009). Another *in vitro* study showed that miR-145 targets insulin receptor substrate-1 and an insulin receptor substrate-1 without its 3′-UTR is no longer down-regulated by miR-145. Further, an insulin receptor substrate-1 lacking the binding site for the miR-145 rescued colon cancer cells from miR145-induced inhibition of growth (La Rocca et al., 2009). In another recent study, Chen et al. reported an inverse correlation between miR-143 and the KRAS protein *in vivo*. In the study, miR-143 directly recognized the 3′-UTR of oncogenic gene, KRAS, and inhibitor of this miRNA stimulated proliferation of epithelial cells through inhibiting constitutive phosphorylation of extracellular signal-regulated kinase 1/2 (Chen et al., 2009). These *in vitro* and *in vivo* studies clearly indicate that miRNAs affect carcinogenesis by targeting key genes and related cancer signaling pathways, thus, it is possible that previously demonstrated chemopreventive benefits of cruciferous vegetables might be, at least in part, due to their ability to modulate miRNAs.

Literature selection criteria

In order to exclude any potential biases, the authors systematically searched for research papers using the Medline/PubMed database from January, 1950 through November, 2016. Only English written publications were included for the review. Once searched, reference lists and citations of all studies were manually examined by more than one author for their

relevance in the context of colorectal cancer. Combinations of search terms used for the sections below are presented in Table 3.

Intact cruciferous vegetables and glucosinolates and miRNAs

As mentioned, it is widely accepted that the major chemopreventive mechanism of cruciferous vegetables is their bifunctional induction of phase I and phase II enzymes by the aryl hydrocarbon receptor signaling pathway (Safe, 2001) and Keap I-Nrf2 signaling pathway, respectively (Wolf, 2001). Modulation of biotranasformation enzymes through cruciferous vegetables is likely due to a class of compounds, glucosinolates (Abdull Razis et al., 2010), and their hydrolyzed products in cruciferous vegetables [(Horn et al., 2002); major types of glucosinolates and cruciferous vegetables are listed in the Table 4]. To the best of our knowledge, however, no study has investigated the potential effects of intact cruciferous vegetables or glucosinolates on miRNA modulation and their implication in colorectal cancer. Nonetheless, a recent work published by Pastrello et al. in 2016 is worthy of attention. It stated that levels of miRNAs in human blood, from a large nutrigenomics cohort study and in a randomized dose-controlled trial, were significantly correlated with the subjects' daily amount of broccoli consumed. In their subsequent *in vitro* validation, one of the miRNAs detected in human blood samples after broccoli consumption down-regulated genes that are frequently up-regulated in lung cancer (Pastrello et al., 2016). Even though it was not directly examined for the effects of cruciferous vegetables on colorectal cancer or related miRNAs involved in colorectal cancer development, the results indicate that (1) realistic consumption of cruciferous vegetable consumption (up to 160 g/day; about 1.6 cups per day) changed the miRNA profile in humans and (2) these impacts were even more favorable to counteract lung cancer development in which the specific miRNA was exposed to glucosinolates and their secondary metabolites (e.g., ITC). Evidence indicates that intact cruciferous vegetables (e.g., broccoli) may exhibit a preventive role against cancers (e.g., lung cancer), but further investigations are warranted if such protection was achieved via miRNA modulation in the context of colorectal cancer.

Table 3. Literature search strategies and combinations of key words for respective sections of the review.[a]

Section of the review	Keywords combinations	References identified through initial search (n)
Intact cruciferous vegetables and glucosinolates and colorectal cancer	microRNA [Title/Abstract] + vegetable [Title/Abstract]	8
	microRNA [Title/Abstract] + glucosinolate [Title/Abstract]	5
	microRNA [Title/Abstract] + cruciferous [Title/Abstract]	10
	microRNA [Title/Abstract] + Brassicaceae [Title/Abstract]	9
Isothiocyanates, secondary metabolites of glucosinolates	microRNA [Title/Abstract] + isothiocyanate [Title/Abstract]	34
	microRNA [Title/Abstract] + sulforaphane [Title/Abstract]	14
	microRNA [Title/Abstract] + PEITC [Title/Abstract]	3
	microRNA [Title/Abstract] + AITC [Title/Abstract]	1
Indol-3-carbinol and oligomers	microRNA [Title/Abstract] + indole-3-carbinol [Title/Abstract]	8
	microRNA [Title/Abstract] + 3,3′-diindolylmethane [Title/Abstract]	7
	microRNA [Title/Abstract] + indolo-[3,2-b]carbazole [Title/Abstract]	0
Selenium	microRNA [Title/Abstract] + selenium [Title/Abstract]	9
Dietary fibers, fermentation products of dietary fibers, and miRNAs	microRNA [Title/Abstract] + fiber [Title/Abstract]	89
	microRNA [Title/Abstract] + dietary fiber [Title/Abstract]	2
	microRNA [Title/Abstract] + short chain fatty acid [Title/Abstract]	2
Total numbers of literature after initial search		**201**

[a] The Medline/PubMed database was utilized for the initial literature search. Only English written publications were included for the review. Initially retrieved studies were screened by more than one author to exclude overlapped results and to examine their relevance in the context of colorectal cancer.
Abbreviations: AITC, Allyl isothiocyanate; PEITC, Penthyl isothiocyanate.

Isothiocyanates, secondary metabolites of glucosinolates

ITCs are present in high concentrations in cruciferous vegetables and have been studied for their chemopreventative properties for many years (Navarro et al., 2011). They are present in intact cruciferous vegetables as glucosinolates, which are released from the vegetable's cells upon chewing and damage. They are then metabolized into ITCs by the enzyme myrosinase, which is also released from the cells (Holst and Williamson, 2004). Different glucosinolates have different metabolic products; for example, glucoraphanin and sinigrin, both of which are found in broccoli, are metabolized to sulforaphane and allyl isothiocyanate, respectively. Additionally, garden cress contains glucotropaeolin, which produces benzyl isothiocyanate. Watercress, however, produces PEITC, which is a metabolite of gluconaturiin (Holst and Williamson, 2004).

Recently, Slaby et al. investigated the effects of ITCs (iberin and sulforaphane) on miRNA expression in epithelial cell lines, NCM460 and NCM356 (Slaby et al., 2013). This *in vitro* study showed that 15 miRNAs (up-regulation of 12 miRNAs and down-regulation of 3 miRNAs) and 18 miRNAs (up-regulation of 15 miRNAs and down-regulation of 3 miRNAs) were modulated by iberin and sulforaphane, respectively. Of modulated miRNAs, miR-23b, and miR-155 were the most significant findings, due to their relevance in colorectal cancer prognosis (Slaby et al., 2013). In particular, in both treatments (i.e., iberin and sulforaphane), miR-23b was up-regulated in comparison to the control. Typically, miR-23b is down-regulated in colorectal cancer and suppresses cancer metastasis and cell proliferation via interacting with its targets [e.g., SMAD3, FZD7, and

Table 4. Major glucosinolates in cruciferous vegetables.

Common name	Scientific name	Glucosinolates	References
Aliphatic glucosinolates			
Broccoli	*Brassica oleracea var. italica*	Glucoraphanin	(Hwang and Kim, 2013; Vale et al., 2015)
Bok choy	*Brassica rapa subsp. Chinensis*	Gluconapin	(He et al., 2003)
Choy sum	*Brassica chinensis var. parachinensis*	Gluconapin	(He et al., 2003)
Chinese cabbage	*Brassica rapa ssp. pekinensis*	Gluconapin	(Kim et al., 2013)
White cabbage	*Brassica oleracea L.*	Glucoiberin	(Ciska et al., 2000; Yi et al., 2015)
		Glucoraphanin	(Yi et al., 2015)
		Sinigrin	(Ciska et al., 2000; Keck and Finley, 2004)
Red cabbage	*Brassica oleracea var. capitata f. rubra*	Glucoiberin	(Ciska et al., 2000)
		Glucoraphanin	(Ciska et al., 2000)
		Progoitrin	(Vale et al., 2015)
		Sinigrin	(Ciska et al., 2000)
Savoy cabbage	*Brassica oleracea var. sabauda*	Glucoiberin	(Ciska et al., 2000)
Kerguelen cabbage	*Pringleaantiscorbutica*	Gluconapin	(Barillari et al., 2005)
Radishes	*Raphanus sativus*	Dehydroerucin	(Ciska et al., 2000; Yi et al., 2016)
Brussels sprouts	*Brassica oleracea var. gemmifera*	Glucoiberin	(Ciska et al., 2000; de Vos and Blijleven, 1988)
		Gluconapin	(Ciska et al., 2000; de Vos and Blijleven, 1988)
		Progoitrin	(Kushad et al., 1999)
		Sinigrin	(de Vos and Blijleven, 1988; Keck and Finley, 2004)
Cauliflower	*Brassica oleracea var. botrytis*	Glucoiberin	(de Vos and Blijleven, 1988; Yi et al., 2015)
		Glucoraphenin	(Yi et al., 2015)
		Sinigrin	(Ciska et al., 2000; Keck and Finley, 2004)
Turnip	*Brassica rapa subsp. rapa*	Gluconapin	(Padilla et al., 2007)
Swede-turnip	*Brassica napobrassica*	Progoitrin	(Fenwick and Heaney, 1983)
Kale	*Brassica oleracea var. sabellica*	Glucoiberin	(Ciska et al., 2000; Yi et al., 2015)
		Gluconapin	(Qian et al., 2015)
		Sinigrin	(Fenwick and Heaney, 1983; Kushad et al., 1999)
Kohlrabi	*Brassica oleracea Gongylodes Group*	Glucoerucin	(Park et al., 2012)
		Glucoiberverin	(Yi et al., 2015)
		Glucoraphanin	(Ciska et al., 2000)
Rape	*Brassica napus ssp. pabularia*	Glucobrassicanapin	(Font et al., 2005)
		Progoitrin	(Ciska et al., 2000; Font et al., 2005)
Horseradish	*Armoracia rusticana*	Sinigrin	(Li and Kushad, 2004)
Rocket (Arugula)	Eruca sativa	Glucoerucin	(Graser et al., 2000)
		Glucoraphanin	(D'Antuono et al., 2008)
Collard	*Brassica oleracea convar. acephala var. medullosa Thell*	Progoitrin	(He et al., 2003)
Cabbage mustard	*Brassica juncea Coss.*	Sinigrin	(He et al., 2003)
Pot herb mustard	*Brassica juncea Coss.*	Gluconapin	(He et al., 2003)
Heterocyclic glucosinolates (Indole)			
Broccoli	*Brassica oleracea var. italica*	Glucobrassicin	(Hwang and Kim, 2013)
Bok choy (Pak choy)	*Brassica rapa subsp. Chinensis*	Neoglucobrassicin	(He et al., 2003)
Choy sum	*Brassica chinensis var. parachinensis*	Neoglucobrassicin	(He et al., 2003)
White cabbage	*Brassica oleracea L.*	Glucobrassicin	(de Vos and Blijleven, 1988; Yi et al., 2015)
Red cabbage	*Brassica oleracea var. capitata f. rubra*	Glucobrassisin	(Ciska et al., 2000)
Savoy cabbage	*Brassica oleracea var. sabauda*	Glucobrassisin	(Ciska et al., 2000)
Brussels sprouts	*Brassica oleracea var. gemmifera*	Glucobrassicin	(de Vos and Blijleven, 1988; Kushad et al., 1999)
Cauliflower	*Brassica oleracea var. botrytis*	Glucobrassicin	(Ciska et al., 2000; de Vos and Blijleven, 1988)
		4-Methoxy-glucobrassicin	(Yi et al., 2015)
Kale	*Brassica oleracea var. sabellica*	Glucobrassicin	(Ciska et al., 2000; Yi et al., 2015)
Kohlrabi	*Brassica oleracea Gongylodes Group*	Glucobrassicin	(Ciska et al., 2000; Park et al., 2012)
Rape	*Brassica napus ssp. pabularia*	4-hydroxy-glucobrassicin	(Ciska et al., 2000)
Collard	*Brassica oleracea convar. acephala var. medullosa Thell*	Glucobrassicin	(He et al., 2003)
Mustard	*Brassica juncea Coss.*	Neoglucobrassicin	(He et al., 2003)
Aromatic glucosinolates			
Chinese cabbage	*Brassica rapa ssp. pekinensis*	Gluconasturtiin	(Kim et al., 2013)
Kerguelen cabbage	*Pringleaantiscorbutica*	Glucotropaeolin	(Barillari et al., 2005)
Watercress	*Nasturtium officinale*	Gluconasturtiin	(Keck and Finley, 2004; Kopsell et al., 2007)
Cauliflower	*Brassica oleracea var. botrytis*	Gluconasturtiin	(Yi et al., 2015)
Kale	*Brassica oleracea var. sabellica*	Glucoraphanin	(Hahn et al., 2016)
Rape	*Brassica napus ssp. pabularia*	Gluconasturtiin	(Ciska et al., 2000)
Horseradish	*Armoracia rusticana*	Gluconasturtiin	(Li and Kushad, 2004)

MAP3K1; (Yuan et al., 2011; Zhang et al., 2011)]. In addition, miR-23b and its family members are thought to be regulators of TGF-β signaling and epithelial-mesenchymal transition, both of which are hallmarks of colorectal cancer progression (Castilla et al., 2011). Thus, it is accepted that miR-23b is a tumor suppressor miRNA in many cancers, including colorectal cancer. In contrast, the other miRNA modulated by ITCs was miR-155 which is up-regulated in a number of neoplastic diseases (Faraoni et al., 2009). Multiple lines of evidence support the fact that miR-155 plays a significant role in

carcinogenesis, acting as an onco-miR [reviewed in (Faraoni et al., 2009)]. In colorectal cancer, miR-155 is up-regulated in tumor tissue (Bakirtzi et al., 2011) and positively correlated with tumor stage and prognosis of colorectal cancer patients (Shibuya et al., 2010). Overall, such changes in miRNA expression modulated by iberin and sulforaphane support the previously observed cancer preventive effects of ITCs.

Even though it was not investigated in colorectal cancer models, sulforaphane induced miR-let-7a which in turn inhibited KRAS expression and cancer stem cell characteristics in pancreatic ductal adenocarcinoma (Appari et al., 2014). Another ITC, PEITC, modulated miR-let-7a and miR-let-7c to counteract the effects of exposure to environmental cigarette smoke in Sprague-Dawley rats (Izzotti et al., 2010). In another *in vivo* study, benzyl isothiocyanate perturbed the miR-221: miR-375 ratio, an important factor in distinguishing pancreatic intraepithelial neoplasia from normal pancreas, to switch hyperproliferative pancreatic cancer cells to a hypoproliferative status (Basu et al., 2011). Thus far, a few *in vitro* studies provide evidence that ITCs modulate miRNAs to counteract against cancer development via multiple mechanisms (e.g., suppression of cancer metastasis and cell proliferation). Additional validation is needed to solidify findings from *in vitro* studies and to examine if effects of ITCs in different *in vitro* models can be recapitulated in *in vivo* models or in humans.

Indol-3-carbinol and oligomers

Indole-3-carbinol (I3C) is another secondary metabolite of glucosinolates; I3C and its oligomers are naturally occurring chemopreventive agents (Aggarwal and Ichikawa, 2005; Weng et al., 2008). An acidic condition can convert I3C into polymeric analogues [e.g., DIM and indolo-[3,2-b]carbazole (ICZ)] and these indole-containing compounds are the major chemopreventive bio-actives present in cruciferous vegetables [reviewed in (Higdon et al., 2007)]. In regard to colorectal cancer, multiple mechanisms of I3C and its oligomers have been proposed thus far. The most extensively investigated mechanism is modulation of biotransformation enzymes by the aryl hydrocarbon receptor signaling (Safe, 2001). In addition, the antiproliferation effect of I3C was demonstrated in HT29 colon cancer cells (Frydoonfar et al., 2002) and SW480 human colon cells (Hudson et al., 2003). Another chemo-preventive mechanism of this class of compound is inducing apoptotic signaling pathways, which have been demonstrated in different colon cancer cell lines such as Colo320 (Zheng et al., 2002), Caco-2 (Bonnesen et al., 2001), and LS-174 (Bonnesen et al., 2001). I3C significantly increased cell cycle arrest in human colon cancer cells (e.g., HCT-116 cells) by modulating the cell cycle regulatory gene (i.e., p21, involved in G_1 phase arrest) and, subsequently, its down-stream target [e.g., cdk4; (Neave et al., 2005)].

Even though it was not directly investigated in colorectal cancer models, evidence indicates that I3C or DIM controls miRNA expression in other types of cancer models: breast cancer cells (Hargraves et al., 2016), pancreatic cancer cells (Li et al., 2009; Paik et al., 2013), and hepatic cancer cells (Wang et al., 2015) as well as rodent lung tissue (Izzotti et al., 2010; Melkamu et al., 2010). For instance, a recent *in vitro* study showed that I3C induces cell-cycle arrest via increasing miR-34a expression in MCF-7 human breast cancer cells (Hargraves et al., 2016). Interestingly, up-regulation of miR-34a was directly mediated by expression of p53 tumor suppressor protein and its down-stream gene p21 which were increased by I3C exposure; this corresponds to a previous finding that a family of miR-34 is a direct transcriptional target of p53 (He et al., 2007). In another study, I3C suppressed miR-21 expression in Panc-1 human pancreatic cancer cells in a dose dependent manner (Paik et al., 2013). Direct up-regulation of PDCD4, a tumor suppressor, by miR-21 suppression was confirmed using a miR-21 mimic model. Changes in miRNA profile by I3C were also observed in carcinogen-exposed mice. The expression of miR-21, miR-31, miR-130a, miR-146b, and miR-377 was significantly up-regulated by vinyl carbamate injection, and those changes were reversed after I3C treatment in lung tissue of mice. PTEN, PDCD4, and reversion-inducing-cystein-rich protein with Kazal motifs were proposed as possible targets of miR-21 (Melkamu et al., 2010).

In a rat study, I3C restored lung miRNAs (i.e., miR-10a, miR-26a, miR-34b, and miR-125a) that were modulated by cigarette smoke (Izzotti et al., 2010). I3C was also reported to inhibit tumorigenicity of hepatocellular carcinoma (HCC) cells via regulation of miR-21 *in vitro* and *in vivo* (Wang et al., 2015). In the study, miR-21, miR-221, and miR-222 expressions were significantly down-regulated by I3C in both SK-Hep-1 and SNU-449 cells, but only miR-21 was able to modulate HCC. This may be due to the direct interaction between miR-21 and PTEN; specifically, I3C down-regulated miR-21 and then miR-21 regulated PTEN through direct binding to its 3′-UTR. The interaction between miR-21 and PTEN was validated in a HCC xenograft mouse model and was also demonstrated elsewhere (Meng et al., 2007), strongly indicating that I3C may inhibit HCC via the miR-21-PTEN axis. Evidence indicates that I3C's oligomers (e.g., DIM) may play a role in cancer development through miRNA modulation. In pancreatic cancer cells, DIM modulated miR-200 and let-7 families are known to stimulate chemo-sensitivity (Li et al., 2009). The same research group reported that DIM up-regulated miR-146a to reduce invasion of Colo357 and Panc-1 pancreatic cancer cell, in part, through down-regulation of epidermal growth factor receptors (EGFR), metastasis-associated protein-2, interleukin-1 receptor-associated kinase 1, and NF-κB (Li et al., 2009).

Taken together, both *in vitro* and *in vivo* studies support the notion that I3C and its acidic condensates (e.g., DIM) might be chemopreventive through modulating various miRNAs and responsible cancer signaling pathways. Albeit these studies are not performed in colorectal cancer models, a few findings are important to note because I3C and its oligomers influenced, via miRNAs, key genes and signaling pathways that are also closely involved with colorectal carcinogenesis. In MCF-7 human breast cancer cells, miR-34a increased with I3C treatment (Hargraves et al., 2016). This miR-34a is known to directly target genes (e.g., Axin 2) involved with the Wnt-β catenin signaling pathway (Kim et al., 2013). It is well known that the canonical Wnt signaling pathway is involved in the development of colorectal cancer (Moon et al., 2004). The other example is miR-21; I3C down-regulated miR-21 in multiple cell lines and rodent tissues such as Panc-1 human pancreatic cancer cells (Paik et al., 2013), mouse lung tissue (Melkamu et al.,

2010), and HCC cells (Wang et al., 2015). In these studies, despite being of different tissues, miR-21 directly targeted and suppressed PTEN. Therefore, it seems reasonable to believe that I3C induces a tumor suppressor gene (i.e., PTEN) by down-regulating miR-21. Of note, PTEN mutation and deletion are one of the most commonly observed pathway changes in colorectal cancer (Danielsen et al., 2015), strongly suggesting that this miR-21-PTEN axis might also be responsible for the chemo-preventive potential of I3C in colorectal cancer models. Given the evidence that (1) different signaling pathways are responsible for different stages of cancer development [e.g., Wnt signaling pathway, apoptosis (You et al., 2002), vs. metastatic signaling pathway, metastasis (Padua and Massague, 2009)] and (2) miRNAs influenced by I3C and its oligomers can control key genes. As discussed above, these bio-actives likely exercise leverage on cancers throughout the stages of carcinogenesis.

Selenium

In addition to the unique phytochemicals found in cruciferous vegetables, selenium (Se) is also noteworthy due to its high abundance (Mahn and Reyes, 2012). Of the cruciferous vegetables, broccoli has the highest Se content (Mahn and Reyes, 2012). According to the latest USDA National Nutrient Database for Standard Reference, broccoli contains approximately 4.4 μg Se per one cup (equivalent to 0.028 μg Se/g broccoli), which is the 5th highest level of Se (https://ndb.nal.usda.gov). Only vegetables that have higher selenium concentrations compared to broccoli are portabella mushroom (0.219 μg Se/g), garlic (0.142 μg Se/g), potato (0.133 Se μg/g), and asparagus (0.039 μg Se/g) per the database confirming that cruciferous vegetables are a good source of Se. Interestingly, the concentration of Se-methylselenocysteine (known as an active anticancer compound) in cruciferous vegetables can be greatly influenced by bio-fortification of Se, thus the actual level of exposure to Se could vary depending upon growing conditions (Avila et al., 2014). Selenium plays many roles in the body systems and is essential for many aspects of human health [reviewed in (Bellinger et al., 2009)]. Thus far, a total of 25 selenoprotien genes have been identified in humans, including glutathione peroxidases; thioredoxin reductases; and selenoproteins P, N, and S (the former being a Se-transport protein, the latter ones being involved in the endoplasmic reticulum) (Reeves and Hoffmann, 2009). These proteins serve various roles in the body, for example, glutathione peroxidases are involved in the antioxidant defense system, while thioredoxin reductases are involved in the thioredoxin redox reactions required for DNA synthesis (Reeves and Hoffmann, 2009).

Selenium is incorporated into selenoproteins as selenocysteine utilizing a specific tRNA (i.e., selenocysteine-tRNA); this specific tRNA is coded within the mRNA transcript's 3′-UTR (Reeves and Hoffmann, 2009). Interestingly, 3′-UTR of mRNAs is frequently reported as a target of miRNA recognition to repress or silence them. Therefore, regulation of miRNAs by Se could be one of the underlying mechanisms to which selenoprotein synthesis is regulated. Recently, Maciel-Dominguez et al. investigated if selenium supply alters gene expression through miRNA regulation in human colon adenocarcinoma cells (Maciel-Dominguez et al., 2013). In the study, a total of 12 miRNAs were modulated after Se depletion which, in turn, influenced expression of 50 genes including selenoproteins such as GPX1, SELW, and SEPSH2. Of the 12 miRNAs, miR-185 showed one of the biggest changes in expression level after Se depletion. Notably, bioinformatics analysis identified target transcripts of miR-185 and, in a subsequent validation, silencing of this miRNA significantly increased expression of its target selenoproteins (i.e., GPX2 and SEPSH2). This Indicates that Se may maintain selenoprotein synthesis via miRNA expression in human colon adenocarcinoma cells.

Effects of Se deficiency on miRNA expression profiles were evaluated by feeding rats a low Se diet over 14 weeks (Xing et al., 2015). As a result, miR-374, miR-16, miR-199a-5p, miR-195, and miR-30e were up-regulated whereas miR-3571, miR-675, and miR-450a were down-regulated in rats with Se deficiency. Furthermore, Se deficiency resulted in cardiac dysfunction via increasing the expression of Wnt and β-catenin. Although this study did not directly assess the effects of Se in colorectal cancer models, results indicate that the level of Se exposure can influence miRNA expression profile as well as key players of the Wnt-β-catenin signaling pathway. No *in vivo* studies have been reported regarding miRNA regulation by Se relating to colorectal carcinogenesis, thus further studies are needed to not only elucidate the mechanisms behind such interactions, but also to understand the downstream effects of selenium intake on colorectal cancer prevention.

Dietary fibers, fermentation products of dietary fibers, and miRNAs

Even though dietary fibers are not unique to cruciferous vegetables, we summarized effects of dietary fibers and their fermentation products on miRNAs because cruciferous vegetables are one of major sources for those. In fact, according to the latest National Nutrient Database for Standard Reference, the average fiber content in cruciferous vegetables is higher than 3.0 g/100 g fresh weight, ranking cruciferous vegetables 9th in fiber-rich foods (https://ndb.nal.usda.gov). In this article, we only addressed studies of naturally occurring plant fibers as related to miRNA and colorectal cancer; however, synthetic fibers (e.g., carboxymethyl cellulose and polyol) as well as animal fibers (e.g., chitin and chitosan) were excluded. For decades, the benefits of dietary fiber for the prevention of colorectal cancer have been recognized through the following preventive mechanisms: (1) promotion of carcinogen dilution in intestinal tract (Nigro et al., 1979), (2) reduction of mean transit time of feces in large bowel (Cummings et al., 1976), and (3) enhancement of beneficial short-chain fatty acid production as substrates of bacterial fermentation (McIntyre et al., 1993). Despite extensive research, results from epidemiological studies are somewhat inconsistent (e.g., Schatzkin et al., 2007); such inconsistency is likely driven by factors such as study design (e.g., different types of colorectal cancer, starting point of intervention, experimental period, and dose).

A few studies have been done thus far involving miRNA modulating effects of secondary metabolites of dietary fibers in colorectal cancer models. In an *in vitro* study, a representative microbial-derived short chain fatty acid (i.e., butyrate), changed

44 miRNA profiles in human colon carcinoma cells (Hu et al., 2011). It is important to note that most miRNAs, which are increased in human tumor tissues, were decreased by butyrate exposure (e.g., miR-17, miR-20a, and miR-106b). Furthermore, these miRNAs targeted the same binding site in 3′-UTRs of mRNA. In the study, the authors suggested dual function of butyrate: (1) increasing histone acetylation by inhibition of histone deacetylases, resulting in increased transcription of p21 and (2) reducing the expression of miR-106b family that inhibits p21 translation thereby increasing p21 translation and inducing cell cycle arrest. Other mechanisms of butyrate include the antiproliferative effects of miR-92a through down-regulation of c-Myc and its downstream cascade (e.g., pri-miR-17–92a and miR-92a; Hu et al., 2015). This antiproliferative mechanism might be highly related to the PTEN signaling pathway; expression of miR-92a is significantly increased in human colorectal cancer tissues and inversely correlated with PTEN expression (Zhang et al., 2014). In addition to the miR-92a-PTEN axis, the other recent study also reported that sodium butyrate exhibits antiproliferative potential via miRNA modulation by targeting an oncogenic gene, NEDD9. Han et al. (2016) showed that, sodium butyrate increased the expression of miR-203 which directly targets NEDD9 to suppress cell proliferation and induce apoptosis in human colorectal cancer cells (i.e., HT-29 and Caco-2 cells).

Davidson et al. examined if a combination of fish oil and fiber (either cellulose or pectin) modulates azoxymethane-induced changes in miRNAs profiles (Davidson et al., 2009). This hypothesis was based on their previous observation that chemo-preventive effects of n-3 polyunsaturated fatty acids were synergistically increased when high fermentable fiber and the fatty acids were provided simultaneously (Crim et al., 2008). Co-administration of fish oil and dietary fibers [both cellulose (nonfermentable) and pectin (fermentable)] increased the expression of five miRNAs: let-7d, miR-15b, miR-107, miR-191, and miR-324; interestingly, there was no significant difference of miRNA expression between the two different fibers, indicating that the degree of fiber fermentation might not be a decisive factor modulating miRNAs and exerting protection against azoxymethane induced cancers. Therefore, further mechanistic studies might be warranted with regard to fiber fermentation and the production of short chain fatty acids in relation to colorectal cancer prevention. Additionally, other carcinogen induced models should be examined besides azoxymethane, considering the varying carcinogenesis mechanisms and related signaling pathways [e.g., azoxymethane (Moen et al., 2016) vs. heterocyclic aromatic amines (Ferguson and Harris, 1996)].

Even though it was performed in epidermal cells, the study of Cha et al. is also interesting to note; in the study, effects of arctiin (a lignin compound) were investigated against UVB-induced damage in HaCaT keratinocyte (Cha et al., 2014). Through the bioinformatic analysis of miRNA expressions, the authors showed that arctiin favorably changed the expression profiles of certain miRNAs, post-UVB treatment. The changed miRNAs are involved in many cancer-related signaling pathways [e.g., cell cycle signaling pathway (miR-125a-5p and miR-29b-1–5p); MAPK signaling pathway (miR-205-3p, miR-3652, miR-513a-5p, and miR-1290)]; therefore, it is worth studying whether the miRNA modulating effects of arctiin are recapitulated in colorectal cancer models considering that some miRNAs, down-regulated by arctiin (e.g., miR-125a-5p and miR-1290), are closely related to colorectal cancer. For example, miR-125-a-5p targeted microtubule-associated tumor suppressor 1 which inhibits ERK2 MAPK pathway and cell proliferation in human colorectal cancer tissue (Ozcan et al., 2016). In another study, miR-1290 induced c-Myc, a key inducer of oncogenic pathways, in serum of colorectal cancer patients (Li et al., 2016).

Although it may be too early to conclude that dietary fibers exert chemo-preventive mechanisms through miRNA modulation, a few *in vitro* studies demonstrated that secondary metabolites of fibers might favorably change its expression profile. In addition to the necessity of *in vivo* and human experiments, a few more factors should be taken into consideration in future studies of dietary fibers: specifically, dietary fibers can be classified into subcategories depending on their biological and physical properties: (1) soluble versus insoluble, (2) viscous versus nonviscous, and (3) fermentable versus nonfermentable. Thus, fiber type-dependent effects on colorectal cancer-related miRNAs should be evaluated. It is well established that short chain fatty acids provide energy for certain intestinal microbiota (Monsma et al., 2000) and that certain microbiota change intestinal miRNA profiles (Dalmasso et al., 2011);however, there is no direct evidence showing interaction(s) between dietary fiber-induced microbiome profiles and miRNA profiles. A better understanding of the complex crosstalk between gut microbiota and cancer signaling pathways would also be informative.

Perspective and future direction

Discovering interactions between small RNA molecules and mRNA transcripts is one of the most recent scientific breakthroughs in biology; it was first proposed to term these small RNA molecules as “miRNA in 2001 (Lagos-Quintana et al., 2001). Since then, a number of studies on this class of noncoding RNAs have been published, but the majority of the studies are in basic science fields, likely due to its infancy. Although it is generally accepted that cruciferous vegetables, glucosinolates, and their secondary metabolites might have potential beneficial effects for colorectal cancer through various mechanisms [e.g., induction of detoxification enzymes (Navarro et al., 2009; Robbins et al., 2010), scavenging free radicals (Zhu et al., 2000), and induction of apoptosis (Bonnesen et al., 2001; Hwang and Lee, 2010)], there is a paucity of information regarding their ability to modulate miRNAs. Therefore, the objective of this review was to report the most recent important results in regards to miRNA regulation by cruciferous vegetables and their bioactives in context of colorectal cancer.

The results of our study found considerable evidence showing that cruciferous vegetables and their metabolites may prevent various cancers, including colorectal cancer, via modulating miRNAs (summarized in the Fig. 1); however, most studies simply observed whether certain miRNAs are either increased or decreased by exposure to bioactives rather than by examining the specific controlling mechanisms (that are listed in the Table 2). Furthermore, the majority of studies have been carried out in *in vitro* cancer cell models as opposed

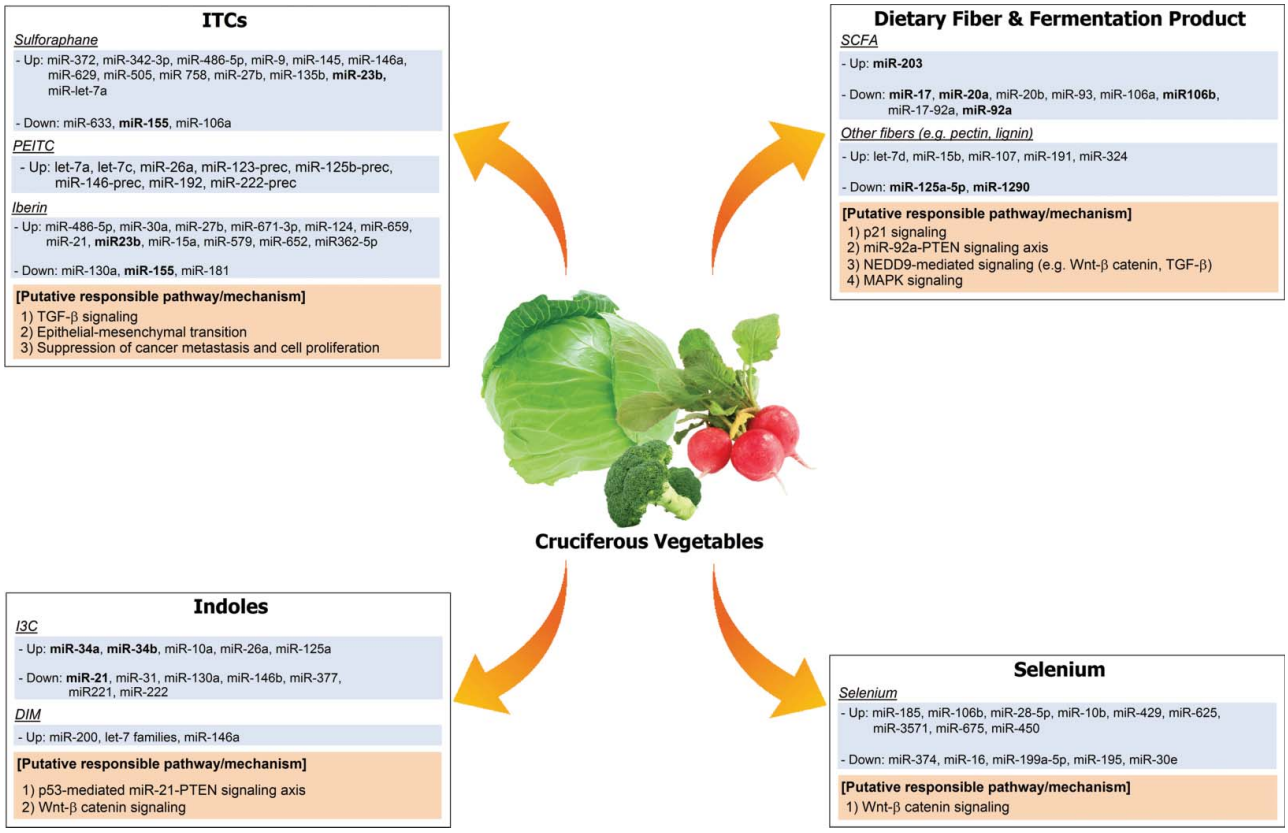

Figure 1. Impacts of secondary metabolites in cruciferous vegetables, Se, and dietary fiber on miRNAs and colorectal cancer related signaling pathways. Up- or down-regulated miRNAs by secondary metabolites in cruciferous vegetable, Se, and dietary fibers were summarized. MiRNAs in bold are indicated as key miRNAs in signaling pathways of colorectal carcinogenesis in the respective references. Abbreviations: DIM, 3,3′-diindolylmethane; PEITC, Penthyl isothiocyanate.

to *in vivo* cancer models. As *in vitro* environments do not always reflect *in vivo* conditions, which can cause misdirection of the actual physiological aspect, additional validation and *in vivo* studies are warranted to gain practical information for further clinical intervention. It seems clear, considering accumulating evidence regarding their interactions with cancer related genes and relevant signaling pathways, that miRNA modulation is an attractive target for the prevention of colorectal cancer. In addition, miRNAs have been characterized as diagnostic and prognostic biomarkers and utilized in cancer therapeutics. Thus, it is very possible that natural agents (not limited to those in cruciferous vegetables) enhance cancer therapeutic efficacy and elicit chemopreventive effects through modulating key miRNAs.

Funding

This work was supported by the University of Arkansas, VPRED Start-up fund.

References

World Cancer Research Fund / American Institute for Cancer Research. (2007). Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective. Washington, DC: AICR.

Abdull Razis, A. F., Bagatta, M., De Nicola, G. R., Iori, R. and Ioannides, C. (2010). Intact glucosinolates modulate hepatic cytochrome P450 and phase II conjugation activities and may contribute directly to the chemopreventive activity of cruciferous vegetables. *Toxicology*. **277**:74–85.

Aggarwal, B. B. and Ichikawa, H. (2005). Molecular targets and anticancer potential of indole-3-carbinol and its derivatives. *Cell Cycle*. **4**:1201–1215.

Antony, M. L., Kim, S. H. and Singh, S. V. (2012). Critical role of p53 upregulated modulator of apoptosis in benzyl isothiocyanate-induced apoptotic cell death. *PLoS One*. **7**:e32267.

Appari, M., Babu, K. R., Kaczorowski, A., Gross, W. and Herr, I. (2014). Sulforaphane, quercetin and catechins complement each other in elimination of advanced pancreatic cancer by miR-let-7 induction and K-ras inhibition. *Int. J. Oncol.* **45**:1391–1400.

Avila, F. W., Yang, Y., Faquin, V., Ramos, S. J., Guilherme, L. R., Thannhauser, T. W. and Li, L. (2014). Impact of selenium supply on Se-methylselenocysteine and glucosinolate accumulation in selenium-biofortified Brassica sprouts. *Food Chem.* **165**:578–586.

Bakirtzi, K., Hatziapostolou, M., Karagiannides, I., Polytarchou, C., Jaeger, S., Iliopoulos, D. and Pothoulakis, C. (2011). Neurotensin signaling activates microRNAs-21 and -155 and Akt, promotes tumor growth in mice, and is increased in human colon tumors. *Gastroenterology*. **141**:1749–1761, e1741.

Barillari, J., Iori, R., Rollin, P. and Hennion, F. (2005). Glucosinolates in the subantarctic crucifer Kerguelen cabbage (*Pringlea antiscorbutica*). *J. Nat. Prod.* **68**:234–236.

Basu, A., Alder, H., Khiyami, A., Leahy, P., Croce, C. M. and Haldar, S. (2011). MicroRNA-375 and MicroRNA-221: Potential noncoding RNAs associated with antiproliferative activity of benzyl isothiocyanate in pancreatic cancer. *Genes Cancer*. **2**:108–119.

Bellinger, F. P., Raman, A. V., Reeves, M. A. and Berry, M. J. (2009). Regulation and function of selenoproteins in human disease. *Biochem. J.* **422**:11–22.

Bonnesen, C., Eggleston, I. M. and Hayes, J. D. (2001). Dietary indoles and isothiocyanates that are generated from cruciferous vegetables can both stimulate apoptosis and confer protection against DNA damage in human colon cell lines. *Cancer Res.* **61**:6120–6130.

Bravo-Egana, V., Rosero, S., Molano, R. D., Pileggi, A., Ricordi, C., Dominguez-Bendala, J. and Pastori, R. L. (2008). Quantitative differential expression analysis reveals miR-7 as major islet microRNA. *Biochem. Biophys. Res. Commun.* **366**:922–926.

Byun, S., Shin, S. H., Park, J., Lim, S., Lee, E., Lee, C., Sung, D., Farrand, L., Lee, S. R., Kim, K. H., Dong, Z., Lee, S. W. and Lee, K. W. (2016). Sulforaphene suppresses growth of colon cancer-derived tumors via induction of glutathione depletion and microtubule depolymerization. *Mol. Nutr. Food Res.* **60**:1068–1078.

Castilla, M. A., Moreno-Bueno, G., Romero-Perez, L., Van De Vijver, K., Biscuola, M., Lopez-Garcia, M. A., Prat, J., Matias-Guiu, X., Cano, A., Oliva, E. and Palacios, J. (2011). Micro-RNA signature of the epithelial-mesenchymal transition in endometrial carcinosarcoma. *J. Pathol.* **223**:72–80.

Cha, H. J., Lee, G. T., Lee, K. S., Lee, K. K., Hong, J. T., Lee, N. K., Kim, S. Y., Lee, B. M., An, I. S., Hahn, H. J., Ahn, K. J., Lee, S. J., An, S. and Bae, S. (2014). Photoprotective effect of arctiin against ultraviolet B-induced damage in HaCaT keratinocytes is mediated by microRNA expression changes. *Mol. Med. Rep.* **10**:1363–1370.

Chen, X., Guo, X., Zhang, H., Xiang, Y., Chen, J., Yin, Y., Cai, X., Wang, K., Wang, G., Ba, Y., Zhu, L., Wang, J., Yang, R., Zhang, Y., Ren, Z., Zen, K., Zhang, J. and Zhang, C. Y. (2009). Role of miR-143 targeting KRAS in colorectal tumorigenesis. *Oncogene*. **28**:1385–1392.

Chendrimada, T. P., Gregory, R. I., Kumaraswamy, E., Norman, J., Cooch, N., Nishikura, K. and Shiekhattar, R. (2005). TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature*. **436**:740–744.

Cheng, A. M., Byrom, M. W., Shelton, J. and Ford, L. P. (2005). Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. *Nucleic Acids Res.* **33**:1290–1297.

Choi, H. J., Lim, D. Y. and Park, J. H. (2009). Induction of G1 and G2/M cell cycle arrests by the dietary compound 3,3′-diindolylmethane in HT-29 human colon cancer cells. *BMC Gastroenterol.* **9**:39.

Chung, Y. K., Chi-Hung Or, R., Lu, C. H., Ouyang, W. T., Yang, S. Y. and Chang, C. C. (2015). Sulforaphane down-regulates SKP2 to stabilize p27(KIP1) for inducing antiproliferation in human colon adenocarcinoma cells. *J. Biosci. Bioeng.* **119**:35–42.

Ciska, E., Martyniak-Przybyszewska, B. and Kozlowska, H. (2000). Content of glucosinolates in cruciferous vegetables grown at the same site for two years under different climatic conditions. *J. Agric. Food Chem.* **48**:2862–2867.

Coates, E. M., Popa, G., Gill, C. I., McCann, M. J., McDougall, G. J., Stewart, D. and Rowland, I. (2007). Colon-available raspberry polyphenols exhibit anti-cancer effects on *in vitro* models of colon cancer. *J. Carcinog.* **6**:4.

Crim, K. C., Sanders, L. M., Hong, M. Y., Taddeo, S. S., Turner, N. D., Chapkin, R. S. and Lupton, J. R. (2008). Upregulation of p21Waf1/Cip1 expression *in vivo* by butyrate administration can be chemoprotective or chemopromotive depending on the lipid component of the diet. *Carcinogenesis*. **29**:1415–1420.

Cummings, J. H., Hill, M. J., Jenkins, D. J., Pearson, J. R. and Wiggins, H. S. (1976). Changes in fecal composition and colonic function due to cereal fiber. *Am. J. Clin. Nutr.* **29**:1468–1473.

D'Antuono, L. F., Elementi, S. and Neri, R. (2008). Glucosinolates in Diplotaxis and Eruca leaves: Diversity, taxonomic relations and applied aspects. *Phytochemistry*. **69**:187–199.

Dalmasso, G., Nguyen, H. T., Yan, Y., Laroui, H., Charania, M. A., Ayyadurai, S., Sitaraman, S. V. and Merlin, D. (2011). Microbiota modulate host gene expression via microRNAs. *PLoS One*. **6**:e19293.

Danielsen, S. A., Eide, P. W., Nesbakken, A., Guren, T., Leithe, E. and Lothe, R. A. (2015). Portrait of the PI3K/AKT pathway in colorectal cancer. *Biochim. Biophys. Acta*. **1855**:104–121.

Davaatseren, M., Hwang, J. T., Park, J. H., Kim, M. S., Wang, S. and Sung, M. J. (2014). Allyl isothiocyanate ameliorates angiogenesis and inflammation in dextran sulfate sodium-induced acute colitis. *PLoS One*. **9**:e102975.

Davidson, L. A., Wang, N., Shah, M. S., Lupton, J. R., Ivanov, I. and Chapkin, R. S. (2009). n-3 Polyunsaturated fatty acids modulate carcinogen-directed non-coding microRNA signatures in rat colon. *Carcinogenesis*. **30**:2077–2084.

de Vos, R. H. and Blijleven, W. G. (1988). The effect of processing conditions on glucosinolates in cruciferous vegetables. *Z. Lebensm. Unters. Forsch.* **187**:525–529.

Fahey, J. W., Zalcmann, A. T. and Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry*. **56**:5–51.

Faraoni, I., Antonetti, F. R., Cardone, J. and Bonmassar, E. (2009). miR-155 gene: A typical multifunctional microRNA. *Biochim. Biophys. Acta*. **1792**:497–505.

Farazi, T. A., Juranek, S. A. and Tuschl, T. (2008). The growing catalog of small RNAs and their association with distinct Argonaute/Piwi family members. *Development*. **135**:1201–1214.

Felton, J. S. and Knize, M. G. (1991). Occurrence, identification, and bacterial mutagenicity of heterocyclic amines in cooked food. *Mutat. Res.* **259**:205–217.

Fenwick, G. R. and Heaney, R. K. (1983). Glucosinolates and their breakdown products in cruciferous crops, foods and feedingstuffs. *Food Chem.* **11**:249–271.

Ferguson, L. R. and Harris, P. J. (1996). Studies on the role of specific dietary fibres in protection against colorectal cancer. *Mutat. Res.* **350**:173–184.

Ferrarini, L., Pellegrini, N., Mazzeo, T., Miglio, C., Galati, S., Milano, F., Rossi, C. and Buschini, A. (2012). Anti-proliferative activity and chemoprotective effects towards DNA oxidative damage of fresh and cooked Brassicaceae. *Br. J. Nutr.* **107**:1324–1332.

Font, R., del Rio-Celestino, M., Cartea, E. and de Haro-Bailon, A. (2005). Quantification of glucosinolates in leaves of leaf rape (*Brassica napus* ssp. pabularia) by near-infrared spectroscopy. *Phytochemistry*. **66**:175–185.

Forman, J. J., Legesse-Miller, A. and Coller, H. A. (2008). A search for conserved sequences in coding regions reveals that the let-7 microRNA targets Dicer within its coding sequence. *Proc. Natl. Acad. Sci. U. S. A.* **105**:14879–14884.

Frydoonfar, H. R., McGrath, D. R. and Spigelman, A. D. (2002). Inhibition of proliferation of a colon cancer cell line by indole-3-carbinol. *Colorectal Dis.* **4**:205–207.

Fukuda, T., Yamagata, K., Fujiyama, S., Matsumoto, T., Koshida, I., Yoshimura, K., Mihara, M., Naitou, M., Endoh, H., Nakamura, T., Akimoto, C., Yamamoto, Y., Katagiri, T., Foulds, C., Takezawa, S., Kitagawa, H., Takeyama, K., O'Malley, B. W. and Kato, S. (2007). DEAD-box RNA helicase subunits of the Drosha complex are required for processing of rRNA and a subset of microRNAs. *Nat. Cell Biol.* **9**:604–611.

Fukuda, Y., Kawasaki, H. and Taira, K. (2005). Exploration of human miRNA target genes in neuronal differentiation. *Nucleic Acids Symp. Ser. (Oxf)*:341–342.

Graser, G., Schneider, B., Oldham, N. J. and Gershenzon, J. (2000). The methionine chain elongation pathway in the biosynthesis of glucosinolates in Eruca sativa (Brassicaceae). *Arch. Biochem. Biophys.* **378**:411–419.

Gregory, R. I., Chendrimada, T. P., Cooch, N. and Shiekhattar, R. (2005). Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. *Cell*. **123**:631–640.

Hahn, C., Muller, A., Kuhnert, N. and Albach, D. (2016). Diversity of Kale (Brassica oleracea var. sabellica): Glucosinolate content and phylogenetic relationships. *J. Agric. Food Chem.* **64**:3215–3225.

Han, J., Lee, Y., Yeom, K. H., Nam, J. W., Heo, I., Rhee, J. K., Sohn, S. Y., Cho, Y., Zhang, B. T. and Kim, V. N. (2006). Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. *Cell*. **125**:887–901.

Han, R., Sun, Q., Wu, J., Zheng, P. and Zhao, G. (2016). Sodium butyrate upregulates miR-203 expression to exert anti-proliferation effect on colorectal cancer cells. *Cell. Physiol. Biochem.* **39**:1919–1929.

Hargraves, K. G., He, L. and Firestone, G. L. (2016). Phytochemical regulation of the tumor suppressive microRNA, miR-34a, by p53-dependent and independent responses in human breast cancer cells. *Mol. Carcinog.* **55**:486–498.

He, H., Liu, L., Song, S., Tang, X. and Wang, Y. (2003). Evaluation of glucosinolate composition and contents in chinese brassica vegetablesed. *Acta. Hortic.* **620**:85–92.

He, L., He, X., Lim, L. P., de Stanchina, E., Xuan, Z., Liang, Y., Xue, W., Zender, L., Magnus, J., Ridzon, D., Jackson, A. L., Linsley, P. S., Chen,

C., Lowe, S. W., Cleary, M. A. and Hannon, G. J. (2007). A microRNA component of the p53 tumour suppressor network. *Nature*. **447**:1130–1134.

Higdon, J. V., Delage, B., Williams, D. E. and Dashwood, R. H. (2007). Cruciferous vegetables and human cancer risk: Epidemiologic evidence and mechanistic basis. *Pharmacol. Res.* **55**:224–236.

Ho, E., Clarke, J. D. and Dashwood, R. H. (2009). Dietary sulforaphane, a histone deacetylase inhibitor for cancer prevention. *J. Nutr.* **139**:2393–2396.

Holst, B. and Williamson, G. (2004). A critical review of the bioavailability of glucosinolates and related compounds. *Nat. Prod. Rep.* **21**:425–447.

Horn, T. L., Reichert, M. A., Bliss, R. L. and Malejka-Giganti, D. (2002). Modulations of P450 mRNA in liver and mammary gland and P450 activities and metabolism of estrogen in liver by treatment of rats with indole-3-carbinol. *Biochem. Pharmacol.* **64**:393–404.

Hu, S., Dong, T. S., Dalal, S. R., Wu, F., Bissonnette, M., Kwon, J. H. and Chang, E. B. (2011). The microbe-derived short chain fatty acid butyrate targets miRNA-dependent p21 gene expression in human colon cancer. *PLoS One*. **6**:e16221.

Hu, S., Liu, L., Chang, E. B., Wang, J. Y. and Raufman, J. P. (2015). Butyrate inhibits pro-proliferative miR-92a by diminishing c-Myc-induced miR-17-92a cluster transcription in human colon cancer cells. *Mol. Cancer*. **14**:180.

Hudson, E. A., Howells, L. M., Gallacher-Horley, B., Fox, L. H., Gescher, A. and Manson, M. M. (2003). Growth-inhibitory effects of the chemopreventive agent indole-3-carbinol are increased in combination with the polyamine putrescine in the SW480 colon tumour cell line. *BMC Cancer*. **3**:2.

Hutvagner, G., McLachlan, J., Pasquinelli, A. E., Balint, E., Tuschl, T. and Zamore, P. D. (2001). A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Science*. **293**:834–838.

Hwang, E. S. and Kim, G. H. (2013). Effects of various heating methods on glucosinolate, carotenoid and tocopherol concentrations in broccoli. *Int. J. Food Sci. Nutr.* **64**:103–111.

Hwang, E. S. and Lee, H. J. (2010). Effects of phenylethyl isothiocyanate and its metabolite on cell-cycle arrest and apoptosis in LNCaP human prostate cancer cells. *Int. J. Food Sci. Nutr.* **61**:324–336.

Iwai, N. and Naraba, H. (2005). Polymorphisms in human pre-miRNAs. *Biochem. Biophys. Res. Commun.* **331**:1439–1444.

Izzotti, A., Calin, G. A., Steele, V. E., Cartiglia, C., Longobardi, M., Croce, C. M. and De Flora, S. (2010). Chemoprevention of cigarette smoke-induced alterations of MicroRNA expression in rat lungs. *Cancer Prev. Res. (Phila)*. **3**:62–72.

Kassie, F., Uhl, M., Rabot, S., Grasl-Kraupp, B., Verkerk, R., Kundi, M., Chabicovsky, M., Schulte-Hermann, R. and Knasmuller, S. (2003). Chemoprevention of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-induced colonic and hepatic preneoplastic lesions in the F344 rat by cruciferous vegetables administered simultaneously with the carcinogen. *Carcinogenesis*. **24**:255–261.

Keck, A. S. and Finley, J. W. (2004). Cruciferous vegetables: Cancer protective mechanisms of glucosinolate hydrolysis products and selenium. *Integr. Cancer Ther.* **3**:5–12.

Kim, E. J., Park, S. Y., Shin, H. K., Kwon, D. Y., Surh, Y. J. and Park, J. H. (2007). Activation of caspase-8 contributes to 3,3′-Diindolylmethane-induced apoptosis in colon cancer cells. *J. Nutr.* **137**:31–36.

Kim, J. K., Gallaher, D. D., Chen, C., Gallaher, C. M., Yao, D. and Trudo, S. P. (2016). Phenethyl isothiocyanate and indole-3-carbinol from cruciferous vegetables, but not furanocoumarins from apiaceous vegetables, reduced PhIP-induced DNA adducts in Wistar rats. *Mol. Nutr. Food Res.* **60**:1956–1966.

Kim, N. H., Cha, Y. H., Kang, S. E., Lee, Y., Lee, I., Cha, S. Y., Ryu, J. K., Na, J. M., Park, C., Yoon, H. G., Park, G. J., Yook, J. I. and Kim, H. S. (2013). p53 regulates nuclear GSK-3 levels through miR-34-mediated Axin2 suppression in colorectal cancer cells. *Cell Cycle*. **12**:1578–1587.

Kim, Y. B., Li, X., Kim, S. J., Kim, H. H., Lee, J., Kim, H. and Park, S. U. (2013). MYB transcription factors regulate glucosinolate biosynthesis in different organs of Chinese cabbage (Brassica rapa ssp. pekinensis). *Molecules*. **18**:8682–8695.

Kopsell, D. A., Barickman, T. C., Sams, C. E. and McElroy, J. S. (2007). Influence of nitrogen and sulfur on biomass production and carotenoid and glucosinolate concentrations in watercress (Nasturtium officinale R. Br.). *J. Agric. Food Chem.* **55**:10628–10634.

Kushad, M. M., Brown, A. F., Kurilich, A. C., Juvik, J. A., Klein, B. P., Wallig, M. A. and Jeffery, E. H. (1999). Variation of glucosinolates in vegetable crops of Brassica oleracea. *J. Agric. Food Chem.* **47**:1541–1548.

La Rocca, G., Badin, M., Shi, B., Xu, S. Q., Deangelis, T., Sepp-Lorenzinoi, L. and Baserga, R. (2009). Mechanism of growth inhibition by MicroRNA 145: The role of the IGF-I receptor signaling pathway. *J. Cell. Physiol.* **220**:485–491.

Lagos-Quintana, M., Rauhut, R., Lendeckel, W. and Tuschl, T. (2001). Identification of novel genes coding for small expressed RNAs. *Science*. **294**:853–858.

Lai, K. C., Hsu, S. C., Kuo, C. L., Ip, S. W., Yang, J. S., Hsu, Y. M., Huang, H. Y., Wu, S. H. and Chung, J. G. (2010a). Phenethyl isothiocyanate inhibited tumor migration and invasion via suppressing multiple signal transduction pathways in human colon cancer HT29 cells. *J. Agric. Food Chem.* **58**:11148–11155.

Lai, K. C., Huang, A. C., Hsu, S. C., Kuo, C. L., Yang, J. S., Wu, S. H. and Chung, J. G. (2010b). Benzyl isothiocyanate (BITC) inhibits migration and invasion of human colon cancer HT29 cells by inhibiting matrix metalloproteinase-2/-9 and urokinase plasminogen (uPA) through PKC and MAPK signaling pathway. *J. Agric. Food Chem.* **58**:2935–2942.

Lai, K. C., Lu, C. C., Tang, Y. J., Chiang, J. H., Kuo, D. H., Chen, F. A., Chen, I. L. and Yang, J. S. (2014). Allyl isothiocyanate inhibits cell metastasis through suppression of the MAPK pathways in epidermal growth factorstimulated HT29 human colorectal adenocarcinoma cells. *Oncol. Rep.* **31**:189–196.

Lam, T. K., Ruczinski, I., Helzlsouer, K. J., Shugart, Y. Y., Caulfield, L. E. and Alberg, A. J. (2010). Cruciferous vegetable intake and lung cancer risk: A nested case-control study matched on cigarette smoking. *Cancer Epidemiol. Biomarkers Prev.* **19**:2534–2540.

Lau, W. S., Chen, T. and Wong, Y. S. (2010). Allyl isothiocyanate induces G2/M arrest in human colorectal adenocarcinoma SW620 cells through down-regulation of Cdc25B and Cdc25C. *Mol. Med. Rep.* **3**:1023–1030.

Lee, Y., Hur, I., Park, S. Y., Kim, Y. K., Suh, M. R. and Kim, V. N. (2006). The role of PACT in the RNA silencing pathway. *EMBO J.* **25**:522–532.

Lee, Y., Jeon, K., Lee, J. T., Kim, S. and Kim, V. N. (2002). MicroRNA maturation: Stepwise processing and subcellular localization. *EMBO J.* **21**:4663–4670.

Lee, Y., Kim, M., Han, J., Yeom, K. H., Lee, S., Baek, S. H. and Kim, V. N. (2004). MicroRNA genes are transcribed by RNA polymerase II. *EMBO J.* **23**:4051–4060.

Leem, S. H., Li, X. J., Park, M. H., Park, B. H. and Kim, S. M. (2015). Genome-wide transcriptome analysis reveals inactivation of Wnt/beta-catenin by 3,3′-diindolylmethane inhibiting proliferation of colon cancer cells. *Int. J. Oncol.* **47**:918–926.

Lewis, B. P., Burge, C. B. and Bartel, D. P. (2005). Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell*. **120**:15–20.

Li, H., Zhang, H., Lu, G., Li, Q., Gu, J., Song, Y., Gao, S. and Ding, Y. (2016). Mechanism analysis of colorectal cancer according to the microRNA expression profile. *Oncol. Lett.* **12**:2329–2336.

Li, X. and Kushad, M. M. (2004). Correlation of glucosinolate content to myrosinase activity in horseradish (*Armoracia rusticana*). *J. Agric. Food Chem.* **52**:6950–6955.

Li, Y., VandenBoom, T. G., 2nd, Kong, D., Wang, Z., Ali, S., Philip, P. A. and Sarkar, F. H. (2009). Up-regulation of miR-200 and let-7 by natural agents leads to the reversal of epithelial-to-mesenchymal transition in gemcitabine-resistant pancreatic cancer cells. *Cancer Res.* **69**:6704–6712.

Liu, C., Teng, Z. Q., Santistevan, N. J., Szulwach, K. E., Guo, W., Jin, P. and Zhao, X. (2010). Epigenetic regulation of miR-184 by MBD1 governs neural stem cell proliferation and differentiation. *Cell Stem Cell*. **6**:433–444.

Liu, X. and Lv, K. (2013). Cruciferous vegetables intake is inversely associated with risk of breast cancer: A meta-analysis. *Breast*. **22**:309–313.

Lytle, J. R., Yario, T. A. and Steitz, J. A. (2007). Target mRNAs are repressed as efficiently by microRNA-binding sites in the 5′ UTR as in the 3′ UTR. *Proc. Natl. Acad. Sci. U. S. A.* **104**:9667–9672.

Maciel-Dominguez, A., Swan, D., Ford, D. and Hesketh, J. (2013). Selenium alters miRNA profile in an intestinal cell line: Evidence that miR-185 regulates expression of GPX2 and SEPSH2. *Mol. Nutr. Food Res.* **57**:2195–2205.

Mahn, A. and Reyes, A. (2012). An overview of health-promoting compounds of broccoli (*Brassica oleracea* var. italica) and the effect of processing. *Food Sci. Technol. Int.* **18**:503–514.

Majumdar, A. P., Kodali, U. and Jaszewski, R. (2004). Chemopreventive role of folic acid in colorectal cancer. *Front. Biosci.* **9**:2725–2732.

Marshall, J. R. (2009). Nutrition and colon cancer prevention. *Curr. Opin. Clin. Nutr. Metab. Care*. **12**:539–543.

McIntyre, A., Gibson, P. R. and Young, G. P. (1993). Butyrate production from dietary fibre and protection against large bowel cancer in a rat model. *Gut*. **34**:386–391.

Melkamu, T., Zhang, X., Tan, J., Zeng, Y. and Kassie, F. (2010). Alteration of microRNA expression in vinyl carbamate-induced mouse lung tumors and modulation by the chemopreventive agent indole-3-carbinol. *Carcinogenesis*. **31**:252–258.

Meng, F., Henson, R., Wehbe-Janek, H., Ghoshal, K., Jacob, S. T. and Patel, T. (2007). MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology*. **133**:647–658.

Miller-Cebert, R. L. B. J., Cebert, E., Shackelford, L. and Verghese, M. (2016). Chemopreventive potential of canola leafy greens and other cruciferous vegetables on Azoxymethane (AOM)-induced colon cancer in Fisher-344 male rats. *Food Nutr. Sci.* **7**:964–976.

Miranda, K. C., Huynh, T., Tay, Y., Ang, Y. S., Tam, W. L., Thomson, A. M., Lim, B. and Rigoutsos, I. (2006). A pattern-based method for the identification of MicroRNA binding sites and their corresponding heteroduplexes. *Cell*. **126**:1203–1217.

Moen, B., Henjum, K., Mage, I., Knutsen, S. H., Rud, I., Hetland, R. B. and Paulsen, J. E. (2016). Effect of dietary fibers on cecal microbiota and intestinal tumorigenesis in azoxymethane treated A/J Min/+ Mice. *PLoS One*. **11**:e0155402.

Monsma, D. J., Thorsen, P. T., Vollendorf, N. W., Crenshaw, T. D. and Marlett, J. A. (2000). In vitro fermentation of swine ileal digesta containing oat bran dietary fiber by rat cecal inocula adapted to the test fiber increases propionate production but fermentation of wheat bran ileal digesta does not produce more butyrate. *J. Nutr.* **130**:585–593.

Moon, R. T., Kohn, A. D., De Ferrari, G. V. and Kaykas, A. (2004). WNT and beta-catenin signalling: Diseases and therapies. *Nat. Rev. Genet.* **5**:691–701.

Motoyama, K., Inoue, H., Takatsuno, Y., Tanaka, F., Mimori, K., Uetake, H., Sugihara, K. and Mori, M. (2009). Over- and under-expressed microRNAs in human colorectal cancer. *Int. J. Oncol.* **34**:1069–1075.

Muralidhar, B., Goldstein, L. D., Ng, G., Winder, D. M., Palmer, R. D., Gooding, E. L., Barbosa-Morais, N. L., Mukherjee, G., Thorne, N. P., Roberts, I., Pett, M. R. and Coleman, N. (2007). Global microRNA profiles in cervical squamous cell carcinoma depend on Drosha expression levels. *J. Pathol.* **212**:368–377.

Navarro, S. L., Li, F. and Lampe, J. W. (2011). Mechanisms of action of isothiocyanates in cancer chemoprevention: An update. *Food Funct.* **2**:579–587.

Navarro, S. L., Peterson, S., Chen, C., Makar, K. W., Schwarz, Y., King, I. B., Li, S. S., Li, L., Kestin, M. and Lampe, J. W. (2009). Cruciferous vegetable feeding alters UGT1A1 activity: Diet- and genotype-dependent changes in serum bilirubin in a controlled feeding trial. *Cancer Prev. Res. (Phila)*. **2**:345–352.

Neave, A. S., Sarup, S. M., Seidelin, M., Duus, F. and Vang, O. (2005). Characterization of the N-methoxyindole-3-carbinol (NI3C)–induced cell cycle arrest in human colon cancer cell lines. *Toxicol. Sci.* **83**:126–135.

Nigro, N. D., Bull, A. W., Klopfer, B. A., Pak, M. S. and Campbell, R. L. (1979). Effect of dietary fiber on azoxymethane-induced intestinal carcinogenesis in rats. *J. Natl. Cancer Inst.* **62**:1097–1102.

Nottrott, S., Simard, M. J. and Richter, J. D. (2006). Human let-7a miRNA blocks protein production on actively translating polyribosomes. *Nat. Struct. Mol. Biol.* **13**:1108–1114.

Okamura, K., Hagen, J. W., Duan, H., Tyler, D. M. and Lai, E. C. (2007). The mirtron pathway generates microRNA-class regulatory RNAs in Drosophila. *Cell*. **130**:89–100.

Ozcan, O., Kara, M., Yumrutas, O., Bozgeyik, E., Bozgeyik, I. and Celik, O. I. (2016). MTUS1 and its targeting miRNAs in colorectal carcinoma: Significant associations. *Tumour Biol.* **37**:6637–6645.

Padilla, G., Cartea, M. E., Velasco, P., de Haro, A. and Ordas, A. (2007). Variation of glucosinolates in vegetable crops of Brassica rapa. *Phytochemistry*. **68**:536–545.

Padua, D. and Massague, J. (2009). Roles of TGFbeta in metastasis. *Cell Res.* **19**:89–102.

Paik, W. H., Kim, H. R., Park, J. K., Song, B. J., Lee, S. H. and Hwang, J. H. (2013). Chemosensitivity induced by down-regulation of microRNA-21 in gemcitabine-resistant pancreatic cancer cells by indole-3-carbinol. *Anticancer Res.* **33**:1473–1481.

Park, W. T., Kim, J. K., Park, S., Lee, S. W., Li, X., Kim, Y. B., Uddin, M. R., Park, N. I., Kim, S. J. and Park, S. U. (2012). Metabolic profiling of glucosinolates, anthocyanins, carotenoids, and other secondary metabolites in kohlrabi (*Brassica oleracea* var. gongylodes). *J. Agric. Food Chem.* **60**:8111–8116.

Parnaud, G., Li, P., Cassar, G., Rouimi, P., Tulliez, J., Combaret, L. and Gamet-Payrastre, L. (2004). Mechanism of sulforaphane-induced cell cycle arrest and apoptosis in human colon cancer cells. *Nutr. Cancer*. **48**:198–206.

Pastrello, C., Tsay, M., McQuaid, R., Abovsky, M., Pasini, E., Shirdel, E., Angeli, M., Tokar, T., Jamnik, J., Kotlyar, M., Jurisicova, A., Kotsopoulos, J., El-Sohemy, A. and Jurisica, I. (2016). Circulating plant miRNAs can regulate human gene expression *in vitro*. *Sci. Rep.* **6**:32773.

Petersen, C. P., Bordeleau, M. E., Pelletier, J. and Sharp, P. A. (2006). Short RNAs repress translation after initiation in mammalian cells. *Mol. Cell.* **21**:533–542.

Pierre, F., Tache, S., Petit, C. R., Van der Meer, R. and Corpet, D. E. (2003). Meat and cancer: Haemoglobin and haemin in a low-calcium diet promote colorectal carcinogenesis at the aberrant crypt stage in rats. *Carcinogenesis*. **24**:1683–1690.

Pillai, R. S., Bhattacharyya, S. N., Artus, C. G., Zoller, T., Cougot, N., Basyuk, E., Bertrand, E. and Filipowicz, W. (2005). Inhibition of translational initiation by Let-7 MicroRNA in human cells. *Science*. **309**:1573–1576.

Place, R. F., Li, L. C., Pookot, D., Noonan, E. J. and Dahiya, R. (2008). MicroRNA-373 induces expression of genes with complementary promoter sequences. *Proc. Natl. Acad. Sci. U. S. A.* **105**:1608–1613.

Pogue, A. I., Cui, J. G., Li, Y. Y., Zhao, Y., Culicchia, F. and Lukiw, W. J. (2010). Micro RNA-125b (miRNA-125b) function in astrogliosis and glial cell proliferation. *Neurosci. Lett.* **476**:18–22.

Poy, M. N., Eliasson, L., Krutzfeldt, J., Kuwajima, S., Ma, X., Macdonald, P. E., Pfeffer, S., Tuschl, T., Rajewsky, N., Rorsman, P. and Stoffel, M. (2004). A pancreatic islet-specific microRNA regulates insulin secretion. *Nature*. **432**:226–230.

Qian, H., Sun, B., Miao, H., Cai, C., Xu, C. and Wang, Q. (2015). Variation of glucosinolates and quinone reductase activity among different varieties of Chinese kale and improvement of glucoraphanin by metabolic engineering. *Food Chem.* **168**:321–326.

Rajendran, P., Dashwood, W. M., Li, L., Kang, Y., Kim, E., Johnson, G., Fischer, K. A., Lohr, C. V., Williams, D. E., Ho, E., Yamamoto, M., Lieberman, D. A. and Dashwood, R. H. (2015). Nrf2 status affects tumor growth, HDAC3 gene promoter associations, and the response to sulforaphane in the colon. *Clin. Epigenetics*. **7**:102.

Reeves, M. A. and Hoffmann, P. R. (2009). The human selenoproteome: Recent insights into functions and regulation. *Cell. Mol. Life Sci.* **66**:2457–2478.

Robbins, M. G., Hauder, J., Somoza, V., Eshelman, B. D., Barnes, D. M. and Hanlon, P. R. (2010). Induction of detoxification enzymes by feeding unblanched Brussels sprouts containing active myrosinase to mice for 2 wk. *J. Food Sci.* **75**:H190–199.

Safe, S. (2001). Molecular biology of the Ah receptor and its role in carcinogenesis. *Toxicol. Lett.* **120**:1–7.

Sakamoto, S., Aoki, K., Higuchi, T., Todaka, H., Morisawa, K., Tamaki, N., Hatano, E., Fukushima, A., Taniguchi, T. and Agata, Y. (2009). The NF90-NF45 complex functions as a negative regulator in the microRNA processing pathway. *Mol. Cell. Biol.* **29**:3754–3769.

Sanz-Pamplona, R., Cordero, D., Berenguer, A., Lejbkowicz, F., Rennert, H., Salazar, R., Biondo, S., Sanjuan, X., Pujana, M. A., Rozek, L.,

Giordano, T. J., Ben-Izhak, O., Cohen, H. I., Trougouboff, P., Bejhar, J., Sova, Y., Rennert, G., Gruber, S. B. and Moreno, V. (2011). Gene expression differences between colon and rectum tumors. *Clin. Cancer Res.* **17**:7303–7312.

Schatzkin, A., Mouw, T., Park, Y., Subar, A. F., Kipnis, V., Hollenbeck, A., Leitzmann, M. F. and Thompson, F. E. (2007). Dietary fiber and whole-grain consumption in relation to colorectal cancer in the NIH-AARP Diet and Health Study. *Am. J. Clin. Nutr.* **85**:1353–1360.

Shibuya, H., Iinuma, H., Shimada, R., Horiuchi, A. and Watanabe, T. (2010). Clinicopathological and prognostic value of microRNA-21 and microRNA-155 in colorectal cancer. *Oncology.* **79**:313–320.

Slaby, O., Sachlova, M., Brezkova, V., Hezova, R., Kovarikova, A., Bischofova, S., Sevcikova, S., Bienertova-Vasku, J., Vasku, A., Svoboda, M. and Vyzula, R. (2013). Identification of microRNAs regulated by isothiocyanates and association of polymorphisms inside their target sites with risk of sporadic colorectal cancer. *Nutr. Cancer*. **65**:247–254.

Stark, K. L., Xu, B., Bagchi, A., Lai, W. S., Liu, H., Hsu, R., Wan, X., Pavlidis, P., Mills, A. A., Karayiorgou, M. and Gogos, J. A. (2008). Altered brain microRNA biogenesis contributes to phenotypic deficits in a 22q11-deletion mouse model. *Nat. Genet.* **40**:751–760.

Tang, L., Zirpoli, G. R., Jayaprakash, V., Reid, M. E., McCann, S. E., Nwogu, C. E., Zhang, Y., Ambrosone, C. B. and Moysich, K. B. (2010). Cruciferous vegetable intake is inversely associated with lung cancer risk among smokers: A case-control study. *BMC Cancer*. **10**:162.

Vale, A. P., Santos, J., Brito, N. V., Fernandes, D., Rosa, E. and Oliveira, M. B. (2015). Evaluating the impact of sprouting conditions on the glucosinolate content of Brassica oleracea sprouts. *Phytochemistry*. **115**:252–260.

Vang, O., Frandsen, H., Hansen, K. T., Sorensen, J. N., Sorensen, H. and Andersen, O. (2001). Biochemical effects of dietary intakes of different broccoli samples. I. Differential modulation of cytochrome P-450 activities in rat liver, kidney, and colon. *Metabolism.* **50**:1123–1129.

Vargas, A. J. and Thompson, P. A. (2012). Diet and nutrient factors in colorectal cancer risk. *Nutr. Clin. Pract.* **27**:613–623.

Vella, M. C., Choi, E. Y., Lin, S. Y., Reinert, K. and Slack, F. J. (2004). The C. elegans microRNA let-7 binds to imperfect let-7 complementary sites from the lin-41 3′UTR. *Genes Dev*. **18**:132–137.

Walters, D. G., Young, P. J., Agus, C., Knize, M. G., Boobis, A. R., Gooderham, N. J. and Lake, B. G. (2004). Cruciferous vegetable consumption alters the metabolism of the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in humans. *Carcinogenesis*. **25**:1659–1669.

Wang, X., He, H., Lu, Y., Ren, W., Teng, K. Y., Chiang, C. L., Yang, Z., Yu, B., Hsu, S., Jacob, S. T., Ghoshal, K. and Lee, L. J. (2015). Indole-3-carbinol inhibits tumorigenicity of hepatocellular carcinoma cells via suppression of microRNA-21 and upregulation of phosphatase and tensin homolog. *Biochim. Biophys. Acta*. **1853**:244–253.

Weng, J. R., Tsai, C. H., Kulp, S. K. and Chen, C. S. (2008). Indole-3-carbinol as a chemopreventive and anti-cancer agent. *Cancer Lett.* **262**:153–163.

Wilfred, B. R., Wang, W. X. and Nelson, P. T. (2007). Energizing miRNA research: A review of the role of miRNAs in lipid metabolism, with a prediction that miR-103/107 regulates human metabolic pathways. *Mol. Genet. Metab.* **91**:209–217.

Wolf, C. R. (2001). Chemoprevention: Increased potential to bear fruit. *Proc. Natl. Acad. Sci. U. S. A.* **98**:2941–2943.

Wu, L., Fan, J. and Belasco, J. G. (2006). MicroRNAs direct rapid deadenylation of mRNA. *Proc. Natl. Acad. Sci. U. S. A.* **103**:4034–4039.

Wu, Q. J., Yang, Y., Vogtmann, E., Wang, J., Han, L. H., Li, H. L. and Xiang, Y. B. (2013). Cruciferous vegetables intake and the risk of colorectal cancer: A meta-analysis of observational studies. *Ann. Oncol.* **24**:1079–1087.

Xing, Y., Liu, Z., Yang, G., Gao, D. and Niu, X. (2015). MicroRNA expression profiles in rats with selenium deficiency and the possible role of the Wnt/beta-catenin signaling pathway in cardiac dysfunction. *Int. J. Mol. Med.* **35**:143–152.

Yang, B., Lin, H., Xiao, J., Lu, Y., Luo, X., Li, B., Zhang, Y., Xu, C., Bai, Y., Wang, H., Chen, G. and Wang, Z. (2007). The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. *Nat. Med.* **13**:486–491.

Yi, G., Lim, S., Chae, W. B., Park, J. E., Park, H. R., Lee, E. J. and Huh, J. H. (2016). Root glucosinolate profiles for screening of radish (*Raphanus sativus* L.) genetic resources. *J. Agric. Food Chem.* **64**:61–70.

Yi, G. E., Robin, A. H., Yang, K., Park, J. I., Kang, J. G., Yang, T. J. and Nou, I. S. (2015). Identification and expression analysis of glucosinolate biosynthetic genes and estimation of glucosinolate contents in edible organs of *Brassica oleracea* subspecies. *Molecules*. **20**:13089–13111.

Yi, R., Doehle, B. P., Qin, Y., Macara, I. G. and Cullen, B. R. (2005). Overexpression of exportin 5 enhances RNA interference mediated by short hairpin RNAs and microRNAs. *RNA*. **11**:220–226.

You, Z., Saims, D., Chen, S., Zhang, Z., Guttridge, D. C., Guan, K. L., MacDougald, O. A., Brown, A. M., Evan, G., Kitajewski, J. and Wang, C. Y. (2002). Wnt signaling promotes oncogenic transformation by inhibiting c-Myc-induced apoptosis. *J. Cell Biol.* **157**:429–440.

Yuan, B., Dong, R., Shi, D., Zhou, Y., Zhao, Y., Miao, M. and Jiao, B. (2011). Down-regulation of miR-23b may contribute to activation of the TGF-beta1/Smad3 signalling pathway during the termination stage of liver regeneration. *FEBS Lett.* **585**:927–934.

Zhang, G., Zhou, H., Xiao, H., Liu, Z., Tian, H. and Zhou, T. (2014). MicroRNA-92a functions as an oncogene in colorectal cancer by targeting PTEN. *Dig. Dis. Sci.* **59**:98–107.

Zhang, H., Hao, Y., Yang, J., Zhou, Y., Li, J., Yin, S., Sun, C., Ma, M., Huang, Y. and Xi, J. J. (2011). Genome-wide functional screening of miR-23b as a pleiotropic modulator suppressing cancer metastasis. *Nat. Commun.* **2**:554.

Zheng, Q., Hirose, Y., Yoshimi, N., Murakami, A., Koshimizu, K., Ohigashi, H., Sakata, K., Matsumoto, Y., Sayama, Y. and Mori, H. (2002). Further investigation of the modifying effect of various chemopreventive agents on apoptosis and cell proliferation in human colon cancer cells. *J. Cancer Res. Clin. Oncol.* **128**:539–546.

Zhu, C., Poulsen, H. E. and Loft, S. (2000). Inhibition of oxidative DNA damage *in vitro* by extracts of brussels sprouts. *Free Radic. Res.* **33**:187–196.