Evidence for dietary regulation of microRNA expression in cancer cells

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MicroRNAs (miRNAs) are an abundant class of short noncoding RNAs that are widely expressed in mammalian cells and are important in post-translational gene regulation, including regulation of cell proliferation, apoptosis, and differentiation processes. miRNAs are involved in cancer initiation and progression and their expression patterns serve as phenotypic signatures of different cancers. Recent evidence suggests that dietary components as diverse as folate, retinoids, and curcumin exert cancer-protective effects through modulation of miRNA expression. miRNAs may be useful as biomarkers of cancer prevention or nutritional status, as well as serve as potential molecular targets that are influenced by dietary interventions.

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INTRODUCTION

MicroRNAs (miRNAs) are an important class of non-coding RNAs, which regulate gene expression post-transcriptionally. Functional studies indicate that miRNAs participate in the regulation of almost every cellular process that has been investigated and that alterations in their expression are observed in and may underlie many different human diseases, including cancer.1 miRNAs are initially part of immature primary transcripts (pri-miRNA) that are transcribed to 60–100 nucleotide hairpin precursor RNAs (pre-miRNA) by the RNAse enzyme, Drosha (Figure 1). The pre-miRNA is transported to the cytoplasm by the nuclear export factor exportin-5. In the cytoplasm, the pre-miRNA is further excised by the RNA polymerase Dicer and unwound by a putative helicase to yield mature miRNAs, whose lengths are ~18–24 nucleotides. Mature miRNAs become part of the RNA-induced silencing complex (RISC) that facilitates 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).2,3 Binding of the miRNA to the mRNA results in a reduced translation rate and/or increased degradation of the mRNA. It is interesting to note that recent preliminary evidence also suggests that miRNAs may increase translation of mRNAs when cells are undergoing cell cycle arrest.4

To date, more than 500 human miRNA genes have been identified (miRBase, http://microrna.sanger.ac.uk/) and it is believed that the human genome encodes about a 1000 miRNAs.5 The specific function of most mammalian miRNAs is unknown.5 However, it is believed that each miRNA has hundreds of evolutionarily conserved targets and several times that number of nonconserved targets.6 It has also been speculated that miRNAs could regulate ~30% of the human genome.7 Disturbances in the expression of miRNAs, processing of miRNA precursors or mutations in the sequence of the miRNA, its precursor, or its target mRNA, may have detrimental effects on cellular function and have been associated with cancer.

miRNAs AND CANCER

Accumulating evidence points to miRNAs in the pathophysiology of all types of human cancers. This is characterized by abnormal levels of expression for mature and/or precursor miRNA transcripts in comparison to
miRNAs have been shown to regulate cell proliferation, differentiation, and apoptosis, all of which are dysregulated during carcinogenesis. Therefore, disturbance of miRNA expression and function may contribute to the initiation and maintenance of tumors.

Genomic, molecular, and functional approaches are being utilized to determine the causes of miRNA deregulation in cancer. For example, it has been reported that more than half of the known human miRNAs are located at fragile sites, as well as sites of loss of heterozygosity, amplification, and common breakpoint regions, which are particular genomic regions prone to alterations in cancer cells. A high-resolution array-based comparative genomics approach for 283 known miRNAs, discovered that a large proportion of miRNA gene containing genomic loci exhibit DNA copy number alterations in breast cancer (72.8%), ovarian cancer (37.1%), and melanoma (85.9%), suggesting that genomic alterations may contribute to the altered miRNA expression in cancer cells.

miRNA microarrays, as well as other molecular techniques, such as real-time quantitative reverse transcription polymerase chain reaction have been used to identify and compare the miRNA expression profiles in normal cells and tissues with those in tumors such as lung, colorectal, breast, thyroid cancers, glioblastomas, and lymphomas. These types of studies demonstrate there are distinct miRNA expression patterns associated with various tumor types and that cancer samples appear to have miRNA expression profiles that are distinct from normal tissues. For instance, the expression of miR-126, miR-143, and miR-145 were significantly decreased in >80% of the tumor samples compared with the corresponding normal tissue, whereas miR-21 was found to be overexpressed in 80% of the tumors. Interestingly, when Lu et al. published a large miRNA expression profile study of >200 miRNAs in primary tumors, cancer cells lines, and normal tissues using a novel bead-based technology, they found that unsupervised hierarchal clustering of the miRNA profiles was able to determine the histologic origin of highly undifferentiated cancer
samples with a significantly higher success rate than profiles obtained by measuring 16,000 protein-coding mRNAs (12 of 17 correct versus 1 of 17 correct, respectively). Furthermore, recent studies have shown that miRNAs are more stable than mRNAs and can be quantitatively recovered from formalin-fixed paraffin-embedded tissues, which constitute the vast majority of archival tissue samples from clinics (correlation coefficient between expression profiles for fresh frozen samples vs. formalin-fixed paraffin-embedded specimens ranging from $R^2 = 0.86 – 0.89$ vs. $R^2 = 0.28$ for mRNA vs. mRNA, respectively). The stability of miRNAs in archived samples is also demonstrated by the authors obtaining good-quality miRNA from 10-year-old formalin-fixed paraffin-embedded colon cancer samples. Thus, miRNA profiling may be a useful tool for molecular diagnosis and prognosis.

The first experimental evidence suggesting that miRNAs might have a functional role in human carcinogenesis and affect a specific molecular target that is associated with carcinogenesis came from the realization that the chromosomal deletion (13q14.3) associated with chronic lymphocytic leukemia (CLL) was found to encompass two miRNAs, miR-15a and miR16-1. Detailed deletion and expression analysis showed that miR-15a and miR-16 were deleted or downregulated in approximately 68% of CLL cases. Subsequent studies demonstrated that miR-15a and miR-16 can inhibit the expression of Bcl2, an important protein for inhibiting apoptosis. Furthermore, overexpression of miR-15A and miR-16 in a megakaryocytic cell line was sufficient to decrease the protein levels of Bcl2 and to activate apoptosis.

The functional significance of miRNAs and cancer development has also been studied in relation to the p53 signaling pathway. p53 is one of the most frequently mutated genes in human cancers and is associated with cell cycle arrest, apoptosis, and DNA repair. Several recent studies have implicated the miR-34 family of miRNAs in the p53 tumor suppressor network. Both in tissue culture and in vivo, the levels of miR-34 were found to be regulated by p53 at the transcriptional level, through direct binding to the promoter region. Furthermore, DNA damage can induce the activation of miR-34 in wild-type animals but not in p53 knockout animals. Activation of miR-34 results in inhibition of cell-cycle progression by reducing the expression of several genes including cyclin E2, cyclin-dependent kinase 4 and hepatocyte growth factor receptor and suggests the possibility that p53 may suppress cell-cycle related genes via induction of miR-34 activity. Similarly, in neuroblastoma tumors, miR-34a (a member of the miR-34 family) directly targets E2F3, which is a potent transcriptionally induced or cell cycle progression activator. Further-more, miR-34a can significantly increase caspase activity and promote apoptotic cell death. In contrast, reduction of miR-34 attenuates p53-mediated cell death. These findings, in combination with the fact that miR-34 is downregulated in several types of human cancer, suggest that miRNAs, in particular miR-34, can affect tumorigenesis by regulating well-known tumor suppressor pathways.

Mutations in the Ras oncogene are present in 15–30% of all human cancers, and overexpression of the Ras oncogene is common in lung cancer. Recent evidence suggests that Ras expression is regulated by the miRNA, let-7. In lung cancer patients, there is an inverse correlation between let-7 (low levels) and Ras protein (high levels) in non-small-cell lung cancers, suggesting that the loss of regulation of Ras by let-7 could play a role in the pathogenesis of lung cancer. Furthermore, two longitudinal studies conducted in lung cancer patients demonstrated that low levels of let-7 correlated with poorer prognosis after surgery. Recent studies have examined the repertoire of let-7 target genes with a role in cancer. The most common molecular targets for let-7 involved genes implicated in cell-cycle regulation; cells transfected with let-7 showed perturbation in the cell cycle. In accordance with the reduced protein levels of cdk6 and cdc25a, cells accumulated in the G1 phase of the cell cycle. Future work is needed to determine the mechanisms underlying the reduction of let-7 in lung cancer and to explore the potential for exogenous let-7 to restore the alterations in cell-cycle control and differentiation, as a possible strategy for cancer treatment.

**DIETARY MODIFICATION OF miRNAs**

A wealth of evidence points to the diet as one of the most important modifiable determinants of cancer risk. A large number of bioactive components have been identified in foods that are protective at different stages of cancer formation. Dietary components have been implicated in many pathways involved in carcinogenesis; including apoptosis, cell-cycle control, inflammation, angiogenesis, and DNA repair. These are also processes that are regulated by miRNAs. While there is some evidence suggesting that dietary modulation of miRNA expression may contribute to the cancer-protective effects of dietary components, as will be described below, this is a largely under-investigated area of science.

**Folate and methyl deficiency**

Dietary folate has been found to modulate miRNA expression in a number of different model systems and this may be related to cancer risk. For example, rats fed a
folate, methionine- and choline-deficient diet develop hepatocellular carcinoma (HCC) at 54 weeks of age in the absence of carcinogen treatment. Comparison of the miRNA profile by microarray analysis of livers from the animals fed the folate/methyl-deficient 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 compared to livers of rats on the normal diet. However, when rats were switched from the deficient to the adequate diet after 36 weeks, expression of miR-122 at 54 weeks was normal and tumors did not develop.

These findings were extended to determine whether the development of HCC was associated with altered expression of miRNAs involved in the regulation of cell proliferation and apoptosis, and to determine targets of aberrantly expressed miRNA that may impact the carcinogenic process. HCC induced by methyl deficiency was characterized by aberrant expression of miRNAs that target known tumor suppressors and oncogenes involved in maintaining the balance between apoptosis and cell proliferation, specifically by profound downregulation of tumor suppressor miRNA-34a, miRNA-16a, miRNA-181a, and miRNA-127. The most remarkable changes, confirmed by RT-PCR, were specific to the downregulation of miRNA-34a and miRNA-127 expression, which occurred early and remained at low levels throughout the hepatocarcinogenic process. The significance of miRNA expression was determined by examining protein levels of the experimentally confirmed targets of these differentially expressed miRNAs. Western blot analysis showed increased protein levels of E2F3 and BCL6, proteins regulated by miRNA-34a and miRNA-127, respectively, in the livers of rats fed a methyl-deficient diet.

Folate deficiency causes a pronounced global increase in miRNA expression in human lymphoblastoid cells in culture. Moreover, returning the folate-deficient cells to complete medium allowed for a return of the miRNA expression profiles to that of control cells. These results suggest that dietary modulation of miRNA expression is reversible and may be related to tumor development.

Alterations in miRNA expression in cell culture have also been observed in human subjects. The miRNA has-miR-222 was identified as being overexpressed under folate-deficient conditions in lymphoblastoid cells in culture, and this finding was confirmed in vivo in human peripheral blood from individuals with low folate status. Utilizing samples from a population-based case-control study of head and neck squamous cell carcinoma, six subjects within the lowest 1% of dietary folate intake had significantly increased expression of has-miR-222 compared to five subjects in the highest 1% of dietary folate intake. These data suggest that miRNA expression might be potential biomarkers of nutritional status in humans. Clearly, additional studies are needed.

Retinoic acid

Retinoic acid is another dietary component that has been shown to modulate miRNA expression. Acute promyelocytic leukemia (APL) is a subtype of acute myelogenous leukemia that is caused by a novel fusion protein resulting from the reciprocal translocation between the retinoic acid receptor-α (RAR-α) on chromosome 17 with the promyelocytic leukemia gene (PML) on chromosome 15. In the absence of adequate retinoic acid, this fusion protein (PML- RAR-α) interferes with myeloid differentiation by forming a corepressor complex that binds to target promoters and causes transcriptional repression of retinoic acid-responsive genes. In contrast, pharmacological doses of all-trans-retinoic acid reverse these effects. These effects appear to be mediated through miRNAs. Utilizing both miRNA microarray analysis of APL cell lines, and quantitative RT-PCR of cells derived from APL patients, it was observed that after treatment with 100 nmol/L all-trans retinoic acid that eight miRNAs were upregulated and one was downregulated; most of which have confirmed targets involved in hematopoietic differentiation and apoptosis. Moreover, the downregulation of Ras and Bcl2 after treatment with 100 nmol/L all-trans-retinoic acid correlates with the activation of known miRNA regulators of those proteins, let-7a and miR-15a/miR-16-1, respectively.

miRNAs are also involved in the retinoic acid-induced neural differentiation. NT-2 cells are human embryonal carcinoma cells that differentiate into neural cells upon treatment with retinoic acid. However, in the presence of siRNA-miR-23, NT-2 cells do not differentiate into the neural cells after treatment with retinoic acid. These results indicate that miR23 plays a critical role during retinoic acid-induced neuronal differentiation. Furthermore, retinoic acid downregulates specific miRNAs (i.e., miR-9/9*, miR-124a, and miR-125B) to induce abnormal development of the spinal cord in a rat model of spina bifida. Therefore, miRNAs also play important roles in differentiation induced by dietary components.

Curcumin

Curcumin, a naturally occurring flavanoid and proapoptotic compound derived from the rhizome of Curcuma longa, has recently been shown to alter the expression profiles of miRNA in human BxPC-3 pancreatic cancer cells. Eleven miRNAs were significantly upregulated and 18 miRNAs were significantly downregulated after 72 h of treatment with 10 μmol/L curcumin in these cells. In particular, curcumin was found to upregulate miRNA-22 and downregulate miRNA-199a*, and the altered expression of these miRNAs observed by microarray profiling
was confirmed by real-time PCR analysis. A major challenge for current miRNA studies is to identify the biologically relevant downstream targets that they regulate. To address this, the expression of two computationally predicted targets for miRNA-22, SP1 transcription factor (SP1) and estrogen receptor 1 (ESR1), were examined using BxPC-3 pancreatic cancer cells. Upregulation of miRNA-22 expression by either treatment with 10 μmol/L curcumin or transfections with miRNA-22 mimics, inhibited the expression of its target genes SP1 and ESR1, while experiments using miRNA-22 antisense enhanced SP1 and ESR1 expression. These findings suggest that modulation of miRNA expression by curcumin may be an important mediator of its anticancer effects in pancreatic cancer cells.

CONCLUSION

Micro RNAs are short, noncoding RNAs that regulate gene expression by sequence-specific base pairing in the 3′ untranslated regions of the target messenger RNA. Recent work has provided new insights into the role of miRNAs in various biological events, including aging and cancer. Studies have demonstrated there are distinct miRNA expression patterns associated with various tumor sites and that cancer tissues appear to have miRNA expression profiles that are distinct from normal tissues. Human cancers commonly exhibit an altered expression profile of miRNAs with either oncogenic (e.g., miR-21) or tumor-suppressive (e.g., let-7) activity. Very few compounds, cancer drugs included, which affect cell growth and/or differentiation, have been shown to affect miRNA expression. Thus, the evidence that folate, retinoids, and curcumin modulate miRNA expression in the models discussed above are quite exciting. Although the miRNA field has grown exponentially in the last decade, much remains to be discovered, especially with respect to their role in carcinogenesis, as well as potential modification by bioactive dietary components. Issues remain about the quantity of dietary components needed to bring about a biological effect, the timing of exposure, and other variables that can influence the response. Importantly, for the future of personalized nutrition, miRNAs may be useful as biomarkers of cancer prevention, early disease, or nutritional status, as well as serve as potential molecular targets that are modulated by dietary interventions.

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