MicroRNA, Nutrition, and Cancer Prevention[1]

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ABSTRACT

MicroRNA (miRNA) are small noncoding RNA molecules that are involved in post-transcriptional gene silencing. Alterations in miRNA expression are observed in and may underlie many different human diseases, including cancer. In fact, miRNA have been shown to affect the hallmarks of cancer, including sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. Genetic and epigenetic alterations may explain aberrant miRNA expression in cancer cells and may also contribute to cancer risk. It is now thought that by circulating through the bloodstream, miRNA can exert their effects at distant sites as well as within the cells of origin. Recent evidence suggests that nutrients and other bioactive food components protect against cancer through modulation of miRNA expression. Moreover, dietary factors have been shown to modify miRNA expression and their mRNA targets in various cancer processes, including apoptosis, cell cycle regulation, differentiation, inflammation, angiogenesis, and metastasis as well as pathways in stress response. Herein, we provide a brief overview of dietary modulation of miRNA expression and its potential role in cancer prevention. Understanding the affect of dietary factors on miRNA expression and function may provide insight on prevention strategies to reduce the burden of cancer. *Adv. Nutr. 2: 472–485, 2011.*

Until about a decade ago, the central dogma of genetics was that RNA is the messenger between genes and the final proteins that they encode. However, recent advances in high-throughput technology for gene expression led to the discovery that most human transcriptional units are ncRNA[2] (1). These RNA molecules do not encode proteins but have important structural, catalytic, or regulatory functions, including regulation of gene expression (1–3). One such ncRNA, miRNA, are small RNA molecules (on average 22 nucleotides in length) that are involved in post-transcriptional gene silencing (4). miRNA arise from intergenic or intragenic (both exonic and intronic) genomic regions that are initially transcribed as immature primary transcripts (pri-miRNA), which are subsequently transcribed to 60–100 nucleotide hairpin pre-miRNA by the RNAse enzyme, Drosha (**Fig. 1**) (5,6). This pre-miRNA is exported to the cytoplasm by the nuclear factor Exportin-5. Once in the cytoplasm, the pre-miRNA is either further processed by the RNA polymerase Dicer and unwound to yield mature miRNA or exported to other cells through the bloodstream. Mature miRNA become part of the RISC that coordinates miRNA-mediated regulation of gene expression through base-pairing between a miRNA and sequence(s) within the 3′ untranslated region of a target mRNA [i.e. between the protein-coding region of the mRNA and its poly(A) tail] (7,8). Binding of the miRNA to the mRNA results in a reduced translation rate and/or increased degradation of the mRNA.

miRNA are largely conserved between species, implying that their gene regulatory function may have ancient origins. Currently >1000 human miRNA sequences are known (9) and it has been speculated that miRNA could regulate ~60% of the human genome (6). miRNA are abundantly present in all human cells and it is thought that each miRNA has hundreds of evolutionarily conserved targets and several times that number of nonconserved targets, suggesting the possibility of regulating an incredible number of targets each (10). Furthermore, more than one miRNA can converge on a single protein-coding gene target. Through regulation of gene expression, miRNA participate in the regulation of almost every cellular process that has been investigated, including cholesterol metabolism (11), postimplantation development (12), insulin synthesis in pancreatic β-cells (13), and hematopoiesis (14), to name a few. Furthermore, miRNA have been shown to be modulated by exercise (15) and

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[2] Abbreviations used: 1,25(OH)$_2$D, 1,25-dihydroxycholecalciferol; BFC, bioactive food component; CAM, chorionallantoic membrane assay; CLL, chronic lymphocytic leukemia; DIM, 3′,3′-diindolylmetane; ECS, environmental cigarette smoke; EGCG, epigallocatechin gallate; EGFR, epidermal growth factor receptor; EMT, epithelial-to-mesenchymal transition; ER, estrogen receptor; HCC, hepatocellular carcinoma; I3C, indole-3-carbinol; miRNA or miR, microRNA; ncRNA, noncoding RNA; PEITC, phenethyl isothiocyanate; PJ, pomegranate juice; pre-miRNA, precursor microRNA; pri-miRNA, precursor primary RNA; PTEN, phosphatase and tensin homolog; RA, retinoic acid; RISC, RNA-induced silencing complex; SNP, single nucleotide polymorphism; VDR, vitamin D receptor.

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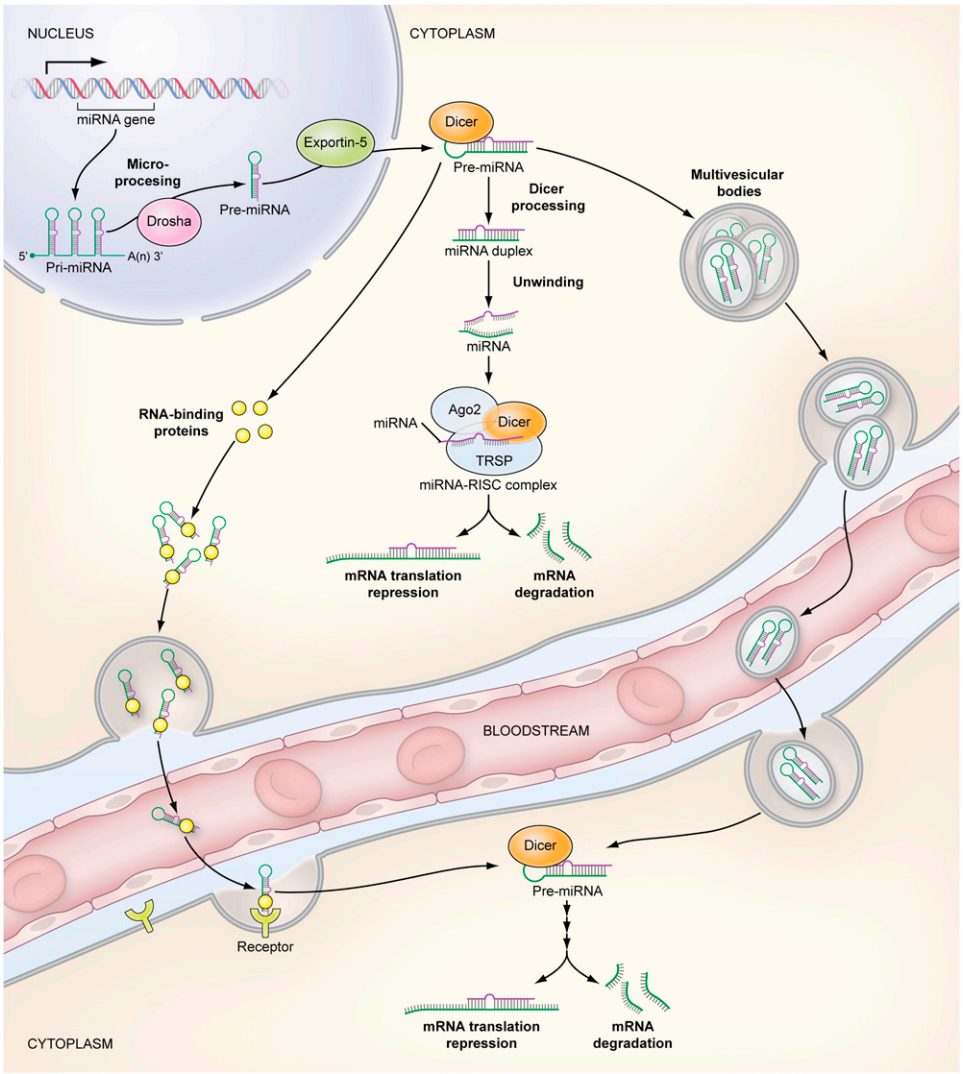

FIGURE 1 Cellular biogenesis, export, and circulation of miRNA. miRNA are processed from precursor molecules via a 2-step mechanism. In the nucleus, the enzyme Drosha processes the pri-miRNA to pre-miRNA hairpins, which are exported to the cytoplasm. Once in the cytoplasm, the pre-miRNA is either further processed by the RNA polymerase Dicer and unwound to yield mature miRNA or exported to other cells through the bloodstream. Mature miRNA are assembled into the RISC. The miRNA-RISC complex regulates post-transcriptional expression by targeting specific mRNA for degradation or translational inhibition. For cell export, pre-miRNA are thought to be packaged into exosomes and/or multivesicular bodies and transported into the bloodstream. These circulating miRNA are thought to be taken up by recipient cells by either endocytosis or receptor binding and processed into mature miRNA to inhibit the expression of target protein-coding genes in the recipient cell. Ago2, Argonaute 2; miRNA, microRNA; pre-miRNA, precursor microRNA; pri-miRNA, precursor primary microRNA; RISC, RNA-induced silencing complex; TRSP, (or TARBP2**)** trans-activation-responsive RNA-binding protein.

environmental chemicals (16) as well as by dietary factors (5,17–21). However, the specific function and mRNA targets of many mammalian miRNA remain unknown (6,22). Characterization of global miRNA expression patterns by microarray technology has recently aided the identification and cataloging of miRNA in normal and diseased tissue, including verifying early observations of tissue and developmental stage-specific expression of miRNA. A detailed understanding of the critical functions of miRNA in health and disease is still emerging.

A relevant, though at present puzzling, feature of miRNA is their remarkable stability, which suggests their utility as biomarkers. For example, miRNA have been efficiently extracted and evaluated from preserved tissue samples, including formalin-fixed and paraffin-embedded material (23). Another emerging aspect of miRNA biology is that miRNA are stable in serum, plasma, and other body fluids (24). It is now thought that miRNA act not only within cells of origin but may also act at other sites within the body by circulating through the bloodstream and targeting other cells (Fig. 1). To accomplish this, pre-miRNA can be packaged into exosomes and/or multivesicular bodies and transported into the bloodstream and thereby taken up by recipient cells and processed into mature miRNA to inhibit the expression of target protein-coding genes in the recipient cell. The presence of circulating miRNA opens up the exciting possibility of analyzing serum miRNA as new noninvasive biomarkers for disease as well as for monitoring the response to interventions.

Alterations in miRNA expression are observed in and may underlie many different human diseases, including cancer (25). miRNA are thought to be involved in cancer initiation and progression and their expression profiles serve as phenotypic signatures of different cancers. Recent evidence suggests that nutrients and other BFC, including folate, selenium, and (n-3) fatty acids, exert cancer protective effects through modulation of miRNA expression. Following a brief summary of the involvement of miRNA in cancer, we

update the most recent knowledge about nutritional modulation of miRNA using examples from the cancer prevention literature where the strongest evidence for this activity can be found.

Current Status of Knowledge

miRNA and cancer

miRNA are thought to influence the pathophysiology of all types of human cancers. Aberrant expression of mature and/or precursor miRNA transcripts have been characterized in several different tumor tissues compared to corresponding normal tissues (26). Studies also demonstrate that there are distinct miRNA expression patterns associated with specific tumor types, as evidenced by a recent study that utilized miRNA profiling to determine the identification of cancers with unknown primary tissue of origin (27). Moreover, miRNA have been associated with tumorigenesis by functioning as tumor suppressors or oncogenes, the archetypical examples being lethal-7 (let-7) and mir-21, respectively (28). Acting in these capacities, miRNA have been shown to affect the hallmarks of cancer, including sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis (**Fig. 2**) (29–32). Therefore, disturbances of miRNA expression and function appear to contribute to the initiation, maintenance, and progression of tumors as well as to invasiveness, metastasis, and even acquisition of drug resistance in cancer (33,34).

CLL was the first human cancer for which miRNA involvement was described (35). In 2002, the chromosomal deletion (13q14.3) associated with CLL was found to encompass 2 miRNA, *miR-15a* and *miR16–1* (35). Through detailed deletion and expression analysis, these investigators demonstrated that miR-15a and miR-16 were deleted or downregulated in ~68% of CLL cases. Additional studies showed that these miRNA negatively regulate Bcl2, a protein that inhibits apoptosis, at the posttranscriptional level (36). Further validation of miR-15a and miR-16 activity as important in protecting against carcinogenesis was obtained when transfection of the *miR-15a/miR-16–1* cluster in a megakaryocytic cell line was sufficient to decrease the protein levels of Bcl2 and activate apoptosis (36).

Genomic, molecular, and functional approaches are being utilized to determine the causes of miRNA deregulation in cancer. For example, early reports suggested that more than one-half of the known human miRNA were located at fragile sites as well as sites of loss of heterozygosity, amplification, and common breakpoint regions, which are genomic regions prone to alterations in cancer cells (37). Other investigators, using a high-resolution, array-based, comparative genomic hybridization approach, discovered that a large proportion of miRNA gene-containing genomic loci exhibit DNA copy number alterations in breast cancer, ovarian cancer, and melanoma, suggesting that genomic alterations likely contribute to the altered miRNA expression in cancer cells (38). Thus, disturbances in the expression of miRNA that result in upregulation may occur as a consequence of gene amplification, gene translocation, or abnormal regulation, whereas loss of function and downregulation of a miRNA could be due to genomic deletion, mutation, epigenetic silencing, abnormal regulation, and/or miRNA processing alterations (26,33). Furthermore, SNP or copy number defects in germline miRNA, its precursor, or its target mRNA may have detrimental effects on cellular function and may contribute to cancer risk (39,40).

Since the first observations of aberrant miRNA in cancer, there has been an explosion of research toward understanding the biology of various miRNA in different cancers. As an example, the role of the let-7 family of miRNA in cancer is briefly highlighted. Decreased expression of let-7 miRNA is associated with increased tumorigenesis, whereas

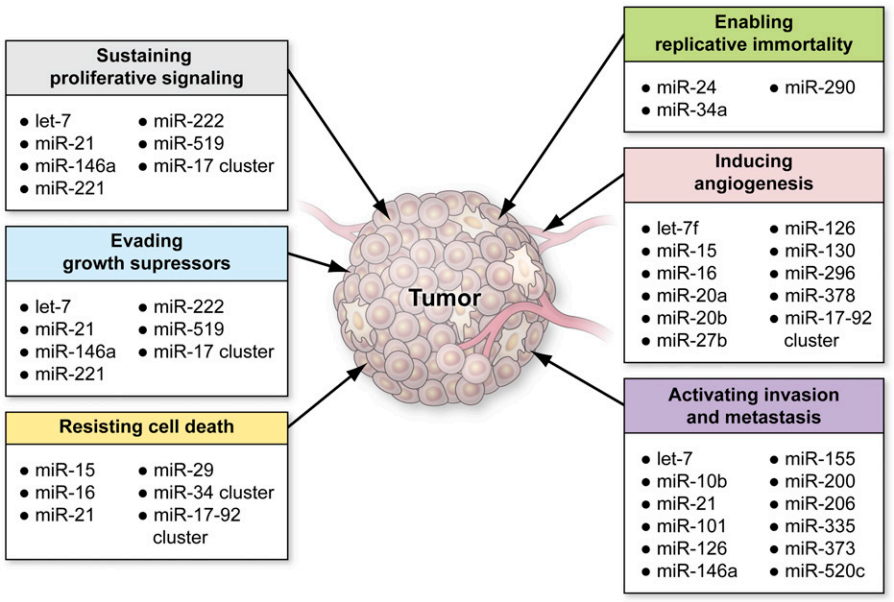

FIGURE 2 Aberrant miRNA expression affects signaling pathways to enhance tumorigenesis. Representative miRNA are depicted that have been shown to act as oncogenes or tumor suppressor genes to affect the 6 common hallmarks of cancer. miRNA, microRNA.

experimental overexpression of let-7 has been shown to inhibit the growth of both cultured tumor cells and tumors in animal models (41). This activity may be related to the ability of let-7 to actively regulate cancer stem cell differentiation. However, the exact role of let-7 in cancer is not yet fully understood.

Although the let-7 family has been found to be downregulated in lung cancer (42), one member of this family, let-7a-3, was found to have increased expression in human lung adenocarcinoma epithelial cells (43). It was found that gene hypomethylation of *let-7a-3* facilitated epigenetic reactivation of the gene and increased expression of let-7a-3 in these cells. Further investigations revealed that the *let-7a-3* gene was heavily methylated in normal human tissues but hypomethylated in some lung adenocarcinomas. These results identify *let-7a-3* as a gene that is epigenetically regulated and functions as a miRNA with oncogenic activity. It is also an example of how miRNA of the same family can have bivalent roles in cancer.

In another report, investigators performed miRNA expression microarray screening using RNA from formalin-fixed, paraffin-embedded breast tissues, including benign, ductal carcinoma in situ, and invasive ductal carcinoma (44). Of 25 differentially expressed miRNA identified, the let-7 family of miRNA were downregulated in human breast cancer tissues at stages of ductal carcinoma in situ and invasive ductal carcinoma compared to the benign stage. An inverse correlation between ERα expression and several members of let-7 family was also observed in the tissues. Bioinformatics analysis revealed that let-7 miRNA sequences matched sequences in the 3′-untranslated regions of ERα, suggesting ERα may be a target of let-7. Confirmation of this activity by overexpressing let-7 miRNA in ER-positive breast cancer MCF-7 cells negatively affected ERα activity, inhibited cell proliferation, and triggered apoptosis. These findings indicated a new regulatory mechanism of let-7 miRNA in ERα-mediated cellular malignant growth of breast cancer.

Observational studies of diabetic cohorts and cancer patients have found that metformin users have lower risks for cancer than patients receiving other diabetes drugs (45). Furthermore, studies in animal tumor models and cancer cell lines have found that metformin prevents tumor development or inhibits cell proliferation (45). An intriguing recent study examined the ability of metformin to modulate the expression of miRNA in MCF-7 breast cancer cells (46). The expression profiles of 88 cancer-related miRNA sequences before and after metformin treatment of MCF-7 cells were quantitatively analyzed using RT-PCR. An 18-fold increase in miRNA let-7a expression compared with untreated control cells was observed, implicating let-7a miRNA as a novel metformin target gene. These observations may explain why the use of metformin has been linked to decreased breast cancer risk.

Interestingly, a number of miRNA that are associated with obesity and diabetes have also been implicated in carcinogenesis. Lower levels of adiponectin and higher levels of leptin contribute to the development of insulin resistance, which is observed in obesity and diabetes. This metabolic disturbance has been found to be associated with aberrant expression of miRNA, such as let7, miR-27, and miR-143, miRNA that are also dysregulated during carcinogenesis, and might provide a mechanistic link between obesity, diabetes, and cancer (47–49). Thus, aberrant miRNA expression might provide novel molecular targets for several cancer prevention modalities agents (i.e. metformin, nutrition) as well as mechanistic insights for the relationship between cellular energetics, diet, and cancer prevention.

Aberrant expression and/or dysregulation of miRNA have been characterized during the early stages of tumor development and thus they are thought to be potential molecular targets for cancer prevention as well as biomarkers of early stage cancer (30–33). A recent study found that patients with early stage, nonsmall cell lung carcinomas in a population of asymptomatic high-risk individuals were identified with 80% accuracy using a panel of 34 miRNA from serum (50). The miRNA signature assigned disease probability accurately in both asymptomatic or symptomatic patients, distinguished between benign and malignant lesions, and captured the onset of the malignant disease in individual patients over time. As for utility in understanding susceptibility, case-control studies have provided evidence for an association of miRNA-SNP and SNP in miRNA-binding sites and cancer risk. As an example, the association between hsa-miR-196a2 rs11614913 polymorphism and cancer risk has been studied but with inconclusive results. A recent meta-analysis of 15 studies that included 9341 cancer cases and 10,569 case-free controls found the hsa-miR-196a2 rs11614913 T > C polymorphism was associated with an significantly increased cancer risk in the variant TC heterozygote [OR = 1.16 (95% CI = 1.02–1.32)], CC homozygote [OR = 1.22 (95% CI = 1.04–1.44)], and TC/CC genotype [OR = 1.18 (95% CI = 1.03–1.34)] as compared to the TT wild-type homozygote (51). Thus, there is increasing evidence suggesting that miRNA are implicated in cancer risk and prevention.

Nutritional modulation of miRNA in cancer models

Several studies have reported on the ability of essential nutrients, phytochemicals, or other BFC to modulate the expression of miRNA in different cancer cells and other model systems. Studies examining the effects of various dietary patterns (i.e. Western diet) or alterations in macronutrient content (i.e. caloric restriction) on miRNA expression and function are few. A recent study concerning the effects of a Western diet [defined as a diet with increased animal fat and lower levels of cholecalciferol and calcium to approximate amounts consumed in Western diets (52)] on EGFR-regulated miRNA in colonic tumorigenesis is one example (53). Earlier reports suggested that tumor promotion by a similar diet required EGFR signals, including MYC and K-Ras (54,55). These investigators recently found that EGFR suppressed miR-143 and miR-145 and that the tumor suppressor effects of these miRNA were uncovered in the

presence of a Western diet that promotes colonic tumorigenesis and upregulates targets of these miRNA (i.e. MYC and K-Ras) that are no longer restrained in the absence of these miRNA (53). Additional in vivo studies examining the influence of macronutrients and/or dietary pattern on miRNA expression and function would likely provide promising insights into nutrition and cancer prevention.

Epidemiological observations might provide insight into the diet, lifestyle, and genetic factors that influence miRNA expression. Such observations for dietary factors are just beginning to be studied. One example concerns the association of miRNA expression with various exposures, including alcohol intake, and clinical features associated with head and neck squamous cell carcinomas (56). In tumor tissue from 169 cases, expression of miR-375 was shown to significantly increase with alcohol consumption and showed higher expression in tumors of pharyngeal and laryngeal origin compared with oral tumors. Studies using appropriate comparison populations as well as individuals at high risk for cancer should be utilized. For example, it might now be plausible to study the association of dietary variables with circulating miRNA in individuals at high risk for certain cancers.

Observations and mechanisms by which dietary factors modulate miRNA expression and function leading to inhibition of cancer cell growth, induction of apoptosis, and other protective processes are highlighted below. Other studies discussed provide examples for the harmful effects of certain dietary factors on miRNA expression and their function in promoting cancer. An increased understanding of the effects of dietary factors on miRNA alterations may provide unique effective prevention strategies for reducing the burden of cancer.

Essential nutrients

Dietary folate has been found to modulate miRNA expression in different model systems and this may be related to the activity of folate in cancer prevention and risk. Human lymphoblastoid cells grown in folate-deficient media induced significant changes in the levels of 24 miRNA, including hsa-miR-222 (57) (**Table 1**). When folate was added back to the media, the miRNA expression profiles returned to that of control cells. These results suggest that dietary modulation of miRNA expression is reversible. Furthermore, miR-222 expression increased in vivo in human peripheral blood from individuals with low-folate status compared to those with the highest folate status (57). These data suggest that aberrant miRNA expression might be potential biomarkers of nutritional status in humans as well as participants in cancer prevention.

Several studies have examined the disruption of miRNA in HCC induced by nutrient deficiency. Rats fed a methyl-deficient diet (a diet devoid of folate and choline and containing low methionine) develop HCC at 54 wk of age in the absence of carcinogen treatment (58). Comparison of the miRNA profile by microarray analysis of livers from the animals fed the methyl-deficient diet to livers of rats on the normal diet showed increased expression of let-7a, miR-21, miR-23, miR-130, miR-190, and miR-17–92 and decreased expression of miR-122 in the methyl-deficient animals (**Table 2**). However, when rats were switched from the deficient to the methyl-adequate diet after 36 wk, expression of miR-122 at 54 wk was normal and tumors did not develop. Decreased expression of miR-122 has been observed in a large set of human HCC samples (59). Thus, the timing of exposure to the methyl-deficient diet has implications for miR-122 expression and liver cancer development.

Additional studies were conducted to determine whether the development of HCC was associated with altered expression of miRNA involved in the regulation of cell proliferation and apoptosis and to determine targets of aberrantly expressed miRNA that influence these carcinogenic processes (60). HCC induced by methyl deficiency was characterized by significant downregulation of miR-34a, miR-16a, miR-127, miR181a, miR-20a, and miR-200b. The significance of aberrant miRNA expression findings was determined by examining protein levels of the experimentally confirmed targets of these differentially expressed miRNA. Western-blot analysis showed increased levels of E2F3 and Bcl6, proteins involved in the balance of apoptosis and cell proliferation and regulated by miR-34a and miR-127, respectively, in the liver of rats fed a methyl-deficient diet (60). These findings suggest that the dysregulation of miRNA expression may be an important contributing factor in the development of HCC.

Hepatic miRNA were found to be dysregulated at early stages of carcinogenesis in C57BL/6 mice fed a choline-deficient and amino acid-defined diet that is known to promote nonalcoholic steatohepatitis-induced HCC after 84 wk (61). Upregulation of oncogenic miR-155, miR-221/222, and miR-21 and downregulation of the abundant liver-specific miR-122 was demonstrated at the early stages of HCC. Reduced protein expression of hepatic PTEN and CCAAT/enhancer binding protein beta (C/EBPβ), respective targets of miR-21 and miR-155, was also found at early stages. It was concluded that upregulation of oncogenic miRNA with concomitant suppression of their tumor suppressor targets is a very early molecular event that could play a causal role in HCC.

RA is an active metabolite of vitamin A involved in cellular differentiation that has also been shown to modulate miRNA expression in various cells, including acute promyelocytic leukemia (62) and neuroblastoma cell lines (63). Eight miRNA were upregulated (miR-15a, miR-15b, miR-16–1, let-7a-3, let-7c, let-7d, miR-223, miR-342, and miR-107) and one was downregulated (miR-181b) in cells derived from acute promyelocytic leukemia patients after incubation with 100 nmol/L all-*trans* retiRA (62). To understand RA regulation of miRNA, the investigators searched for RA-modulated transcription factor binding sites in the upstream genomic region of the *let-7a-3/let-7b* cluster and identified a functional proximal NFκB binding site as essential for the transactivation of the *let-7a-3/let-7b* cluster. Thus, RA-induced NFκB binds to the promoter of the *let-7a-3* gene and activates its transcription. Moreover, the

TABLE 1 Representative dietary components that can modulate microRNA (miRNA) in various cell lines[1]

Dietary component	Cell type	Concentration	miRNA upregulated	miRNA downregulated	Reference
Essential nutrients					
Folate	Human lymphoblast TK-6 cells	Folate-deficient RPMI 1640 (Invitrogen) with dialyzed FBS to eliminate folic acid in the serum	miR-222		(57)
RA	Human promyelocytic cell line NB4	100 nmol/L	miR-15a, miR-15b, miR-16–1, let-7a-3, let-7c, let-7d, miR-223, miR-342 and miR-107	miR-181b	(62)
RA	Human neuroblastoma cell lines SK-N-BE, LAN5 and SHSY-5Y	5 μmol/L	miR-10a and miR-10b		(63)
1,25$(OH)_2$D	Human myeloid leukemia cell lines HL60 and U937	1–10 nmol/L		miR181a and miR181b	(64)
1,25$(OH)_2$D	Normal human breast epithelial cell line MCF12F	250 nmol/L	let-7b	miR-26b, miR-200c, miR-200b, and miR-182	(65)
Sodium selenite	Human prostate cancer cell line LNCaP	2.5 μmol/L	miR-34b and miR-34c		(69)
Phytochemicals					
EGCG	Human HCC cell line HepG2	100 μmol/L	let-7a, miR-16 and miR-221	miR-18a, miR-34b, miR-193b, miR-222 and miR-342	(70)
Curcumin	Human pancreatic carcinoma cell line BxPC-3	10 μmol/L	miR-22	miR-199a*	(73)
Curcumin	Human breast adenocarcinoma cell line MCF-7	10–60 μmol/L	miR-15a and miR-16		(74)
Curcumin	Human lung adenocarcinoma cell line A549	5–30 μmol/L		miR-186*	(75)
DIM	Human breast cancer cell line MCF-7	30–45 μmol/L	miR-21		(81)
DIM	Human pancreatic cancer cell lines MiaPaCa-2, Panc-1 (gemcitabine-resistant) and L3.6pl (gemcitabine-sensitive)	25 μmol/L BioResponse-DIM with higher bioavailability	miR-200b, miR-200c, let-7b, and let-7e		(83)
DIM	Human pancreatic cancer cell lines Panc-1, Colo357	25 μmol/L BioResponse-DIM with higher bioavailability	miR-146a		(84)
Isoflavones	Human pancreatic cancer cell lines, MiaPaCa-2, Panc-1 (gemcitabine-resistant), and L3.6pl (gemcitabine-sensitive)	25 μmol/L Isoflavone mixture G2535 (70.54% genistein, 26.34% daidzin, and 0.31% glycitein)	miR-200b, miR-200c, let-7b, and let-7e		(83)
Isoflavones	Human pancreatic cancer cell lines Panc-1, Colo357	25 μmol/L Isoflavone mixture G2535 (70.54% genistein, 26.34% diadzin, and 0.31% glycitein)	miR-146a		(84)
Genistein	Human uveal melanoma cell line C918	25–200 μmol/L		miR-27a	(85)
Genistein	Human prostate cancer cell line PC-3	50 μmol/L		miR-221 and miR-222	(87)
PJ	Human prostate cancer cell line DU145	5% filtered PJ	miR-335, miR-205, miR-200 family, and miR-126	miR-21 and miR-373	(89)

(Continued)

TABLE 1 (*Continued*)

Dietary component	Cell type	Concentration	miRNA upregulated	miRNA downregulated	Reference
Resveratrol	Human acute monocytic leukemia cell line THP-1	50 μmol/L	miR-663	miR-155	(91)
Resveratrol	Human colon adenocarcinoma cell line SW480	50 μmol/L	miR-146b-5p, miR-1, and miR-663		(92)
Resveratrol	Human prostate adenocarcinoma cells LNCaP	50 μmol/L	miR-150 and miR-296–5p	miR-7, miR-17, miR-18b, miR-20a, miR-20b, miR-92b miR-106a and miR106b	(93)
Other bioactive food components					
Oleic acid	Human hepatocellular liver carcinoma cell line HepG2	50 μmol/L	miR-21		(96)
SCFA butyrate	Human colon cancer cell line HCT-116	1 mmol/L		miR-17, miR-20a, miR-20b, miR-93, miR-106a and miR-106b	(101)

[1] DIM, 3′,3′-diindolylmetane; EGCG, epigallocatechin gallate; HCC, hepatocellular carcinoma; 1,25$(OH)_2$D, 1,25-dihydroxycholecalciferol; PJ, pomegranate juice; RA, retinoic acid.

downregulation of K-Ras and Bcl2 after treatment with 100 nmol/L all-*trans*-RA correlates with the activation of known miRNA regulators of those proteins, let-7a and miR-15a/miR-16–1, respectively (62).

miRNA contributing to RA-induced differentiation in neuroblastoma has been a noteworthy area of investigation. Recently, miR-10a and miR-10b expression was found to be increased in SK-N-BE, LAN5, and SHSY-5Y neuroblastoma cells following RA treatment (63). One of the predicted downregulated miR-10a/b targets was nuclear receptor corepressor 2, a corepressor of gene transcription, which is known to suppress neurite outgrowth. Through various validation strategies, including ectopic overexpression of the implicated miRNA, it was concluded that miR-10a/b is important in the process of neural cell differentiation through targeting of nuclear receptor corepressor 2, which in turn induces a cascade of primary and secondary transcriptional alterations, including the downregulation of MYCN, a potent oncoprotein in neuroblastoma (63).

Human myeloid leukemia cells exposed to 1,25$(OH)_2$D, the active metabolite of vitamin D, acquire characteristics of normal monocytes and arrest in the G(1) phase of the cell cycle, in part due to the upregulation of p27(Kip1) and p21(Cip1) (64). These regulators of cell cycle progression may be targets of specific miRNA. Exposure of HL60 and U937 cells to low (1–10 nmol/L) concentrations of 1,25$(OH)_2$D decreased the expression of miR-181a and miR-181b, which resulted in the accumulation of p27 (Kip1) and p21(Cip1) and cell cycle arrest. Moreover, expression of pre-miR-181a in these cells increased the cellular miR-181a levels and reduced the 1,25$(OH)_2$D-induced increase in p27(Kip1) at both mRNA and protein levels, suggesting a role for miR-181a in myeloid cancers. Other cells have been examined for the ability of 1,25$(OH)_2$D to modulate miRNA. For example, a significant protective role for 1,25$(OH)_2$D against cellular stress in breast epithelial cells was found to be mediated by altered miRNA expression, in particular reduced expression of miR-182 (65). The results highlight the effects of 1,25$(OH)_2$D as a protective hormone against cellular stress; this effect is mediated through the maintenance of miRNA expression.

In addition to bone mineralization and maintenance of calcium balance, 1,25$(OH)_2$D exerts a number of physiologic actions that are mediated via its nuclear receptor (VDR), a ligand-regulated transcription factor that binds to specific sequences (vitamin D response elements) in its target genes and modulates their expression. It is interesting to note that the VDR has been found to be modulated by miRNA. A putative miR-125b recognition element was recently identified in the 3′-untranslated region of human VDR mRNA (66). Target activity was verified by electrophoretic mobility shift assays, which demonstrated overexpression of miR-125b significantly decreased the endogenous VDR protein level in MCF-7 cells to 40% of control (66). These data suggest that increased expression of miR-125b in cancer cells could inhibit the beneficial effects of 1,25 $(OH)_2$D by inhibiting VDR expression.

TABLE 2 Representative dietary components that can modulate microRNA (miRNA) using in vivo models[1]

Dietary component	Animal model	Concentration	miRNA Upregulated	miRNA Downregulated	Reference
Essential nutrients					
Folate, methionine, choline	Male weaning Fisher 344 rats	Low in L-methionine (0.18%), devoid of choline and folic acid (Dyets, Inc.)	let-7a, miR-21, miR-23, miR-130, miR-190 and miR-17–92	miR-122a	(58)
Folate, methionine, choline	Male weaning Fisher 344 rats	Low in L-methionine (0.18%), devoid of choline and folic acid (Dyets, Inc.)		miR-34a, miR-16a, miR-127, miR-181a, miR-20a, and miR-200b	(60)
Choline	C57BL/6 mice	Lombardi's choline-deficient (0 g/kg), low-methionine (1.7 g/kg), and amino acid-defined diet (Dyets, Inc.)	miR-155, miR-221/222, and miR-21	miR-122	(61)
Vitamin E	Weaned male Fisher 344 rats	Diets deficient in vitamin E (VE-free vitamin premix, E15313–2;ssniff Spezialdiaeten)		miR-122a and miR-125b	(67)
Phytochemicals					
EGCG	Athymic nude mice implanted with androgen-sensitive human prostate carcinoma CWR22Rν1 cells	EGCG for 6 wk (1 mg/d 3×/wk)	miR-330	miR-21	(71)
Curcumin	Chicken-embryo-metastasis CAM assay (RKO and HCT116 cells inoculated into 10-d-old chicken embryos)	20 μmol/L		miR-21	(76)
I3C	Adult male Sprague-Dawley rats	2.5 g/kg diet; mean intake 62.5 mg/rat/d	miR-34b, miR-26a, miR-125a-p, and miR-10a		(78)
I3C	Female A/J mice	70 μmol I3C/g diet		miR-21, miR-31, miR-130a, miR-146b and miR-377	(79)
PEITC	Adult male Sprague-Dawley rats	500 mg/kg diet; mean intake 9.6 mg/rat/d	miR125b, miR-26a, miR-146-p, let-7a, let-7c, miR-192, miR-99b, miR-123-p, and miR-222-p		(78)
Other bioactive food components					
(n-3) PUFA	Weanling male Sprague-Dawley rats	11.5 g fish oil/100 g diet; total fat content diet 15%	let-7d, miR-15b, miR-107, miR-191 and miR-324–5p		(98)
(n-3) PUFA	Weanling male Sprague-Dawley rats	11.5 g fish oil/100 g diet; total fat content diet 15%	miR-19b, miR26b, miR-27b, and miR-203		(99)

[1] CAM, chorionallantoic membrane assay; EGCG, epigallocatechin gallate; I3C, indole-3-carbinol; PEITC, phenethyl isothiocyanate.

Vitamin E is another essential nutrient that can influence miRNA expression. miRNA expression was found altered after rats were fed diets deficient in vitamin E (α tocopherol, < 1 mg/kg diet; γ tocopherol, < 1 mg/kg diet) for 6 mo compared to rats fed a vitamin E-sufficient diet (α tocopherol, 12 mg/kg diet; γ tocopherol, 24 mg/kg diet) (67). Vitamin E deficiency resulted in reduced concentrations of hepatic miR-122a and miR-125b, the targets of which are thought to be involved in lipid metabolism, inflammation, and HCC. Thus, appropriate vitamin E status may exert regulatory properties through miRNA expression to ultimately influence processes involved in cancer prevention.

Insight concerning the molecular mechanisms of selenium in cancer prevention continues to be an active area of research following the publication of the Selenium and Vitamin E Cancer Prevention Trial results (68). Treatment of LNCaP human prostate cancer cells with sodium selenite elicited rapid (within 4–8 h) transcriptional activation of p53 with concomitant upregulation of p53-target genes, including genes of the miR-34 family (69). The findings suggest that selenium-induced growth arrest and anticancer activity might be mediated in part by miRNA (69).

Phytochemicals

The tea polyphenol EGCG has many interesting cancer protective activities, some of which might be mediated by changes in miRNA expression. Using microarray analysis, EGCG treatment of HepG2 human HCC cells was found to upregulate the expression of 13 miRNA and downregulate the expression of 48 miRNA (70). Quantitative real-time PCR confirmed 8 of the differentially expressed miRNA (Table 1). Additional studies focused on the activity of miR-16 and its target, the antiapoptotic protein Bcl2. Results suggested that EGCG treatment increased miR-16 expression that downregulated Bcl2 and induced apoptosis in these cells. miRNA were also found to be regulated by EGCG in prostate tumors isolated from mice. In this study, a significant downregulation of androgen-regulated miR-21 and upregulation of a tumor suppressor, miR-330, in tumors of mice treated with EGCG was identified (71). The results suggested that androgen receptor-regulated miRNA can be modulated by EGCG.

Curcumin (diferuloylmethane), a component of the spice *Curcuma longa* (or turmeric), has been found to modulate cancer signaling pathways, possibly through miRNA expression (72). Curcumin was found to upregulate 11 and downregulate 18 miRNA following 72 h of incubation in human pancreatic cancer cells (73). Upregulation of miR-22 by curcumin was confirmed by real-time PCR and found to suppress the expression of its targets, Sp1 transcription factor and ER,α. The results suggest that curcumin may influence pancreatic cell proliferation through miRNA regulation. In another study, curcumin upregulated the expression of miR-15a and miR-16 in MCF-7 cells (74). Both miR-15a and miR-16 inhibited the expression of Bcl2, thereby inducing apoptosis. This proapoptotic activity was confirmed by silencing miR-15a and miR-16 with anti-miR-15a and anti-miR-16 oligonucleotides, which restored the expression of Bcl2 (74). Curcumin has also been shown to promote apoptosis in human lung adenocarcinoma cells through miR-186* signaling (75). In this circumstance, caspase-10, a member of the cysteine-aspartic acid protease family involved in death receptor signaling, was found to be a target of miR-186*. In primary tumors generated in vivo using a chicken-embryo-metastasis CAM, curcumin was shown to inhibit activator protein-1 (AP-1) binding upstream of the pri-miR-21, which reduced miR-21 expression and induced expression of the tumor suppressor Pdcd4, a target of miR-21 (76). Furthermore, curcumin inhibited metastasis in the CAM assay. These investigators concluded that curcumin inhibited tumor growth, invasion, and in vivo metastasis through inhibition of transcriptional regulation of miR-21 (76). Thus, curcumin appears to modulate miRNA that target proliferation, apoptosis, invasion, and metastasis depending on the cellular context.

In a recent study, exposure to ECS caused extensive downregulation of miRNA expression in the lungs of rats, resulting in increased expression of many genes and proteins involved in processes such as stress response, apoptosis, proliferation, angiogenesis, and gene transcription (77). These investigators next tested the ability of various chemopreventive agents to influence the expression of miRNA in ECS-free or ECS-exposed rats (78). I3C and PEITC, both of which are found in cruciferous vegetables and display anticancer activity, were among the agents examined. Interestingly, it was found that these agents did not alter miRNA expression to any appreciable extent in lung tissue of ECS-free rats but did modulate miRNA expression in ECS-exposed rats. PEITC and I3C treatment reversed many of the aberrantly expressed miRNA observed in the ECS-exposed lung tissue, including those involved in stress response (miR-125b), cell proliferation (let-7a, let-7c, and miR-222-p), cell apoptosis (miR-99b), and p53 functions (miR-34b). Thus, these dietary factors may protect against tissue-specific aberrant miRNA expression and signaling during ECS exposure. In a carcinogenesis model, I3C was found to reverse vinyl carbamate-induced deregulation of miRNA levels in lung tissues of female A/J mice (79). Lung tissue of the mice supplemented with I3C was found to have reduced levels of miR-21, miR-31, miR-130a, miR-146b, and miR-377 relative to the level in mice treated with the carcinogen. Additional studies verified potential targets for the oncogenic effect of miR-21and the chemoprotective activity of I3C.

Following consumption, I3C rapidly undergoes a condensation reaction in the stomach, which leads to the formation of many oligomeric compounds, including DIM (80). Several studies highlight DIM activity in cancer prevention and recent observations include its capacity to modulate miRNA. For example, treatment of MCF-7 breast cancer cells with DIM increased miR-21 expression and reduced expression of the cell-cycle promotion phosphatase Cdc25A, resulting in inhibition of growth (81). BioResponse-DIM (82), which has greater bioavailability than DIM, and a soy isoflavone mixture were both found to influence

chemotherapeutic drug resistance through modulation of miRNA (83). In this study, the involvement of miRNA in the acquisition of drug resistance characteristics, including EMT phenotype, was explored in gemcitabine-resistant pancreatic cancer cells. The expression of miR-200b, miR-200c, let-7b, let-7c, let-7d, and let-7e was significantly downregulated in gemcitabine-resistant pancreatic cancer cells, which displayed EMT characteristics, including elongated fibroblastoid morphology, lower expression of epithelial marker E-cadherin, and higher expression of mesenchymal markers such as vimentin and zinc finger E-box-binding homeobox 1 (ZEB1) (83). Members of the miR-200 and let-7 families were upregulated following treatment with either the DIM formulation or the soy isoflavone mixture in both gemcitabine-resistant and gemcitabine-sensitive pancreatic cancer cells. Moreover, treatment with the DIM formulation or the isoflavone mixture reversed the EMT phenotype through upregulation of miR-200 and downregulation of its targets. Additional studies in this laboratory found that treatment with either the DIM formulation or the soy isoflavone mixture increased miR-146a in pancreatic cancer cells, causing a downregulation of EGFR, metastasis-associated protein2 (MTA-2), interleukin-1 receptor-associated kinase 1 (IRAK-1), and NFκB, with consequent inhibition of pancreatic cancer cell invasion (84). Thus, the cancer prevention activity of BFC from cruciferous vegetables and soybeans appears to include modulation of miRNA and their mRNA targets.

Genistein, a dietary isoflavone from soybeans, has been shown to be a potent cancer prevention agent in several model systems. In addition to the studies examining the isoflavone mixture described above (83,84), genistein in isolation has also influenced miRNA expression and activity. The growth inhibitory action of genistein was associated with inhibition of miR-27a expression as well as increased gene expression of the miR-27a target zinc finger and BTB domain containing 10 (*ZBTB10*) in human uveal melanoma cells (85). Another study reported that genistein augmented the apoptotic effect of exogenous miR-16 in murine CLL cells, suggesting the use of dietary compounds in combination with miRNA for cancer treatment (86). Genistein has also been shown to regulate expression of the imprinted tumor suppressor gene ARHI by altering miRNA expression in prostate cancer cells (87). miRNA-221 and miR-222 levels decreased ~30 and 55%, respectively, and ARHI mRNA levels were induced 2-fold after genistein treatment to these cells. Therefore, a correlation between the antitumor activity of genistein and miRNA-mediated regulatory mechanisms appear to be present in different cell systems.

PJ has been shown to inhibit the progression of prostate cancer (88). In an intriguing study, the effects of PJ on cell death, adhesion, and migration were thought to be related to miRNA expression in prostate cancer cells in culture (89). Antiinvasive miRNA such as miR-335, miR-205, miR-200, and miR-126 were upregulated, whereas proinvasive miRNA such as miR-21 and miR-373 were downregulated in prostate cancer cells treated with 5% PJ for 12 h. Some of the predicted targets of these miRNA, including E-cadherin (miR-200) and intercellular adhesion molecule-1 (miR-21), were also found to be modulated by PJ. Furthermore, mimics for the proinvasive miRNA-21 and an inhibitor of the antiinvasive miRNA-335 reversed the ability of PJ to inhibit cell migration in prostate cancer cells. These findings support the need for additional study of the mechanism of action of pomegranate and its components for deterring prostate cancer progression.

Resveratrol, a polyphenolic compound from the stilbene family, is found in grapes, red wine, peanuts, and some berries and has many healthful properties, including antiinflammatory and anticancer activity (90). Recent reports suggest that miRNA participate in some of the activities described for resveratrol. For example, human THP-1 monocytes were treated with resveratrol before challenging them with LPS stimulation to test whether the antitumor and antiinflammatory effects of resveratrol rely on the modulation of expression of miRNA (91). Resveratrol was found to impair the upregulation of oncogenic proinflammatory miR-155 by LPS. This finding was thought to be in part through resveratrol upregulation of miR-663, which targets the transcripts of the transcription factors junB and junD with consequent decreased junB and junD levels as well as reduced AP-1 activity. Following 14 h of resveratrol treatment to SW480 colon cancer cells, the levels of 22 miRNA were significantly increased and those of 26 miRNA were significantly decreased (92). The magnitude of the changes in miRNA levels, however, were generally minimal, with a few exceptions, such as a 17- and 11-fold upregulation of miR-146b-5p and miR-1, respectively, suggesting that the resveratrol effects on miRNA populations are not global but rather miRNA specific. Using an in silico analysis procedure, the miRNA modulated by resveratrol were found to putatively target genes encoding Dicer1, tumor-suppressor factors such as PDCD4 or PTEN, as well as key effectors of the TGFβ signaling pathway (92). Experimentally, resveratrol was found to upregulate miR-663 and decrease its target transcript, TGFβ1. Differential miRNA expression in prostate cancer cells treated with resveratrol has also been reported (93). These investigators found that 23 miRNA were significantly downregulated and 28 miRNA were significantly upregulated after resveratrol treatment and selected miRNA were verified by real-time PCR. Using the TargetScan database (94), they also identified putative mRNA targets for the resveratrol-modulated miRNA. A computational approach to examine the possible pathways collectively regulated by the resveratrol-modulated miRNA was also performed (93). These studies suggest that the protective properties of resveratrol may arise at least in part from its capability to modulate the composition of miRNA populations in cells and suggest that the manipulation of the levels of key miRNA may help to potentiate the antiinflammatory and anticancer effects of resveratrol.

Other BFC

Olive oil is enriched in the MUFA oleic acid. The role of oleic acid consumption in carcinogenesis is not clear.

Recently, oleic acid was found to inhibit the expression of PTEN, a phosphoinositide phosphatase that acts as a tumor suppressor gene and is frequently mutated/deleted in human cancers (95) through upregulation of miRNA-21 in human HCC cells, suggesting a procarcinogenic capacity for this fatty acid (96). Additional studies are needed to determine the influence of dietary fatty acids on miRNA dysregulation in diseases of the liver, including HCC development.

High intakes of (n-3) PUFA (e.g. fish oil and flaxseed oil) have been inversely correlated with the development of certain cancers, but the mechanisms by which dietary PUFA contribute to the prevention of cancer has not been fully established (97). The influence of corn oil or fish oil with pectin or cellulose on miRNA expression in azoymethane-induced colon cancer in rats has been examined (98). At an early stage of cancer progression, the expression of 5 tumor suppressor miRNA (let-7d, miR-15b, miR-107, miR-191, and miR-324–5p) were significantly affected by diet-carcinogen interactions. Interestingly, the fish oil-fed animals exhibited the smallest number of differentially expressed miRNA (98). These same investigators recently performed an integrated miRNA and mRNA expression analysis to elucidate the combined effects of dietary bioactive agents and carcinogen on the biological function of miRNA in the rat colon (99). Four computational approaches were utilized to test the hypothesis that miRNA and their post-transcriptionally regulated mRNA targets are differentially modulated by carcinogen and diet treatment. Diet and carcinogen exposure were found to modulate a number of miRNA (i.e. miR-16, miR-19b, miR-21, miR26b, miR27b, miR-93, and miR-203) linked to oncogenic signaling pathways. Furthermore, gene expression analyses showed that oncogenic signals PTK2B, PDE4B, and TCF4 were suppressed by the fish oil plus pectin diet at both the mRNA and protein levels (99). This study provides the most comprehensive analysis to date of the integrated expression of miRNA and mRNA targets modulated by diet during carcinogenesis.

Another class of important dietary factors implicated in the prevention of colorectal cancer is the SCFA that are formed by microbial anaerobic fermentation of dietary fiber in the large intestine (100). The SCFA butyrate has been shown to modulate expression of genes involved in cell cycle regulation, including p21. The effects of butyrate on growth arrest and miRNA expression in HCT-116 colon cancer cells was recently investigated (101). Butyrate changed the expression of 44 miRNA in these cells, many of which were aberrantly expressed in colon cancer tissues. Several of these changes were confirmed using real-time qPCR, including the expression of miR-17, miR-20a, miR-20b, miR-93, miR-106a, and miR-106b, which were all decreased in response to butyrate treatment. Furthermore, miRNA expression has been found to be increased in human colon cancer samples, suggesting that the beneficial effects of butyrate may be mediated by suppressing the miRNA that are upregulated in colon cancer. An exogenous miR-106b mimic reduced the butyrate-induced p21 protein expression compared with cells treated with butyrate. Thus, it appears that SCFA derived from diet-microbe interactions in the gut may regulate host gene expression involved in intestinal homeostasis as well as carcinogenesis through modulation of miRNA.

Conclusions

miRNA are small ncRNA that appear to regulate gene expression in every cellular process that has been investigated. They regulate gene expression through interaction with 3′ untranslated regions of target mRNA of protein-coding genes, resulting in inhibition of mRNA translation or degradation of the mRNA transcript. A current emphasis, as well as a challenge, for miRNA studies is to identify the biologically relevant downstream targets that they regulate. Much of this work has provided new insights into the role of miRNA in various biological events, including cancer pathways. miRNA have been shown to participate in cancer development by regulating the common hallmarks of cancer. Thus, disturbances of miRNA expression and function appear to contribute to the initiation, promotion, and progression of tumors. Other emerging topics concerning the biology of miRNA include the influence of RNA-binding proteins on miRNA biogenesis, activity, and stability and the role of circulating miRNA as potential biomarkers or as intercellular signaling molecules. Advances in these areas are likely to provide additional molecular insights toward understanding cancer biology. More focused functional studies are also needed to characterize the precise activity of specific miRNA in the development of cancers.

Since our initial review of the topic (5), there has been an explosion of research on nutritional modulation of miRNA in the cancer context, which we have highlighted in this review. We have described the activity of dietary modulation of miRNA expression and their mRNA targets in various cancer processes, including apoptosis, cell cycle regulation, inflammation, and metastasis as well as pathways in stress response. Insights from these studies will likely lead to a better understanding of molecular mechanisms responsible for the influence of diet in cancer prevention. Dietary modulation of miRNA in the context of other diseases has also emerged, including studies examining diet modulation of miRNA in obesity (102) and heart disease (103). Many of the studies about diet modulation of miRNA that are highlighted in this review are descriptive, i.e. cells or animals are treated with a BFC and miRNA expression and downstream target protein expression are observed. Thus, there is a need for experimental functional studies, both in vitro and in vivo, including studies to understand the complex regulation of miRNA and their targets and how dietary factors participate in these processes. The use of transgenic mice with specific loss or gain of miRNA genes may be of utility in probing certain functions.

Interpreting the in vitro data with regard to the concentration used and the timing of exposure must be done with care. The rationale for the various time and dose exposures should be apparent in studies so that a better understanding

of the physiological relevance can be determined. For in vivo studies, issues of effective concentrations and timing prevail, as do questions about the frequency of dietary exposure needed to sustain any beneficial miRNA response. More studies are needed to determine the appropriate timing of dietary exposure that is critical for maintaining "normal" miRNA expression for cancer prevention or disrupting aberrant miRNA expression during early stages of cancer development. The importance of temporal influence of diet is highlighted by a recent study in mice, which suggested that maternal dietary exposure altered miRNA expression in adult offspring (104), implicating critical windows of vulnerability to dietary exposure.

Clearly, much remains to be discovered with regard to nutritional modulation of miRNA expression, including cell or tissue specific responses, the quantity of BFC needed to bring about the desired biological effect, the timing of exposure, and other variables that can influence the response, including interactions with other dietary factors. Furthermore, interactions between BFC, SNP in miRNA, and cancer risk are open avenues of investigation. Importantly, miRNA may be useful as blood biomarkers of cancer prevention, cancer susceptibility, or dietary exposure, as well as function as potential molecular targets that are modulated by dietary interventions.

Acknowledgments

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Literature Cited

1. Birney E, Stamatoyannopoulos JA, Dutta A, Guigo R, Gingeras TR, Margulies EH, Weng Z, Snyder M, Dermitzakis ET, Thurman RE, et al. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature. 2007;447: 799–816.
2. Eddy SR. Non-coding RNA genes and the modern RNA world. Nat Rev Genet. 2001;2:919–29.
3. Costa FF. Non-coding RNAs: lost in translation? Gene. 2007;386:1–10.
4. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet. 2008;9:102–14.
5. Davis CD, Ross SA. Evidence for dietary regulation of microRNA expression in cancer cells. Nutr Rev. 2008;66:477–82.
6. Sayed D, Abdellatif M. MicroRNAs in development and disease. Physiol Rev. 2011;91:827–87.
7. Doench JG, Sharp PA. Specificity of mircroRNA target selection in translational repression. Genes Dev. 2004;18:504–11.
8. Pillai RS, Artus CG, Filipowicz W. Tethering of human Ago proteins to mRNA mimics the miRNA-mediated repression of protein synthesis. RNA. 2004;10:1518–25.
9. miRBase: the microRNA database. release 17; April 2011 [cited 2011 Sept 15]. Available from: http://www.mirbase.org.
10. Bentwich I, Avniel A, Karov Y, Aharonov R, Gilad S, Brad O, Barzilai A, Einat P, Einav U, Meiri E, et al. Identification of hundreds of conserved and nonconserved human microRNAs. Nat Genet. 2005;37: 766–70.
11. Moore KJ, Rayner KJ, Suárez Y, Fernández-Hernando C. microRNAs and cholesterol metabolism. Trends Endocrinol Metab. 2010;21: 699–706.
12. Suh N, Blelloch R. Small RNAs in early mammalian development: from gametes to gastrulation. Development. 2011;138:1653–61.
13. Melkman-Zehavi T, Oren R, Kredo-Russo S, Shapira T, Mandelbaum AD, Rivkin N, Nir T, Lennox KA, Behlke MA, Dor Y, et al. miRNAs control insulin content is pancreatic β-cells via downregulation of transcriptional repressors. EMBO J. 2011;30:835–45.
14. O'Connell RM, Zhao JL, Rao DS. MicroRNA function in myeloid biology. Blood. 2011;118:2960–9.
15. Baggish AL, Hale A, Weiner RB, Lewis GD, Systrom D, Wang F, Wang TJ, Chan SY. Dynamic regulation of circulating microRNA during acute exhaustive exercise and sustained aerobic exercise training. J Physiol. 2011;589:3983–94.
16. Hou L, Wang D, Baccarelli A. Environmental chemicals and microRNAs. Mutat Res. 2011;714:105–12.
17. Saini S, Majid S, Dahiya R. Diet, microRNAs and prostate cancer. Pharm Res. 2010;27:1014–26.
18. Li Y, Kong D, Wang Z, Sarkar FH. Regulation of microRNAs by natural agents: an emerging field in chemoprevention and chemotherapy research. Pharm Res. 2010;27:1027–41.
19. Link A, Balaguer F, Goel A. Cancer chemoprevention by dietary polyphenols: promising role for epigenetics. Biochem Pharmacol. 2010;80: 1771–92.
20. Huang J, Plass C, Gerhäuser C. Cancer chemoprevention by targeting the epigenome. Curr Drug Targets. Epub 2010 Dec 15.
21. Parasramka MA, Ho E, Williams DE, Dashwood RH. MicroRNAs, diet, and cancer: new mechanistic insights on the epigenetic actions of phytochemicals. Mol Carcinog. Epub 2011 Jul 7.
22. Thomson DW, Bracken CP, Goodall GJ. Experimental strategies for microRNA target identification. Nucleic Acids Res. 2011;39:6845–53.
23. Li J, Smyth P, Flavin R, Cahill S, Denning K, Aherne S, Guenther SM, O'Leary JJ, Sheils O. Comparison of miRNA expression patterns using total RNA extracted from matched samples of formalin-fixed paraffin-embedded (FFPE) cells and snap frozen cells. BMC Biotechnol. 2007;7:36.
24. Cortez MA, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK, Calin GA. Medscape. MicroRNAs in body fluids-the mix of hormones and biomarkers. Nat Rev Clin Oncol. 2011;8:467–77.
25. Munker R, Calin GA. MicroRNA profiling in cancer. Clin Sci (Lond). 2011;121:141–58.
26. Ferdin J, Kunej T, Calin GA. Non-coding RNAs: identification of cancer-associated microRNAs by gene profiling. Technol Cancer Res Treat. 2010;9:123–38.
27. Ferracin M, Pedriali M, Veronese A, Zagatti B, Gafà R, Magri E, Lunardi M, Munerato G, Querzoli G, Maestri I, et al. MicroRNA profiling for the identification of cancers with unknown primary tissue-of-origin. J Pathol. 2011;225:43–53.
28. Kent OA, Mendell JT. A small piece in the cancer puzzle: microRNAs as tumor suppressors and oncogenes. Oncogene. 2006;25:6188–96.
29. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646–74.
30. Ruan K, Fang X, Ouyang G. MicroRNAs: novel regulators in the hallmarks of human cancer. Cancer Lett. 2009;285:116–26.
31. Negrini M, Nicoloso MS, Calin GA. MicroRNAs and cancer: new paradigms in molecular oncology. Curr Opin Cell Biol. 2009;21:470–9.
32. Lee SK, Calin GA. Non-coding RNAs and cancer: new paradigms in oncology. Discov Med. 2011;11:245–54.
33. Di Leva G, Croce CM. Roles of small RNAs in tumor formation. Trends Mol Med. 2010;16:257–67.
34. Gandellini P, Profumo V, Folini M, Zaffaroni N. MicroRNAs as new therapeutic targets and tools in cancer. Expert Opin Ther Targets. 2011;15:265–79.
35. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci USA. 2002;99:15524–9.
36. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci USA. 2005; 102:13944–9.
37. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M, et al. Human microRNA

genes are frequently located at fragile sties and genomic regions involved in cancers. Proc Natl Acad Sci USA. 2004;101:2999–3004.
38. Zhang L, Huang J, Yang N, Greshock J, Megraw MS, Giannakakis A, Liang S, Naylor TL, Barchetti A, Ward MR, et al. microRNAs exhibit high frequency genomic alterations in human cancer. Proc Natl Acad Sci USA. 2006;103:9136–41.
39. Duan S, Mi S, Zhang W, Dolan ME. Comprehensive analysis of the impact of SNPs and CNVs on human microRNAs and their regulatory genes. RNA Biol. 2009;6:412–25.
40. Slaby O, Bienertova-Vasku J, Svoboda M, Vyzula R. Genetic polymorphisms and microRNAs: new direction in molecular epidemiology of solid cancer. J Cell Mol Med. Epub 2011 Jun 21.
41. Barh D, Malhotra R, Ravi B, Sindhurani P. MicroRNA let-7: an emerging next-generation cancer therapeutic. Curr Oncol. 2010;17:70–80.
42. Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, Harano T, Yatabe Y, Nagino M, Nimura Y, et al. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res. 2004;64:3753–6.
43. Brueckner B, Stresemann C, Kuner R, Mund C, Musch T, Meister M, Sültmann H, Lyko F. The human let-7a-3 locus contains an epigenetically regulated microRNA gene with oncogenic function. Cancer Res. 2007;67:1419–23.
44. Zhao Y, Deng C, Wang J, Xiao J, Gatalica Z, Recker RR, Xiao GG. Let-7 family miRNAs regulate estrogen receptor alpha signaling in estrogen receptor positive breast cancer. Breast Cancer Res Treat. 2011; 127:69–80.
45. Li D. Metformin as an antitumor agent in cancer prevention and treatment. J Diabetes. Epub 2011 Feb 21.
46. Oliveras-Ferraros C, Cufí S, Vazquez-Martin A, Torres-Garcia VZ, Del Barco S, Martin-Castillo B, Menendez JA. Micro(mi)RNA expression profile of breast cancer epithelial cells treated with the anti-diabetic drug metformin: induction of the tumor suppressor miRNA let-7a and suppression of the TGFβ-induced oncomiR miRNA-181a. Cell Cycle. 2011;10:1144–51.
47. Lin Q, Gao Z, Alarcon RM, Ye J, Yun Z. A role of miR-27 in the regulation of adipogenesis. FEBS J. 2009;276:2348–58.
48. Bao B, Wang Z, Li Y, Kong D, Ali S, Banerjee S, Ahmad A, Sarkar FH. The complexities of obesity and diabetes with the development and progression of pancreatic cancer. Biochim Biophys Acta. 2011;1815: 135–46.
49. Ali AS, Ali S, Ahmad A, Bao B, Philip PA, Sarkar FH. Expression of microRNAs: potential molecular link between obesity, diabetes and cancer. Obes Rev. Epub 2011 Jul 19.
50. Bianchi F, Nicassio F, Marzi M, Belloni E, Dall'olio V, Bernard L, Pelosi G, Maisonneuve P, Veronesi G, Di Fiore PP. A serum circulating miRNA diagnostic test to identify asymptomatic high-risk individuals with early stage lung cancer. EMBO Mol Med. 2011;3:495–503.
51. Chu H, Wang M, Shi D, Ma L, Zhang Z, Tong N, Huo X, Wang W, Luo D, Gao Y, et al. Hsa-miR-196a2 Rs11614913 polymorphism contributes to cancer susceptibility: evidence from 15 case-control studies. PLoS ONE. 2011;6:e18108.
52. Newmark HL, Yang K, Lipkin M, Kopelovich L, Liu Y, Fan K, Shinozaki HA. Western-style diet induces benign and malignant neoplasms in the colon of normal C57Bl/6 mice. Carcinogenesis. 2001;22: 1871–5.
53. Zhu H, Dougherty U, Robinson V, Mustafi R, Pekow J, Kupfer S, Li YC, Hart J, Goss K, Fichera A, et al. EGFR signals downregulate tumor suppressors miR-143 and miR-145 in Western diet-promoted murine colon cancer: role of G1 regulators. Mol Cancer Res. 2011;9:960–75.
54. Dougherty U, Cerasi D, Taylor I, Kocherginsky M, Tekin U, Badal S, Aluri L, Sehdev A, Cerda S, Mustafi R, et al. Epidermal growth factor receptor is required for colonic tumor promotion by dietary fat in the azoxymethane/dextran sulfate sodium model: roles of transforming growth factor-alpha and PTGS2. Clin Cancer Res. 2009;15:6780–9.
55. Singh J, Hamid R, Reddy BS. Dietary fat and colon cancer: modulating effect of types and amount of dietary fat on ras-p21 function during promotion and progression stages of colon cancer. Cancer Res. 1997; 57:253–8.
56. Avissar M, McClean MD, Kelsey KT, Marsit CJ. MicroRNA expression in head and neck cancer associates with alcohol consumption and survival. Carcinogenesis. 2009;30:2059–63.
57. Marsit CJ, Eddy K, Kelsey KT. MicroRNA responses to cellular stress. Cancer Res. 2006;66:10843–8.
58. Kutay H, Bai S, Datta J, Motiwala T, Pogribny I, Frankel W, Jacob ST, Ghoshal K. Downregulation of miR-122 in the rodent and human hepatocellular carcinomas. J Cell Biochem. 2006;99:671–8.
59. Gramantieri L, Ferracin M, Fornari F, Veronese A, Sabbioni S, Liu CG, Calin GA, Giovannini C, Ferrazzi E, Grazi GL, et al. Cyclin G1 is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. Cancer Res. 2007;67:6092–9.
60. Tryndyak VP, Ross SA, Beland FA, Pogribny IP. Down-regulation of the microRNAs miR-34a, miR-127, and miR-200b in rat liver during hepatocarcinogenesis induced by a methyl-deficient diet. Mol Carcinog. 2009;48:479–87.
61. Wang B, Majumder S, Nuovo G, Kutay H, Volinia S, Patel T, Schmittgen TD, Croce C, Ghoshal K, Jacob ST. Role of microRNA-155 at early stages of hepatocarcinogenesis induced by choline-deficient and amino acid-defined diet in C57BL/6 mice. Hepatology. 2009;50:1152–61.
62. Garzon R, Pichiorri F, Palumbo T, Visentini M, Aqeilan R, Cimmino A, Wang H, Sun H, Volinia S, Alder H, et al. MicroRNA gene expression during retinoic acid-induced differentiation of human acute promyelocytic leukemia. Oncogene. 2007;26:4148–57.
63. Foley NH, Bray I, Watters KM, Das S, Bryan K, Bernas T, Prehn JH, Stallings RL. MicroRNAs 10a and 10b are potent inducers of neuroblastoma cell differentiation through targeting of nuclear receptor corepressor 2. Cell Death Differ. 2011;18:1089–98.
64. Wang X, Gocek E, Liu CG, Studzinski GP. MicroRNAs181 regulate the expression of p27Kip1 in human myeloid leukemia cells induced to differentiate by 1,25-dihydroxyvitamin D3. Cell Cycle. 2009;8:736–41.
65. Peng X, Vaishnav A, Murillo G, Alimirah F, Torres KE, Mehta RG. Protection against cellular stress by 25-hydroxyvitamin D3 in breast epithelial cells. J Cell Biochem. 2010;110:1324–33.
66. Mohri T, Nakajima M, Takagi S, Komagata S, Yokoi T. MicroRNA regulates human vitamin D receptor. Int J Cancer. 2009;125:1328–33.
67. Gaedicke S, Zhang X, Schmelzer C, Lou Y, Doering F, Frank J, Rimbach G. Vitamin E dependent microRNA regulation in rat liver. FEBS Lett. 2008;582:3542–6.
68. Lippman SM, Klein EA, Goodman PJ, Lucia MS, Thompson IM, Ford LG, Parnes HL, Minasian LM, Gaziano JM, Hartline JA, et al. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). JAMA. 2009;301:39–51.
69. Sarveswaran S, Liroff J, Zhou Z, Nikitin AY, Ghosh J. Selenite triggers rapid transcriptional activation of p53, and p53-mediated apoptosis in prostate cancer cells: Implication for the treatment of early-stage prostate cancer. Int J Oncol. 2010;36:1419–28.
70. Tsang WP, Kwok TT. Epigallocatechin gallate up-regulation of miR-16 and induction of apoptosis in human cancer cells. J Nutr Biochem. 2010;21:140–6.
71. Siddiqui IA, Asim M, Hafeez BB, Adhami VM, Tarapore RS, Mukhtar H. Green tea polyphenol EGCG blunts androgen receptor function in prostate cancer. FASEB J. 2011;25:1198–207.
72. Reuter S, Gupta SC, Park B, Goel A, Aggarwal BB. Epigenetic changes induced by curcumin and other natural compounds. Genes Nutr. 2011;6:93–108.
73. Sun M, Estrov Z, Ji Y, Coombes KR, Harris DH, Kurzrock R. Curcumin (diferuloylmethane) alters the expression profiles of microRNAs in human pancreatic cancer cells. Mol Cancer Ther. 2008;7:464–73.
74. Yang J, Cao Y, Sun J, Zhang Y. Curcumin reduces the expression of Bcl-2 by upregulating miR-15a and miR-16 in MCF-7 cells. Med Oncol. 2010;27:1114–8.
75. Zhang J, Du Y, Wu C, Ren X, Ti X, Shi J, Zhao F, Yin H. Curcumin promotes apoptosis in human lung adenocarcinoma cells through miR-186* signaling pathway. Oncol Rep. 2010;24:1217–23.
76. Mudduluru G, George-William JN, Muppala S, Asangani IA, Kumarswamy R, Nelson LD, Allgayer H. Curcumin regulates miR-

21 expression and inhibits invasion and metastasis in colorectal cancer. Biosci Rep. 2011;31:185–97.
77. Izzotti A, Calin GA, Arrigo P, Steele VE, Croce CM, De Flora S. Downregulation of microRNA expression in the lungs of rats exposed to cigarette smoke. FASEB J. 2009;23:806–12.
78. Izzotti A, Calin GA, Steele VE, Cartiglia C, Longobardi M, Croce CM, De Flora S. Chemoprevention of cigarette smoke-induced alterations of MicroRNA expression in rat lungs. Cancer Prev Res (Phila). 2010;3: 62–72.
79. Melkamu T, Zhang X, Tan J, Zeng Y, Kassie F. Alteration of microRNA expression in vinyl carbamate-induced mouse lung tumors and modulation by the chemopreventive agent indole-3-carbinol. Carcinogenesis. 2010;31:252–8.
80. Minich DM, Bland JS. A review of the clinical efficacy and safety of cruciferous vegetable phytochemicals. Nutr Rev. 2007;65:259–67.
81. Jin Y. 3,3′-Diindolylmethane inhibits breast cancer cell growth via miR-21-mediated Cdc25A degradation. Mol Cell Biochem. 2011 Jul 15. [Epub ahead of print]
82. Anderton MJ, Manson MM, Verschoyle R, Gescher A, Steward WP, Williams ML, Mager DE. Physiological modeling of formulated and crystalline 3,3′-diindolylmethane pharmacokinetics following oral administration in mice. Drug Metab Dispos. 2004;32:632–8.
83. Li Y, VandenBoom TG II, Kong D, Wang Z, Ali S, Philip PA, Sarkar FH. 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. 2009;69:6704–12.
84. Li Y, Vandenboom TG II, Wang Z, Kong D, Ali S, Philip PA, Sarkar FH. miR-146a suppresses invasion of pancreatic cancer cells. Cancer Res. 2010;70:1486–95.
85. Sun Q, Cong R, Yan H, Gu H, Zeng Y, Liu N, Chen J, Wang B. Genistein inhibits growth of human uveal melanoma cells and affects microRNA-27a and target gene expression. Oncol Rep. 2009;22:563–7.
86. Salerno E, Scaglione BJ, Coffman FD, Brown BD, Baccarini A, Fernandes H, Marti G, Raveche ES. Correcting miR-15a/16 genetic defect in New Zealand Black mouse model of CLL enhances drug sensitivity. Mol Cancer Ther. 2009;8:2684–92.
87. Chen Y, Zaman MS, Deng G, Majid S, Saini S, Liu J, Tanaka Y, Dahiya R. MicroRNAs 221/222 and genistein-mediated regulation of ARHI tumor suppressor gene in prostate cancer. Cancer Prev Res (Phila). 2011;4:76–86.
88. Pantuck AJ, Leppert JT, Zomorodian N, Aronson W, Hong J, Barnard RJ, Seeram N, Liker H, Wang H, Elashoff R, et al. Phase II study of pomegranate juice for men with rising prostate-specific antigen following surgery or radiation for prostate cancer. Clin Cancer Res. 2006;12:4018–26.
89. Wang L, Alcon A, Yuan H, Ho J, Li QJ, Martins-Green M. Cellular and molecular mechanisms of pomegranate juice-induced antimetastatic effect on prostate cancer cells. Integr Biol (Camb). 2011;3:742–54.
90. Smoliga JM, Baur JA, Hausenblas HA. Resveratrol and health: a comprehensive review of human clinical trials. Mol Nutr Food Res. 2011; 55:1129–41.
91. Tili E, Michaille JJ, Adair B, Alder H, Limagne E, Taccioli C, Ferracin M, Delmas D, Latruffe N, Croce CM. Resveratrol decreases the levels of miR-155 by upregulating miR-663, a microRNA targeting JunB and JunD. Carcinogenesis. 2010;31:1561–6.
92. Tili E, Michaille JJ, Alder H, Volinia S, Delmas D, Latruffe N, Croce CM. Resveratrol modulates the levels of microRNAs targeting genes encoding tumor-suppressors and effectors of TGFβ signaling pathway in SW480 cells. Biochem Pharmacol. 2010;80:2057–65.
93. Dhar S, Hicks C, Levenson AS. Resveratrol and prostate cancer: promising role for microRNAs. Mol Nutr Food Res. 2011;55:1219–29.
94. TargetScan Human. release 5.2; June 2011 [cited 2011 Sept 15]. Available from: www.targetscan.org.
95. Li L, Ross AH. Why is PTEN an important tumor suppressor? J Cell Biochem. 2007;102:1368–74.
96. Vinciguerra M, Sgroi A, Veyrat-Durebex C, Rubbia-Brandt L, Buhler LH, Foti M. Unsaturated fatty acids inhibit the expression of tumor suppressor phosphatase and tensin homolog (PTEN) via microRNA-21 up-regulation in hepatocytes. Hepatology. 2009;49:1176–84.
97. Riediger ND, Othman RA, Suh M, Moghadasian MH. A systemic review of the roles of n-3 fatty acids in health and disease. J Am Diet Assoc. 2009;109:668–79.
98. Davidson LA, Wang N, Shah MS, Lupton JR, Ivanov I, Chapkin RS. n-3 Polyunsaturated fatty acids modulate carcinogen-directed noncoding microRNA signatures in rat colon. Carcinogenesis. 2009;30: 2077–84.
99. Shah MS, Schwartz SL, Zhao C, Davidson LA, Zhou B, Lupton JR, Ivanov I, Chapkin RS. Integrated microRNA and mRNA expression profiling in a rat colon carcinogenesis model: effect of a chemoprotective diet. Physiol Genomics. 2011;43:640–54.
100. Canani RB, Costanzo MD, Leone L, Pedata M, Meli R, Calignano A. Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. World J Gastroenterol. 2011;17:1519–28.
101. Hu S, Dong TS, Dalal SR, Wu F, Bissonnette M, Kwon JH, Chang EB. The microbe-derived short chain fatty acid butyrate targets miRNA-dependent p21 gene expression in human colon cancer. PLoS ONE. 2011;6:e16221.
102. Park JH, Ahn J, Kim S, Kwon DY, Ha TY. Murine hepatic miRNAs expression and regulation of gene expression in diet-induced obese mice. Mol Cells. 2011;31:33–8.
103. Mukhopadhyay P, Pacher P, Das DK. MicroRNA signatures of resveratrol in the ischemic heart. Ann N Y Acad Sci. 2011;1215:109–16.
104. Zhang J, Zhang F, Didelot X, Bruce KD, Cagampang FR, Vatish M, Hanson M, Lehnert H, Ceriello A, Byrne CD. Maternal high fat diet during pregnancy and lactation alters hepatic expression of insulin like growth factor-2 and key microRNAs in the adult offspring. BMC Genomics. 2009;10:478.