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Commentary

MicroRNAs in cancer management and their modulation by dietary agents

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ABSTRACT

MicroRNAs (miRNAs) represent a class of small (21–23 nucleotides) non-coding RNAs that emerged as key post-transcriptional gene regulators, implicated in numerous physiological and pathological processes. Currently, a main focus of miRNA research is related to the roles of miRNAs in cancer development. The biogenesis and modes of action of miRNAs have not been completely elucidated; however, miRNA-mediated translational repression is involved in the regulation of almost every cellular process. Thus, pathological alterations in miRNA expression signatures are commonly associated with disease development. This review specifically focuses on miRNAs in cancer, with an emphasis on their use as potential biomarkers for cancer diagnosis and prognosis. Then, we discuss the potential use of synthetic antisense or miRNA mimetic oligonucleotides and dietary agents to modulate miRNA expression for chemotherapy and chemoprevention of cancer, respectively.

1. Introduction

The concept of post-transcriptional regulation of gene expression by antisense RNAs was introduced in 1993 when Lee et al. published the first report of a small RNA (lin-4) with antisense complementarity to the 3′UTR of lin-14 m RNA; importantly, lin-4 displayed translational inhibition potential in *Caenorhabditis elegans* [1]. The micro RNA (miRNA) era began with the detection of the small non-coding RNA let-7. Currently, the Sanger database miRbase 17.0 (http://www.mirbase.org) contains 1424 mature human miRNA sequences that may regulate at least one-third of all human protein-coding genes [2].

Abbreviations: ACTR1A, ARP1 actin-related protein 1 homolog A centractin alpha; AGTR1, angiotensin II receptor type 1; AICDA, activation-induced cytidine deaminase; AKT, v-akt murine thymoma viral oncogene homolog; ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; APAF1, apoptotic peptidase activating factor 1; APL, acute promyelocytic leukemia; ARHI, age-related hearing impairment; ASO, antisense oligonucleotides; ATRA, all-trans retinoic acid; BACH1, BTB and CNC homology basic leucine zipper transcription factor; BAK1, BCL2-antagonist/killer 1; BCL2, B-cell CLL/lymphoma 2; BIM, BCL2-interacting mediator of cell death; CAGR, cancer-associated genomic region; CCN, cyclin; CDC2, Cell division control protein 2 homolog; CDC25A, cell division cycle 25A; CDK, cyclin-dependent kinase; CDKN, CDK inhibitor; CEBPB, CCAAT/enhancer binding protein (C/EBP) beta; CLL, chronic lymphoid leukemia; c-Kit, transcription factor; CREB, cAMP responsive element binding protein; DGCR8, DiGeorge syndrome critical region 8; DIM, 3,3′-diindolylmethane; DNMT, DNA methyltransferase; E2F, E2F transcription factor; EGCG, epigallocatechin gallate; EGFR, epidermal growth factor receptor; ERBB/HER, epidermal growth factor receptor class avian erythroblastosis oncogene B; ERK, extracellular signal-regulated kinase; ESF1, ESF1 nucleolar pre-rRNA processing protein homolog; ESR1, estrogen receptor 1; ETS1, v-ets erythroblastosis virus E26 oncogene homolog 1; Fas, tumor necrosis factor receptor superfamily member 6; FDA, food and drug administration; FOX, forkhead box; HAT, histone acetyltransferase; HDAC, histone deacetylase; HMGA2, high mobility group AT-hook 2; HOX, homeobox; I3C, indole-3-carbinol; IGF1R, insulin-like growth factor 1 receptor; IGF2, insulin-like growth factor 1; IL6R, interleukin 6 receptor; IRS1, insulin receptor substrate 1; JUN, jun proto-oncogene; KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; LATS2, LATS large tumor suppressor homolog 2; LDOC1, leucine zipper downregulated in cancer; MAFK, v-maf musculoaponeuritic fibrosarcoma oncogene homolog K; MATR3, matrin 3; MCL1, myeloid cell leukemia sequence 1; MCM2, minichromosome maintenance complex component 2; MET, met proto-oncogene tyrosine kinase; miRNA, microRNA; mirtron, pri-miRNA-containing intron; MNT, MAX binding protein; MSH2, mutS homolog 2; mTOR, mammalian target of rapamycin; MYOD, myogenic differentiation; NFIB, nuclear factor I/B; NF-κB, nuclear factor kappa B; NRAS, neuroblastoma RAS viral (v-ras) oncogene homolog; PACT, protein kinase R activating protein; PDCD4, programmed cell death 4; PDCD6IP, programed cell death 6 interacting protein; PML-RARα, promyelocytic-retinoic receptor alpha; PRIM1, primase DNA polypeptide 1; PTEN, phosphatase and tensin homolog; RAS, proto-oncogene; RAB, member of the RAS oncogene family; RAR, retinoic acid receptor; RARE, retinoic acid response elements; Rho, rhodopsin; RISC, RNA-induced silencing complex; RNA Pol, RNA polymerase; RXR, retinoid X receptor; SHIP1, SH2 domain-containing inositol phosphatase 1; SNAI2, snail homolog 2 (Slug); SNP, single nucleotide polymorphism; SOCS, suppressor of cytokine signaling; SP1, specificity protein 1; TCL1, T-cell leukemia/lymphoma 1A; TGFBR, transforming growth factor beta receptor; TIMP3, Tissue inhibitor of metalloproteinases 3; TM6SF1, transmembrane 6 superfamily member 1; TP, tumor protein; TP53INP1, TP53 inducible nuclear protein 1; TPM1, tropomyosin 1; TRBP (TARBP), trans-activator RNA binding protein; TSG, tumor suppressor gene; UTR, untranslated region; VIM, vimentin; WT1, Wilms tumor 1; ZBTB10, zinc finger and BTB domain containing 10; ZEB, zinc finger E-box binding homeobox.

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miRNAs are a family of 19- to 24-nucleotide non-protein-coding RNAs that post-transcriptionally regulate mRNA function. miRNAs are involved in many fine-tuned biological processes; however, miRNA genes and the mechanisms by which miRNAs are processed are hotspots for pathological aberrations. Analyses of the patterns of these alterations reveal promising cancer biomarkers as well as therapeutic targets, such as synthetic antisense oligonucleotides or miRNA mimetic molecules. Since studies show that dietary agents have an influence on miRNA expression patterns, the cancer-preventing potential of these compounds will be discussed.

2. Biogenesis and *modus operandi* of microRNAs

Genes coding for miRNAs are located either in intergenic regions or in defined transcription units. Approximately 50% of the miRNA genes are found in introns or exons of both protein-coding and long non-coding transcripts and are consequently co-transcribed with their host gene.

Although miRNAs located in Alu repeats are transcribed by RNA polymerase (RNA Pol) III, miRNA genes are usually transcribed by RNA Pol II into polycistronic primary transcripts (pri-miRNAs) with lengths of approximately 1–10 kb (Fig. 1). Pri-miRNAs are characterized by a 5′-methyl cap structure, a poly(A) tail at the 3′ end and at least one hairpin structure of approximately 70 nucleotides. In the canonical miRNA pathway, a complex consisting of the double-stranded RNA-specific endoribonuclease III Drosha, the binding protein Pasha and the DiGeorge syndrome critical region 8 protein (DGCR8) processes pri-miRNAs into 70- to 100-nucleotide pre-miRNAs. In addition to a stem-loop structure, pre-miRNAs bear 3′-dinucleotide overhangs. An alternative mechanism of pri-miRNA processing occurs through the mirtron pathway. In this pathway, pre-miRNA structures are generated from pri-miRNA-containing introns (mirtrons) by the nuclear splicing machinery. The exportin-5/Ran-GTPase heterocomplex transports pre-miRNA from the nucleus to the cytoplasm, where it undergoes further maturation. The pre-miRNA is subsequently processed by Dicer III into a 19- to 24-nucleotide double-stranded

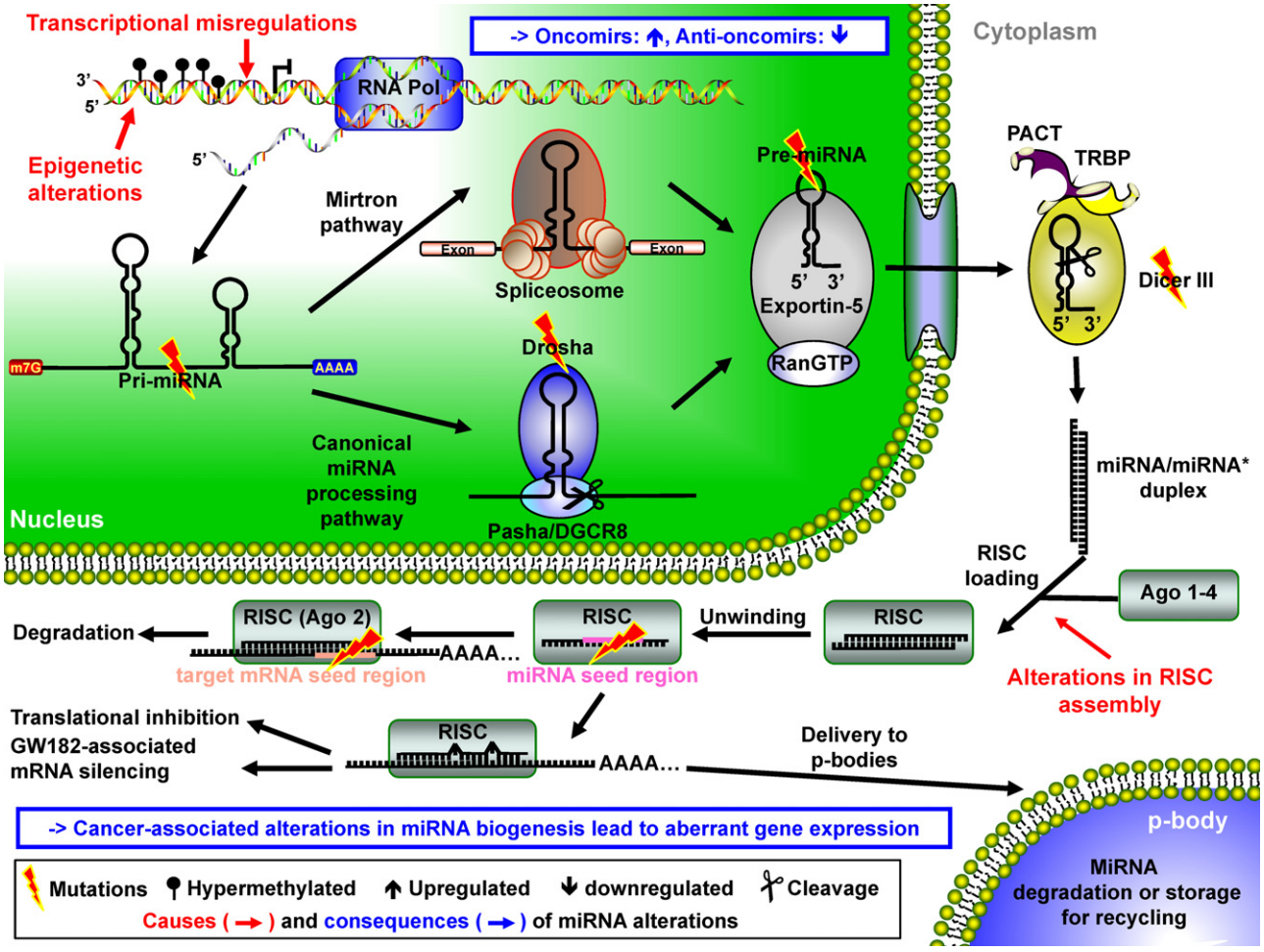

Fig. 1. miRNA biogenesis and cancer-associated alterations in microRNA pathways. First, a microRNA gene is transcribed into a primary miRNA (pri-miRNA) by an RNA polymerase (RNA Pol). miRNA genes are usually transcribed by RNA Pol II; however, miRNAs located in Alu repeats are transcribed by RNA Pol III. The pri-miRNA is further modified through either canonical processing or the alternative mirtron pathways into a pre-miRNA. In the next step, the pre-miRNA is exported from the nucleus by exportin-5 in a RanGTP-dependent mechanism into the cytoplasm, where it is cleaved by Dicer III. The resulting miRNA/miRNA* duplex is then loaded into the RISC complex, where it is unwound. The remaining single-stranded miRNA interacts with the target mRNA. In general, perfect complementarity between miRNA and mRNA sequences and the presence of the Ago2 endonuclease leads to mRNA degradation. Remarkably, incomplete complementarity can either induce deadenylation and degradation or lead to GW182-associated translational inhibition (*e.g.*, by preventing circularization or inhibiting the initiation of translation). Moreover, RISC complexes with captured target mRNAs can be delivered to parking bodies, where mRNA is degraded or stored for recycling. In cancer cells, mutations can occur in intermediate stages of miRNA (*i.e.*, pri- and pre-miRNAs), in the mature miRNA seed region, in the target mRNA sequence or in miRNA-processing proteins. Moreover, alterations in the expression pattern of miRNA-regulating transcription factors, abrogation of RISC assembly and aberrations in epigenetic mechanisms involved in the regulation of the expression of miRNA genes can enhance oncomir expression and repress tumor suppressor miRNA expression. Consequently, alterations in the miRNA biogenesis process lead to aberrant gene expression. DGCR8, DiGeorge syndrome critical region 8; PACT, protein kinase R activating protein; RISC, RNA-induced silencing complex; RNA Pol, RNA polymerase; TRBP, trans-activator RNA binding protein.

miRNA/miRNA* (*, passenger strand) duplex with 3′-dinucleotide overhangs. In human cells, Dicer interacts with the trans-activator RNA binding protein (TRBP) and the protein kinase R (PKR) activating protein (PACT) [2].

miRNAs are unable to silence their target genes alone (Fig. 1). Rather, mature miRNAs require assembly into the multi-protein effector RNA-induced silencing complex (RISC). The essential core components of the RISC are members of the Argonaute (Ago) protein family (Ago 1–4). In general, Ago proteins contain two conserved RNA binding domains: a PAZ domain that binds the single-stranded 3′ end of miRNAs and a PIWI domain that structurally resembles ribonuclease H and that interacts with the phosphorylated 5′ end of the miRNA guide strand [3]. Noteworthy, the slicer protein Ago 2 is the only family member with endonuclease activity [4].

RISC assembly is initialized by the ATP-dependent incorporation of the miRNA/miRNA* duplex into the Ago complex (RISC loading). Subsequently, the miRNA duplex is unwound, and the miRNA* passenger strand is discarded from the RISC complex through either an Ago 2 slicer-dependent mechanism or slicer-independent unwinding [2]. The remaining mature single-stranded miRNA determines the specificity of the RISC complex for its target mRNA by interacting with the 3′-untranslated region (UTR) of the transcript. RISC target recognition is primarily determined by base pairing of nucleotides in the 'seed' region and is enhanced by additional interactions in the middle of the 3′ region [5].

How miRNAs induce translational repression or accelerate mRNA turnover remains an ongoing debate. Perfect or near perfect (no internal mismatches or bulges) complementarity between a miRNA and the targeted 3′-UTR and the presence of the endonuclease Ago 2 in the RISC complex are prerequisites for specific cleavage of target mRNA [6]. The resulting mRNA fragments are degraded through the normal mRNA turnover pathway. Alternatively, imperfect miRNA/mRNA complementarity and the interaction between the RISC and the RNA binding protein GW182 either prevent the mRNA circularization associated with translational inhibition or induce mRNA degradation *via* the normal decay pathway, in which deadenylation leads to decapping and exonuclease cleavage of the mRNA [7]. RISC-mediated mRNA repression may also interfere with the cap binding of eIF4E or inhibit the late translation initiation step, resulting in translational inhibition [8]. Moreover, the RISC complex has been postulated to act on post-initiation steps by reducing the elongation rate of the ribosomal machinery or triggering the proteolysis of the newly synthesized peptide [2]. Finally, RISC complexes with captured target mRNAs are found in processing or parking bodies (p-bodies), where mRNAs either undergo degradation or are temporarily stored for later recycling [9].

In conclusion, miRNA-mediated inhibition of protein synthesis allows a high degree of flexibility in translational activity, the ability to immediately respond to changes and prevents the expression of high levels of potentially harmful proteins (*i.e.*, proteins involved in apoptosis), and gene expression imbalances. Taken together, these properties allow fine tuning of biological processes including development, tissue differentiation, cell metabolism, cell cycle regulation, apoptosis, senescence and cell migration [10–12]. Thus, short non-coding RNAs provide "canalization" for the development of particular cell types *via* a strictly determined pathway.

3. Aberrant miRNA expression and carcinogenesis

The link between miRNAs and cancer pathogenesis has emerged from the discovery that genes encoding miRNAs are frequently located in cancer-associated genomic regions (CAGR) [13]. Indeed, approximately 50% of all annotated human miRNA genes are located in amplification or chromosomal rearrangement hotspots, common breakpoint regions in or near oncogenes, tumor suppressor genes (TSGs) or fragile sites. As miRNAs are frequently expressed as polycistronic transcripts, deregulation of one member of the cluster is accompanied by deregulation of the other cluster members. An increasing number of miRNAs have been reported to be dysregulated in various cancers (Table 1). Herein, we discuss the most thoroughly studied miRNA alterations associated with carcinogenesis.

Remarkably, Calin et al. reported for the first time the abnormal expression of the miR-15a/miR-16-1 cluster in B-cell chronic lymphocytic leukemia (CLL). Both miRNAs are located at chromosomal position 13q14.3, which is frequently deleted in CLL, lymphomas and prostate cancer [14,15]. The anti-apoptotic B-cell lymphoma (BCL) 2 gene is an identified target of miR-16-1, and the downregulation of the mir-15a/miR-16-1 cluster increases BCL2 expression, which is associated with cell survival and the promotion of carcinogenesis (Table 1). Additional genes that affect cell growth, cell cycle, development, tumor suppression and apoptosis, such CDC2, ETS1, JUN, and MCL-1, were reported to be modulated by this cluster [16]. The overexpression of the miR-15a/miR-16-1 cluster in the chronic myeloid leukemia MEG-01 cell line inhibits the growth of MEG-01 tumor engraftments in nude mice, underlining the major tumor suppression function of these miRNAs [17].

miRNA expression patterns vary between healthy and pathological tissues as well as among different cancer types. miRNAs are generally downregulated in cancer cells; however, increased expression of a few cancer type-specific miRNA genes is associated with carcinogenesis [13,18–20]. Therefore, miRNome-wide alterations appear more likely to be involved in carcinogenesis than changes in a single miRNA gene that regulates one oncogene or TSG.

miRNAs of the oncomir subclass are mostly responsible for the regulation of genes with tumor suppressor functions and are overexpressed in cancer cells. For example, the polycistronic cluster miR-17–92 ('oncomir-1′) is often overexpressed in lymphomas and CLL cells, thus inhibiting the expression of the proapoptotic gene BCL2-interacting mediator of cell death (BIM) as well as the TSG phosphatase and tensin homolog (PTEN), leading to enhanced cell survival and proliferation. In addition, co-expression of the miR-17–92 cluster leads to c-MYC-induced tumor development [21]. miR-155, another commonly deregulated oncogenic miRNA, is normally involved in B-cell development and regulation of inflammation [22]. Costinean et al. report that the restricted ectopic expression of miR-155 in B-cells results in a transition into polyclonal pro-B-cell leukemia, showing that this single miRNA is sufficient for malignant transformation. The overexpression of miR-155 is not limited to leukemia cells and has also been identified in Hodgkin's, large B-cell and Burkitt's lymphoma, as well as in breast and lung cancer [23]. Kaposi's sarcoma-associated herpes virus- or Epstein-Barr virus-specific orthologs of miR-155 are expressed in lymphoma and leukemia cells and may thus contribute to neoplasia [24,25]. Validated targets of miR-155 include the tumor protein 53 inducible nuclear protein (TP53INP) 1 gene, which is a double-strand break-mediated inducer of apoptosis, and the TSG suppressor of cytokine signaling (SOCS) 1 [26,27]. Interestingly, Skalsky et al. reported that miR-155 regulates the expression of two transcription factors, LDOC1 (leucine zipper, downregulated in cancer 1) and BACH1 (BTB and CNC homology 1, basic leucine zipper transcription factor 1), which are implicated in the transcriptional regulation of NF-κB (nuclear factor-kappa B) and MAFK (v-maf musculoaponeurotic fibrosarcoma oncogene homolog K), respectively [24].

Interestingly, numerous studies describe the upregulation of miR-21 expression in various cancer types (*e.g.*, pancreatic, colon, breast, prostate, lung, stomach and squamous cell cancer) [20,28].

Table 1
List of miRNA genes/clusters with oncogenic or tumor suppressor functions in various tumor types.

Deregulation	Name/cluster	Cancer type	Targets
–	Let-7a-2	Breast, colon, lung, ovary, stomach	KRAS, NRAS, CDK6, CDC25A, HMGA2, c-MYC
–	miR-9-3	Breast	TP53
–	miR-15a/miR-16-1	Leukemia (CLL), lung, prostate	BCL2, CCND1, CCND2, CCNE1 CDC2, ETS1, JUN, MCL1, MSH2, PDCD6IP, RAB6B, WT1, RAB21, ACTR1A, PRIM1
–	miR-16	Liver	BCL2
–	miR-23a/b	Lymphoma, prostate	c-MYC
–	miR-26a	Liver	CCND2, CCNE2
–	miR-29b-1-miR-29a, miR-29b-2-miR-29c	Bile ducts, breast, leukemia (AML, CLL), liver, lung, lymphoma	MCL1, CDK6, TCL1, DNMT1, DNMT3
–	miR-31	Breast	RhoA
–	miR-34a, miR-34b, miR-34c	Bladder, breast, cervix, colon, kidney, leukemia, lung	CCNE2, CCND1 TP53, CDK4, CDK6, MET, MYC, CREB, E2F3, BCL2
–	miR-98	Breast	E2F2, c-MYC
–	miR-101	Liver	MCL1
–	miR-122	Liver	CCNG1, TP53
–	miR-125B	Breast, prostate	BAK1, ERBB, LIN28
–	miR-128	Brain	E2F3a
–	miR-143	Colon, rectum	KRAS
–	miR-145	Breast, colon, lung, prostate	IRS1, c-MYC, EGFR, IGF1R
–	miR-195	Liver	CCND1, E2F3
–	miR-200, miR-200b	Pancreas, prostate	ZEB1, ZEB2
–	miR-204	Bile ducts	BCL2, MCL1
–	miR-205	Breast	HER3
–	miR-320	Bile ducts	BCL2, MCL1
–	miR-330	Prostate	ESF1
–	miR-331	Prostate	HER2/neu
–	miR-512-5p	Stomach	MCL1
+	lin-28	Ovary	IGF2
+	miR-10b	Brain	RhoC
+	miR-17–92	Brain, breast, colon, leukemia, lymphoma, lung, stomach	BIM, PTEN, CDKN1A, N-MYC
+	miR-18a	Liver	ESR1
+	miR-21	Breast, colon, esophagus, lung, pancreas, prostate, skin, stomach	PTEN, TPM1, EGFR, PDCD4, BCL2, TIMP3, CDK6, CDKN1A, FAS, IL6R, SOCS5, APAF1, NFIB
+	miR-22	Breast	ESR1
+	miR-25	Stomach	CDKN1C
+	miR-27a	Breast	FOXO1
+	miR-92	Leukemia	TP63
+	miR-93	Stomach	CDKN1A
+	miR-96	Breast	FOXO1
+	miR-106b-25, miR-106b	Esophagus, leukemia stomach	CDKN1A, TP73
+	miR-155	Breast, colon, leukemia (CLL, AML), lymphoma (Burkitt's and Hodgkin's), lung, prostate	BACH1, LDOC1, SOCS1, TP53INP1, CEBPB, MATR3, TM6SF1, AGTR1, AICDA, SHIP1
+	miR-182	Breast, melanocytes	FOXO1, FOXO3
+	miR-210	Breast, melanocytes	MNT
+	miR-221	Stomach	CDKN1B, CDKN1C
+	miR-372, miR-373	Breast, testis	LATS2

–, downregulated; +, upregulated; ACTR1A, ARP1 actin-related protein 1 homolog A centractin alpha; AGTR1, angiotensin II receptor type 1; AICDA, activation-induced cytidine deaminase; AML, acute myeloid leukemia; APAF1, apoptotic peptidase activating factor 1; BACH1, BTB and CNC homology, basic leucine zipper transcription factor; BAK1, BCL2-antagonist/killer 1; BCL2, B-cell CLL/lymphoma 2; BIM, BCL2-interacting mediator of cell death; CCN, cyclin; CDC2, cell division control protein 2 homolog; CDC25A, cell division cycle 25A; CDK, cyclin-dependent kinase; CDKN, CDK inhibitor; CEBPB, CCAAT/enhancer binding protein (C/EBP) beta; CLL, chronic lymphoid leukemia; CREB, cAMP responsive element binding protein; DNMT, DNA methyltransferase; E2F, E2F transcription factor; EGFR, epidermal growth factor receptor; ERBB/HER, epidermal growth factor receptor class avian erythroblastosis oncogene B; ESF1, ESF1 nucleolar pre-rRNA processing protein homolog; ESR1, estrogen receptor 1; ETS1, v-ets erythroblastosis virus E26 oncogene homolog 1; Fas, tumor necrosis factor receptor superfamily member 6; FOX, forkhead box; HMGA2, high mobility group AT-hook 2; IGF1R, insulin-like growth factor 1 receptor; IGF2, insulin-like growth factor 1; IL6R, interleukin 6 receptor; IRS1, insulin receptor substrate 1; JUN, jun proto-oncogene; KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; LATS2, LATS large tumor suppressor homolog 2; LDOC1, leucine zipper; downregulated in cancer; MATR3, matrin 3; MCL1, myeloid cell leukemia sequence 1; MET, met proto-oncogene tyrosine kinase; MNT, MAX binding protein; MSH2, mutS homolog 2; NFIB, nuclear factor I/B; NRAS, neuroblastoma RAS viral (v-ras) oncogene homolog; PDCD6IP, programmed cell death 6 interacting protein; PRIM1, primase DNA polypeptide 1; PTEN, phosphatase and tensin homolog; RAB, member of the RAS oncogene family; Rho, rhodopsin; SHIP1, SH2 domain-containing inositol phosphatase 1; SOCS, suppressor of cytokine signalling; TCL1, T-cell leukemia/lymphoma 1A; TIMP3, Tissue inhibitor of metalloproteinases 3; TM6SF1, transmembrane 6 superfamily member 1; TP, tumor protein; TP53INP1, TP53 inducible nuclear protein 1; TPM1, tropomyosin 1; WT1, Wilms tumor 1; ZEB, zinc finger E-box binding homeobox.

Accordingly, conditional miR-21 overexpression in mice leads to a pre-B malignant lymphoid-like phenotype whereas miR-21 repression induces apoptosis and tumor regression [29]. Indeed, overexpression of miR-21 causes the repression of the TSG PTEN, leading to phosphoinositide-3 kinase (PI3K) upregulation, which in turn promotes the v-akt murine thymoma viral oncogene homolog (AKT)/mammalian target of rapamycin (mTOR) pathway and cell proliferation [26,30].

miRNAs that have a protective and tumor suppressive role, referred to as anti-oncomirs, are commonly downregulated in cancer cells. Interestingly, the most well-known tumor suppressor miRNAs are the abovementioned miR-15a and miR-16-1, which are involved in regulating the expression of approximately 14% of all human genes. Moreover, miR-125b is consistently downregulated in breast and prostate cancer and likely acts as an oncosuppressor miRNA in normal cells [31]. miR-125b targets the

epidermal growth factor receptor family member and oncogene avian erythroblastosis oncogene B (ERBB), confirming its role in tumor suppression. Ectopic overexpression of miR-125a/b in ERBB-dependent breast cancer cell lines reduces ERBB expression, leading to the inhibition of extracellular signal-regulated kinase (ERK) 1/2 and AKT phosphorylation. Consequently, these breast cancer cells show reduced anchorage-dependent growth, cell mobility and invasion potential [32]. In contrast, silencing miR-125a/b induces the phosphorylation of ERK1/2 and AKT, thus activating the mTOR pathway and enhancing cell survival. miR-125a/b homologs target LIN28 mRNA, acting as a translational enhancer for insulin growth factor (IGF) 2, myogenic differentiation (MYOD) 1 and ARBP/36B4 ribosomal protein mRNA [33]. Indeed, LIN28 facilitates the transformation of cancer cells, and its overexpression is associated with disease progression of various tumor types. The pro-apoptotic gene BCL2-antagonist/killer (BAK) 1 was confirmed to be a target of miR-125b, further emphasizing the role of this miRNA in cancer development [34]. Ectopic overexpression of miR-145 in colon cancer cells leads to post-transcriptional downregulation of insulin receptor substrate (IRS) 1. This gene encodes a docking protein for insulin-like growth factor receptor and the insulin receptor and triggers mitogenic, anti-apoptotic and anti-differentiation signals [35]. In contrast, miR-145 is downregulated in breast and lung cancer and deleted in prostate cancer [28]. PI3K/AKT and p53 pathways, two of the main players in carcinogenesis, regulate the expression of miR-145, which is involved in the posttranscriptional regulation of the proto-oncogene c-MYC [36]. Markedly, some miRNAs can play either oncogenic or tumor suppressive roles in the context of different cell types and gene expression patterns [37].

Similar to protein-coding genes, miRNA functions are also influenced by point mutations (Fig. 1); however, site-restricted errors in the sequence of mature miRNA seed regions seem to be rare [37]. In contrast, point mutations in the 3′-UTR region of the miRNA target mRNA may be responsible for reductions in or loss of target specificity or may affect miRNA target recognition sensitivity, leading to aberrant miRNA-mediated mRNA repression. For example, a point mutation in the 3′-UTR binding site of SOCS-1 negatively influences its miR-155-mediated repression in breast cancer cells [27]. Single nucleotide polymorphisms (SNPs) or chromosomal alterations in miRNA target sites may affect miRNA/mRNA interactions, impairing post-transcriptional gene regulation. For example, various cancer types are positive for a chromosomal rearrangement in the high mobility group AT-hook (HMGA) 2 locus that separates the open reading frame from the 3′UTR. As a consequence, HMGA2 escapes from let-7 miRNA-associated repression, is overexpressed and promotes cancer development [38]. Point mutations could also affect RISC complex assembly and compromise miRNA-mediated mRNA silencing [2]. Furthermore, tumor-specific mutations in miRNA sequences may influence precursor and mature miRNA stability or play a role in regulating miRNA expression levels [39]. Imbalances in the expression pattern of miRNA-regulating transcription factors may incorrectly induce transcription of pri-miRNAs involved in well-established tumor suppressive or oncogenic pathways [40]. For example, the oncogenic transcription factor c-MYC and the tumor suppressor TP53 regulate the expression of the oncogenic miR-17–92 cluster and miR-34a, respectively [41,42].

Approximately half of all known human miRNA genes are associated with a CpG island (Fig. 1). Consequently, aberrant DNA methylation-associated epigenetic silencing may also affect the miRNA network [19]. The miRNA-203 locus is known to be methylated more frequently in T-cell lymphoma than in normal T lymphocytes [43]. DNA hypermethylation of miR-9-1, miR-124a and miR-127 is often detected in breast, colorectal and bladder cancer, respectively [44–46].

Finally, impairments in the miRNA processing steps could cause cancer-specific changes in miRNA expression patterns (Fig. 1). Indeed, Dicer or Drosha expression levels are frequently altered in numerous cancers [47,48]. In addition, the RISC-loading complex trans-activation-responsive RNA-binding protein (TARBP) 2 is frequently mutated, leading to Dicer destabilization and attenuation of miRNA processing [37]. Similarly, the interaction of Drosha with the oncogenic ALL1 fusion protein leads to Drosha dysfunction, which in turn affects pri-miRNA selection and processing [49].

In conclusion, the expression of miRNAs is often deregulated in cancer cells, with numerous miRNAs being overexpressed in one type of cancer and downregulated in another. For example, miR-205 is upregulated in lung, bladder and pancreatic cancers. In contrast, it is significantly downregulated in prostate cancer and esophageal squamous cell carcinoma [37]. These observations reveal that it is not possible to generalize cancer-associated miRNA. Nonetheless, cancer-specific miRNA expression signatures may prove useful as a diagnostic and therapeutic tool.

4. MicroRNAs as cancer biomarkers for diagnostics, prognostics and predictive pharmacodynamics

Molecular cancer diagnosis is no longer limited to karyotyping and analysis of chromosomal copy numbers or structure alterations. The increasing knowledge in the field of carcinogenesis now allows the early detection of malignant cells at the genomic (*i.e.*, SNP risk allele analysis), transcriptomic (*i.e.*, multi-gene expression test) and proteomic (*i.e.*, molecular imaging) levels. Accordingly, the analysis of reversible epimutations such as transcriptional silencing of TSGs by promoter hypermethylation or monitoring of miRNA expression signatures that are associated with tumorigenesis could be highly informative tools for cancer management.

In general, cancer cells are less differentiated and have lower miRNA expression levels than normal differentiated cells; this is especially true for blood cancer cells [50]. Genome-wide miRNA expression profiling allows the identification of cell-specific changes in miRNA signatures. For example, in CLL patient cells, miR-213 and miR-220 are downregulated whereas miR-190 and miR-133 are upregulated compared with healthy samples [51]. miR-331, miR-29a, miR-195, miR-34a and miR-29c are highly expressed in CLL cells. In acute lymphocytic leukemia (ALL), enhanced expression of miR128b, miR-204, miR-218, miR-331 and miR-181b-1 has been reported [52]. miR-128a is overexpressed in ALL when compared with AML whereas miR-223 and let-7b are downregulated [53]. These exemplary results emphasize that even within individual malignancies, differential lineage-specific miRNA expression profiles could be used as a highly accurate tool to distinguish subtypes with different mechanisms of tumorigenesis [18].

Interestingly, short non-coding RNAs are highly resistant to degradation, fast and simple to extract from fresh cells and even retroactively from FFPE-fixed samples. Highly stable circulating miRNAs are detected in a cell-free form in blood serum or plasma, and miRNA release patterns are specific to the cancer state [54].

Taken together, these characteristics indicate that miRNAs represent highly attractive non-invasive and high-throughput putative biomarkers for cancer identification. For example, mapping of 217 miRNAs allowed the classification of 12 of 17 poorly differentiated and histologically indistinguishable tumors of unknown origin whereas an expression analysis of 16,000 mRNAs failed to accurately identify the same tumors [18]. The central role of miRNAs in development and gene regulation associated with cancer-specific expression signatures has opened up great opportunities in cancer diagnosis and prognosis. Nevertheless, there are significant discrepancies among reported miRNA signatures. These variations may arise from patient-specific clinicopathological

characteristics (*e.g.*, genomic alterations) or simply from the heterogeneity of the neoplasm as a result of contamination with stromal cells or surrounding tissue [55]. A major focus will be the development of miRNA biomarker signatures that are specific for each cancer type and accurately reflect the abovementioned variables.

Although chemotherapy is often used to treat cancer, malignant cells frequently develop drug resistance, leading to treatment failure. Interestingly, miRNA expression signatures have been linked to several clinicopathological variables such as tumor stage, receptor status, patient survival, disease recurrence, and treatment resistance. According to the personalized medicine model, miRNA-associated molecular taxonomy could thus help to predict the likelihood of patients developing resistance against a particular treatment. For example, a breast cancer study revealed that miR-451 and mir-27 are both implicated in the development of doxorubicin resistance [55]. Another publication showed that the overexpression of miR-125b in breast cancer cells is responsible for paclitaxel resistance [34]. Therefore, the analysis of miRNAs that affect drug sensitivity represents an important and potentially fruitful area of investigation for the clinical management of cancer treatment and to provide a mechanistic understanding of the factors that contribute to drug resistance.

5. Therapeutic approaches in miRNA targeting

Because miRNAs influence the expression of numerous genes and thus finely tune critical points in disease pathways, restoration of native miRNA expression signatures is a promising therapeutic goal that could either be used as a direct anti-cancer treatment or as part of a combination therapy that increases the sensitivity of tumor cells to traditional chemotherapeutics.

Chemically modified antisense oligonucleotides (ASO), also called anti-microRNAs, are widely used to repress overexpressed miRNAs (*e.g.*, oncomirs). These single-stranded ASO RNA or DNA molecules have a sequence complementary to the endogenous target miRNA and can carry chemical modifications on their backbone (*e.g.*, phosphorothioate), 2′-sugar modifications (*e.g.*, 2′-O-methyl) or changes in nucleotide linkages (*e.g.*, phosphorothioates) [56]. Chemical modifications of ASOs are essential to reduce nuclease degradation, enhance target affinity, activate RNase H, attenuate toxicity, promote protein binding, improve aqueous solubility and thus *in vivo* delivery, and delay plasma clearance [57]. ASOs are, in theory, able to target miRNAs and interfere with several steps of their production, processing and function. Although ASO-mediated degradation of intermediary pri- or pre-miRNA may be feasible, it is often less successful or simply impossible because of spatial or structural restraints. The most straightforward and apparently most effective ASOs are complementary to the mature miRNA [57].

Intracellular delivery of exogenous therapeutic RNA or DNA molecules to their target remains a great challenge. *Ex vivo*, cells are transfected with synthetic ASOs through the use of cationic lipids, electroporation or chemical modifications such as cholesterol conjugation or locked nucleic acid; however, for clinical application in cancer therapy, delivery is more problematic because the target malignant cells are dispersed throughout the entire body. Since one miRNA has multiple targets, the effects of ASO-mediated miRNA repression must be evaluated. Multiple strategies to increase cell-specific delivery, such as cross-linking of ASOs with cholesterol, glycans, peptides or folate, which allows binding to cell surfaces, are under examination. Alternatively, ASOs could be enclosed in or on nanoparticles or liposomes or fused with structured pieces of RNA (aptamers) that bind cell receptors. Promising results have been achieved by intravenous or local administration of chimeric ASOs (*e.g.*, to the lung by inhalation) [58]; however, phagocytic immune cells (*e.g.*, macrophages and monocytes) complicate the systemic delivery of miRNAs by removing RNA from the bloodstream. In addition, heavily vascularized and highly metabolic organs such as the liver are more receptive to ASOs in liposomes or nanoparticles [57].

Antagomirs were the first miRNA inhibitors demonstrated to work in mammals. This class of ASOs with a 2′-O-methyl-modified ribose sugar, terminal phosphorothioates and cholesterol group at the 3′-end likely target the mature miRNA [59]. Administration by intravenous injection inhibited target miRNA expression in several organs; however, because antagomirs require high doses to be effective, they probably induce off-target effects and toxicity. Therefore, the clinical use of these ASOs is highly unlikely. ASOs containing locked nucleic acid (LNA) chemistry appear at the moment to be the most promising approach to repress miRNAs. LNAs are bicyclic high-affinity RNA analogues that contain conformationally locked nucleotide monomers with methylene bridges connecting the 2′-oxygen and 4′-carbon atoms of the ribose ring. Accordingly, a miR-122 inhibitor using LNAs is in clinical phase II trials for anti-cancer and anti-inflammatory treatments [60].

The use of microRNA decoys or sponges is another strategy to repress oncogenic miRNAs. In this strategy, high copy number vectors stably express transcripts that carry microRNA target sites. Decoy transcripts saturate the endogenous miRNAs that share a common seed region, leading to the upregulation of natural target genes of the oncogenic miRNA. In addition to these artificial miRNA sponges, the first evidence for naturally occurring miRNA decoys in plants has been published [61]. Viral vectors can be used to deliver sponge transgenes; however, this system carries substantial risks for insertional mutagenesis. Alternatively, the use of non-integrating adeno-associated viruses as well as the availability of several serotypes and development of self-complementary genomes could allow more efficient tissue targeting and cell transduction, respectively [56]. The first decoy consisted of an adenoviral vector with two sites for the muscle-specific miR-133 inserted in the 3′-UTR of a green fluorescent protein reporter gene [62]. Remarkably, sponges offer several advantages compared with ASOs, such as the potential to simultaneously repress several miRNAs using a vector coding for multiple miRNA target sequences; however, further investigations are needed to determine the potential clinical applications of sponges as anti-cancer therapies.

As mentioned above, the global expression of miRNAs is generally reduced in cancer cells; this is particularly true of miRNAs with tumor suppressor functions. Similar to the idea of gene therapy, in which missing or aberrant genes are substituted by exogenous DNA sequences, miRNA replacement strategies offer the potential to restore miRNA expression and function through the use of miRNA mimics. This therapeutic approach would affect many pathways simultaneously, likely reversing the carcinogenic cell state and achieving a clinical benefit. In contrast to protein-coding genes, synthetic miRNA mimics are much smaller, already active, and operate in the cytoplasm. Therefore, they can potentially be delivered systemically. Finally, the fact that one miRNA can regulate up to 1000 mRNAs and therefore modulate various pathways further enhances the therapeutic potential of miRNA mimics. Because miRNA mimetic RNA molecules have the same sequence and target the same mRNAs, in cancer cells, they behave like the endogenously repressed miRNAs. Consequently, off-target effects are rather unlikely [63]. For example, cell culture assays show that overexpression of miR-34a in cancer cells induces cell cycle arrest, apoptosis and senescence. Accordingly, systemic delivery of synthetic miR-34a in a lipid-containing formulation leads to accumulation of miR-34a in lung tumors in mice, repression of miR-34a target genes and inhibition of tumor growth [63].

The question of miRNA mimic toxicity for normal cells remains a topic of ongoing debate. Theoretically, exogenous miRNAs could overload the RISC, alter the expression patterns of endogenous miRNAs and thus reduce the viability of normal cells or promote oncogenesis; however, this toxicity was never observed *in vivo*, suggesting that miRNA delivery to normal tissue is well tolerated. The molecular bases for this tolerance are still unclear, and hypotheses are purely speculative. It is assumed that normal cells, which are not addicted to oncogenic pathways, can recover from the treatment. Another possibility is that in contrast to cancer cells, normal cells can likely regulate the presence of miRNA mimics through an unknown mechanism. Moreover, miRNA mimics restore pathways in cancer cells that are already functional in normal cells [63]. Nevertheless, it is of primary importance to avoid the introduction of miRNA mimics with cancer promoting effects such as those observed with miR-182 mimics, which promote metastasis in melanoma [64]. As an alternative to miRNA analogues, miRNA expression could be restored through the use of vector constructs that overexpress a particular miRNA. Viral vector constructs with either constitutively active or tissue-specific inducible promoters allow selective miRNA overexpression [65]. Despite these promising results *in vitro* and *in vivo*, the concept of miRNA replacement as a tumor suppressor agent requires further investigation.

Taken together, these data demonstrate that many approaches for systemic delivery of artificial exogenous miRNAs, which is necessary for either miRNA silencing or miRNA restoration, are under investigation (*i.e.*, nanoparticles). Nevertheless, sponge- and vector-mediated miRNA replacement technologies offer more advantages for their use in scientific research whereas the use of small RNA or DNA molecules for miRNA repression or replacement is more promising from the perspective of therapeutic miRNAs. In conclusion, solving the delivery problem is of major interest for the clinical application of miRNA in disease treatment. Alternatively, reversal of methylation-associated miRNA silencing by DNA demethylating agents such as 5-aza-2′-deoxycytidine could restore native miRNA expression patterns. This compound has also been shown to induce differentiation, senescence, autophagy and apoptosis [66]. Given the involvement of miRNAs in all of these biological processes, these effects may be mediated by restoring miRNA expression through DNA demethylation. The bioavailability, therapeutic benefits, toxicity and side effects of systemic delivery of miRNA analogues or DNA demethylation-induced miRNA expression must be carefully evaluated. Since no RNA interference-based drugs have been approved until now by the Food and Drug Administration (FDA) for sale, the use of miRNA analogues is limited to research applications and may never go beyond research.

6. Impact of natural compounds on miRNA expression

In 2007, Newman and Cragg updated their 2003 publication in which they summarized the newly discovered anti-cancer drugs of the last few decades. They cited approximately 155 FDA-approved anti-cancer agents, of which approximately 34% were of natural origin or directly derived from products of natural origin [67]. Many of these anti-cancer drugs have an impact on cell cycle or cell proliferation and/or induce cell death pathways. Because of the high regulatory potential and the important role of miRNA alterations in carcinogenesis, it is of key interest to identify natural compounds that affect miRNA expression and evaluate their anti-cancer and cancer prevention activities. Accordingly, the impact of natural compounds on miRNA expression could increase the sensitivity of cancer cells to conventional chemotherapeutic agents and thereby improve cancer treatment. In this section, we summarize experimental evidence demonstrating the effect of dietary agents on miRNA modulation, which may contribute to their chemopreventive potential (Table 2).

6.1. Curcumin

The multi-target drug curcumin (diferuloylmethane) is extracted from the rhizome of *Curcuma longa*. Its strong therapeutic anti-carcinogenic potential has received much attention, and curcumin is currently being tested in clinical studies for colorectal, rectal and pancreatic cancer and for multiple myeloma, adenocarcinoma and osteosarcoma [68]. In addition, curcumin is a negative regulator of inflammation, detoxification and metastasis

Table 2
Effects of dietary compounds on miRNA expression patterns in cancer cells.

Compound	Affected miRNAs (and validated target genes)	Comments
ATRA	↑ let-7 family, −10a (*HOXB1, HOXB3*), −15a, −15b, −16-1, −34a, −107 (*CDKN6*), −125b, −223, −342 ↓ −181b	
Butyrate	↓ −17, −17–92, −18b–106a, −20a, −25–106b, −93, −106a, −106b (*CDKN1A*)	
Curcumin	↑ −15a (*BCL2*), −16 (*BCL2*), −103, −22 (*SP1, ESR1*), −27a, −181b, −181d, −186*, −81a ↓ −7, −15b, −25, −26a, −92, −93, −98–140, 146b, −148a, −195, −196a, −199*, −204, −374, −510	Inhibition of cell proliferation and induction of apoptosis
Curcumin analog	↓ −21 (*PTEN*), −200 (*PTEN*)	
DIM	↑ −200 (*ZEB1*, *SNAI2*, *VIM*)	
EGCG	↑ let-7 family (*RAS*), −20a (*E2F*, *TGFBR2*), −16, −221 (*c-Kit*), −330 ↓ −21, −125b (*TGFBR3*)	Induction of cell death
Genistein	↑ −1296 (*MCM2*) ↓ −27a (*ZBTB10*), −221 (*ARHI*), −222 (*ARHI*)	Inhibition of cell colony formation and invasion
I3C	↑ −21, −31, −34a (*TP53*), −103a, −146b, −337 ↓ −1, −143	
Resveratrol	↑ −1, −30c-1, −146b-5p, −194-2, −206, −323, −340, −363*-5p, −494, −497, −506, −560, −572, −574, −615, −622, −638, −639, −659, −663 (*AP1*), −801 ↓−16-1, −17, −17–92, −21 (*PTEN*, *PDCD4*), −23b, −25, −26a, −29c, −30a-3p, −30d, −30e-5p, −92a-2, −100-1/2, −102, −103-2, −146a, −181a2, −196a1, −205, −424, −594, −629, −631, −657	

↑, upregulated; ↓, downregulated; ARHI, age-related hearing impairment; ATRA, all-trans retinoic acid; BCL2, B-cell CLL/lymphoma 2; CDKN6, cyclin dependent kinase 6; c-Kit, transcription factor; DIM, 3,3′-diindolylmethane; EGCG, epigallocatechin gallate; ESR1, estrogen receptor 1; HOX, homeobox; I3C, indole-3-carbinol; MCM2, minichromosome maintenance complex component 2; PDCD4, programmed cell death 4; PTEN, phosphatase and tensin homolog; RAS, proto-oncogene; SNAI2, snail homolog 2 (Slug); SP1, specificity protein 1; TGFBR, transforming growth factor beta receptor; TP53, tumor protein 53; VIM, vimentin; WT1, Wilms tumor 1; ZBTB10, zinc finger and BTB domain containing 10; ZEB1, zinc finger E-box binding homeobox 1.

pathways [69,70]. In contrast, curcumin induces CDKN2A and TP53 gene expression, leading to cell cycle arrest and apoptosis and autophagy, respectively [70,71].

An initial study by Sun et al. in 2008 reported the effect of curcumin on miRNA expression signatures in pancreatic cancer cells [72]. Curcumin treatment induces downregulation of 18 miRNAs (*e.g.*, miR-199*) and upregulation of 11 miRNAs, including miR-22, which targets specificity protein (SP) 1 and estrogen receptor (ESR) 1 translation. A study of the effect of curcumin on miRNA expression patterns in lung cancer cells reports the upregulation of four miRNAs and the downregulation of two miRNAs (*i.e.*, miR-186* and miR-136). Interestingly, by studying the effects of curcumin on miRNA expression, Zhang et al. revealed another miRNA-mediated pro-apoptotic mechanism: they reported that curcumin represses the expression of miR-186*, which is involved in the pathogenesis of the multi-drug-resistant lung cancer cell line A549/DDP. Curcumin is thought to both inhibit lung cancer cell proliferation and induce apoptosis through the regulation of various specific miRNAs; for example, curcumin downregulates the potential oncomir miR-186* [73]. Moreover, curcumin induces apoptosis in MCF-7 cells by upregulating the expression of miR-15a and miR-16, leading to the downregulation of the anti-apoptotic BCL2 gene, which is often overexpressed in cancer cells [74].

How curcumin influences miRNA expression remains speculative; however, curcumin is known to bind to DNA methyltransferase (DNMT) 1 and to block histone deacetylases (HDACs) and histone acetyltransferases (HATs), thus promoting DNA demethylation and histone acetylation and deacetylation, respectively [75]. Consequently, curcumin likely induces DNA demethylation and histone acetylation, thus activating the expression of various epigenetically-silenced miRNAs.

Although curcumin has a high *in vitro* activity, a low bioavailability drastically limits its effects *in vivo.* Therefore, new strategies for curcumin delivery to and in malignant cells need to be established. Accordingly, the use of a synthetic curcumin analogue (CDF-difluorinated-curcumin) represses miR-200 and miR-21 expression, leading to induction of PTEN expression in pancreatic cancer cells [76].

6.2. All-trans retinoic acid

The vitamin A metabolite all-trans retinoic acid (ATRA) plays an important role in HOX gene-mediated axis determination during embryogenesis [77]. The presence of ATRA induces dimerization of retinoic acid receptor (RAR) and retinoid X receptor (RXR). The resulting heterodimer (RAR:RXR) subsequently binds to DNA in regions called retinoic acid response elements (RAREs) and transactivates genes involved in growth and differentiation [78]. ATRA (Tretinoin, Vesanoid®) is given in combination with chemotherapy to patients suffering from acute promyelocytic leukemia (APL) characterized by the promyelocytic-retinoic receptor alpha (PML-RARα) fusion gene, which interferes with cell differentiation and blocks blood cell maturation.Profiling of miRNA expression in ATRA-treated APL cell lines revealed the upregulation of miR-15a, miR-15b, miR-16-1 miR-223, miR-342, miR-107 and several let-7 family members whereas miR-181b was downregulated. Most of these miRNAs have validated targets that are involved in hematopoietic differentiation and apoptosis [79]. miR-107 targets the cell cycle regulator cyclin-dependent kinase 6 in pancreatic cancer cells, leading to inhibition of cell proliferation [80]. Moreover, ATRA treatment induces miR-34a expression, causing cell growth arrest and differentiation of neuroblastoma cells [81]. *In vitro* induction of the differentiation of the malignant embryonal cancer cell line Tera-2 by retinoic acid led to the upregulation of miR-125b and let-7 miRNAs. miR-125b is mostly absent in breast cancer cell lines, potentially explaining the resistance of breast cancer cells to differentiation [28]. Although ATRA mostly induces tumor suppressor miRNAs, it also promotes the expression of oncomirs. Indeed, treatment of pancreatic cancer cells with ATRA promotes metastatic and invasion behavior by inducing the expression of miR-10a, which mediates repression of HOXB1 and HOXB3 genes, which are important for normal development [82].

6.3. Epigallocatechin gallate

The polyphenol epigallocatechin gallate (EGCG) is the most abundant polyphenol in green tea (*Camellia sinensis*). This catechin exerts profound biochemical and pharmaceutical activities, including anti-oxidative, anti-inflammatory and anti-tumor properties, by inducing cell cycle arrest and apoptosis [83]. Thus, green tea has been proposed as a preventive treatment for various cancer types (*e.g.*, breast, prostate, and colon) [84].

Ahn et al. reported in 2010 that treatment of the hepatocellular cancer cell line HepG2 with EGCG leads to the downregulation of miR-125b expression and concomitantly to the upregulation of genes involved in cell proliferation (*e.g.*, transforming growth factor, beta receptor (TGFBR) 3) [83]. Another study reports EGCG-induced upregulation of miR-16 in hepatocellular carcinoma HepG2 cells, which triggers the downregulation of the cell survival gene BCL2, leading to cell death. The same study shows the induction of both the let-7 family, which suppresses the expression of the proto-oncogene RAS, the miR-20a-targeting E2F1 transcription factor and TGFBR2, and miR-221, which inhibits c-Kit [85]. In prostate cancer xenograft tissue from EGCG-treated nude mice, miR-21 expression is downregulated whereas miR-330 expression is upregulated. miR-330 functions as an oncomir by inducing apoptosis in prostate cancer cells [86].

miRNA expression can be affected by epigenetic changes, such as CpG island hypermethylation. Considering that EGCG is commonly reported to function as a DNA demethylating agent, aberrant methylation-associated silencing of the anti-oncomir mir-127, which targets the proto-oncogene BCL6, could be reversed by EGCG treatment [46].

6.4. Genistein

The isoflavone genistein, which is isolated from the soybean, has been found to be a potent antitumor agent through its modulation of estrogen receptor binding in targeted tissues. Genistein influences the miRNA signatures of cancer cells. Indeed, genistein decreases miR-221 and miR-222 overexpression in the PC3 prostate cancer cell line, leading to the upregulation of the expression of the tumor suppressor age-related hearing impairment (ARHI) and the inhibition of cell proliferation, colony formation and invasion [87]. Genistein treatment of human uveal melanoma suppresses miR-27a expression, inducing the expression of the putative Sp transcription factor repressor ZBTB10 and growth inhibition [88]. Finally, genistein upregulates miR-1296 expression, leading to the repression of minichromosome maintenance complex component (MCM) 2. Consistent with the role of this gene in the formation of replication foci and the recruitment of replication-related proteins, the inhibition of MCM2 induces cell cycle arrest [89].

6.5. Resveratrol

Resveratrol (trans-3,5,4′-trihydroxystilbene) is another polyphenolic non-flavonoid bioactive compound that can be derived from plants and fruits, such as blueberries, peanuts or red grapes. *In vitro* analyses show that resveratrol possesses anti-tumor

properties mediated in part through the activation of the Fas/CD95 signaling pathway, which leads to cell death induction. Because of its potential to affect NF-κB signaling, resveratrol also negatively modulates inflammatory pathways. This natural compound was reported to activate the NAD-dependent histone deacetylase sirtuin 1 and inhibits the Wnt pathway; however, low *in vivo* bioavailability restricts its application to tumors that allow direct contact with the compound (*e.g.*, skin and gastrointestinal cancer). Resveratrol is also in pre-clinical studies for human cancer prevention [90].

In addition to the long list of targets of resveratrol, which could explain its chemopreventive properties, resveratrol modulates the miRNA expression signatures of cancer cells. For example, Tili et al. show that resveratrol upregulates the expression of miR-663, which targets multiple genes involved in the immune response. Accordingly, resveratrol decreases JunB and JunC levels and AP-1 activity and impairs the expression of the oncomir miR-155 [91]. Resveratrol mediates the upregulation of 22 miRNAs (*e.g.*, miR-146-5p and miR-1) and the inhibition of 26 miRNAs, including the oncomirs miR-17, miR-21, miR-25, miR-92a-2 and the miR-17–92 cluster, in the colon adenocarcinoma cell line SW480 [90]. Interestingly, mir-21 targets transcripts encoding key regulators of cell proliferation and apoptosis such as PTEN and PDCD4 [92].

6.6. Indoles

Cruciferous vegetables such as broccoli and cabbage are recognized as foods with cancer preventive properties. One of the compounds isolated from these vegetables is indole-3-carbinol (I3C), which is catabolized in the acidic environment of the stomach into a mixture of biologically active compounds. The major form of this mixture is the dimeric product 3,3′-diindolylmethane (DIM), which has been examined for chemoprotective properties in various cancers [5]. The I3C anticarcinogenic phytochemical alters estrogen metabolism and induces G1 growth arrest and apoptosis in human prostate cancer cell lines [93]. The compound I3C is the subject of on-going research regarding its potential antioxidant effects.

Melkamu et al. show that chemically-induced deregulation of miRNA expression during mouse lung carcinogenesis (*i.e.*, upregulation of miR-21, miR-31, miR-130a, miR-146 and miR-377) can be prevented by I3C [94]. Furthermore, cigarette smoke-induced alterations in lung tissue miRNA expression patterns and the consequences of these changes are partially reversed by I3C; for example, the ability of p53 to target miR-34a is restored [95]. DIM treatment of pancreatic cancer cell lines induces reexpression of miR-200, thus reducing the expression of the ZEB1, slug and vimentin genes, which are involved in drug resistance [96]. The repression of miR-21 and induction of its target genes, which are involved in the regulation of cell survival, may be responsible for the chemopreventive activity of I3C.

6.7. Butyrate

The fatty acid butyrate is one of the metabolic end products of unabsorbed dietary fibers that have been bacterially fermented in the gut; alternatively, butyrate can be obtained directly from butter. Butyrate is known to induce cell cycle arrest, differentiation and apoptosis in numerous cancer models [97,98].

Butyrate plays a major role in the regulation of miRNAs. Indeed, Hu et al. reported significant changes in the expression of 44 miRNAs (*e.g.*, miR-20b, miR-93 and miR-106b) in HCT-116 cells in response to butyrate treatment, including members of the oncogenic miR-17–92, miR-18b-106a and miR-25-106b clusters. A comparative analysis of tumor and normal tissues shows that miRNAs affected by butyrate are mainly overexpressed in cancer cells as compared with normal cells. One of these miRNAs, miR-106b, targets the cell cycle regulator CDKN1A [99]. Taken together, these data suggest that the butyrate-induced biological effects mentioned above are at least partially linked to the modulation of the expression of miRNAs involved in the regulation of cell cycle (*i.e.*, miR-106b).

6.8. Daily nutrition

Besides purified natural compounds, daily nutrition can have an influence on miRNA expression patterns. Vitamins such as A, D, E and B as well as selenium and fatty acids have a regulatory effect on miRNAs implicated in the regulation of tumor suppressor- and cancer-associated pathways [5]. Davis et al. reported that folate/methyl-deficient diets could induce hepatocellular carcinoma in rats as a result of aberrant expression of miR-34a, miR-16a, miR-181a and miR-127, which target tumor suppressor genes and oncogenes involved in maintaining the balance between apoptosis and cell proliferation (*e.g.*, E2F3 and Bcl-6). Interestingly, culturing lymphoblastoid cells under folate-deficient conditions causes global increases in miRNA expression (*e.g.*, miR-222), and culturing cells in folate-containing medium reverses this aberrant miRNA expression pattern [100]. Folate plays an essential role in the remethylation of homocysteine to methionine, which is converted back to *S*-adenosyl methionine (SAM), completing the SAM cycle. Considering that SAM is the methyl group donor for DNMT-mediated DNA methylation and that folate-deficiency leads to SAM depletion, these observations suggest that DNA demethylation is responsible for the induction of epigenetically-silenced miRNAs.

7. Conclusions

miRNAs exert pleiotropic effects on cell physiology by regulating the transcript levels of numerous genes. Therefore, miRNA dysregulation plays a role in carcinogenesis by promoting cell growth, invasion and metastasis and by inhibiting differentiation and apoptosis. Dysregulated miRNA expression is also implicated in drug resistance. From a clinical standpoint, the analysis of cancer type-specific miRNA signatures holds great promise as a tool for identifying biomarkers for accurate, non-invasive and early cancer detection as well as for prognosis and prediction of treatment response. However, most of the published miRNA expression studies examined the expression of only a few miRNAs in a small, clinicopathologically homogeneous tumor dataset. Hence, further studies are necessary to validate particular miRNA candidates and to extend these analyses to large, prospective and genome-wide miRNome studies. Because miRNAs ensure fine-tuning of mRNA translation, the miRNA system is highly sensitive to individual genomic variables and environmental changes such as infections. These biological variations must be elucidated and considered in the evaluation of miRNA expression patterns to prevent the identification of false-positives. To apply miRNA signatures as potential markers for cancer evaluation, it is critical to elucidate miRNA functions and to determine the functional consequences of cancer type-specific differences in miRNA expression. Further investigation of the causes and consequences of miRNA dysregulation will be valuable in enhancing the understanding of cancer pathogenesis; these studies will likely lead to the discovery of novel molecular targets for the development of new anti-cancer therapies. From this perspective, miRNAs are an emerging therapeutic target for synthetic agents and chemopreventive target for dietary agents; however, the clinical application of ASO or miRNA decoys for cancer treatment remains limited, as studies have focused on the fundamental understanding of miRNA expression patterns in cancer. Further studies focusing on target delivery and selectivity

are necessary to design efficient therapies with minimal off-target effects and minimal effects on normal tissues, respectively. Alternatively, dietary agents dramatically affect miRNA expression and have promising anti-cancer and chemopreventive effects; however, most of the studies of dietary agents are highly descriptive and only report the effects of these agents on miRNA expression levels. Mechanistic studies of the consequences of treatment with naturally occurring compounds would provide new insights into the advantages and disadvantages of this approach. Further difficulties include issues such as bioavailability, selective targeting and generation of the appropriate active metabolite; these difficulties are not miRNA-specific. To solve these problems, the scientific community has tried to imitate natural compounds by generating synthetic compounds with enhanced bioavailability or encapsulating compounds in nanoparticles or liposomes. Despite these challenges, an increasing understanding of the critical aspects involved in targeting miRNAs is emerging. Given the potential of dietary agents, it is not unreasonable to propose the development of individualized medicine approaches to cancer management involving additional miRNA-targeting anti-cancer therapies or chemopreventive treatments using dietary agents.

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