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Regulating miRNA by Natural Agents as a New Strategy for Cancer Treatment

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Abstract

MicroRNAs (miRNAs) are small single-strand non-coding endogenous RNAs that regulate gene expression by multiple mechanisms. Recent evidence suggests that miRNAs are critically involved in the pathogenesis, evolution, and progression of cancer. The miRNAs are also crucial for the regulation of cancer stem cells (CSCs). In addition, miRNAs are known to control the processes of Epithelial-to-Mesenchymal Transition (EMT) of cancer cells. This evidence suggests that miRNAs could serve as targets in cancer treatment, and as such manipulating miRNAs could be useful for the killing CSCs or reversal of EMT phenotype of cancer cells. Hence, targeting miRNAs, which are deregulated in cancer, could be a promising strategy for cancer therapy. Recently, the regulation of miRNAs by natural, nontoxic chemopreventive agents including curcumin, resveratrol, isoflavones, (–)-epigallocatechin-3-gallate (EGCG), lycopene, 3,3'-diindolylmethane (DIM), and indole-3-carbinol (I3C) has been described. Therefore, natural agents could inhibit cancer progression, increase drug sensitivity, reverse EMT, and prevent metastasis through modulation of miRNAs, which will provide a newer therapeutic approach for cancer treatment especially when combined with conventional therapeutics.

Keywords

Cancer stem cell; epithelial to mesenchymal transition; microRNA; natural agent

1. INTRODUCTION

MicroRNAs (miRNAs) are small single-stranded non-coding endogenous RNAs that are known to regulate gene expression by multiple mechanisms. These tiny molecules comprising of only 19–25 nucleotides (~22nt) in length control post-transcriptional regulation of genes [1, 2]. Although few of them were first described in 1993, recently many of them have been identified. Furthermore, the insights into the basic functional abilities of more important miRNAs have been revealed. They are active in humans, animals, and plants [1]. It is known that miRNAs are significantly involved in the control of not only normal

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

biological activities but pathological activities as well [3]. Recent evidence suggests that miRNAs control the pathogenesis, evolution and progression of cancer. Additionally, these molecules are critical in the regulation of cancer stem cells (CSCs) making them potential targets for overcoming drug resistance. The miRNAs are also proposed to assist in the progression of EMT (epithelial to mesenchymal transition) of cancer cells, which is implicated in cancer metastasis. This evidence suggests that miRNAs could serve as targets to treat cancers by eradicating CSCs or by reversal of EMT phenotype [4].

Recently, targeting miRNAs by natural nontoxic agents has been described [4]. Therefore, natural agents could inhibit cancer progression, increase drug sensitivity, cause reversal of EMT, and prevent metastasis through the modulation of miRNAs, which provides a newer therapeutic approach for cancer treatment especially when combined with conventional therapies [5–9].

2. REGULATION OF GENE EXPRESSION BY miRNAs

From the report of the first miRNA, let-4, we were able to gain new insights into the mechanism of action of this and other novel RNAs. The miRNAs act by binding to 3'-untranslated region (UTR) of messenger RNAs (mRNAs). Initially, transcripts complementary to 3'-UTR of mRNA were found to be capable of governing translation via a RNA-RNA interaction [1]. The RNA-RNA interaction results in the inhibition of protein translation or degradation of target mRNA, leading to decreased levels of target proteins. Similar to other genes, miRNA genes are also located in DNA strands [10]. Under the modulation of RNA polymerase, miRNA genes are initially transcribed to produce primary miRNA (pri-miRNA). The pri-miRNA contains more than hundreds of base pair of nucleotides [10, 11]. By modulation of Drosha, pri-miRNA is cleaved and becomes precursor miRNA (pre-miRNA). The pre-miRNA consists of ~70 nucleotides [12–17]. The pre-miRNA is then transported to cytoplasm and dissociated to single strand [11, 18, 19]. The single strand, which has complementary sequence, functionally binds to 3'UTR of target mRNA. The mature miRNA combines with RISC (RNA-induced silencing complex) which directs the miRNA binding to 3'UTR of target mRNA [11, 18, 19]. In this way, miRNAs regulate gene expression through targeting mRNA cleavage or translational inhibition [4].

3. THE ROLE OF miRNAs IN CANCER

The ability of the miRNA to influence development and differentiation allows for their potential in the development and progression of cancer [3, 4, 20]. Furthermore, it has been established that the miRNA undergoes several alterations in their levels in cancers, indicating the role of miRNA in cancer progression and metastasis [21]. The role of the miRNA may differ depending upon their levels. Some miRNAs may have oncogenic potential while others may act as tumor suppressor genes thereby controlling the biologic manifestations of cancers (Fig. 1). The miRNAs are also known to control the differentiation of physiologic embryonic stem cells [22–24]. Importantly, they also regulate CSC and EMT-phenotypic cells, implicating their role in drug resistance and cancer metastasis [22, 25–29].

3.1. The miRNAs with an Oncogenic Potential

Several miRNAs have been proposed to have a critical role as oncogenes. These include miR-21. The expression level of this miRNA is much higher in various cancers compared to normal cells [30–35]. Several studies have suggested that miR-21 is oncogenic because the miR-21 has an anti-apoptotic role in human cancer cells. Inhibition of this miRNA leads to increased apoptosis through activation of caspases [32]. Moreover, the inhibition of miR-21 suppressed cell proliferation, decreased the expression of Bcl-2, and increased apoptotic cell

death [33]. These phenomena have been found in various cancers [34–37]. By mechanistic studies, several targets of miR-21 have been identified and confirmed. The targets of miR-21 are tumor suppressor such as *Pdcd4* [38], NF- κ B inhibitor such as *LRRFIP1*, or bone morphogenetic protein receptor II (*BMPRII*) [3, 20, 39, 40] and of course many other targets.

The miR-17-92 cluster is another miRNA showing oncogenic activity in various cancers [41–43]. This class of miRNA includes miR-17, miR-20a, miR-19b-1, miR-18a, miR-19a, and miR-92-1. Animal studies have shown that the introduction of miR-17-92 cluster and c-myc accelerated the growth of tumor [41]. Further studies have demonstrated that c-myc could increase the expression of miR-17-92 cluster, leading to the accelerated angiogenesis and tumor development [44]. Moreover, another member of myc family, N-myc, could also increase the expression of miR-17-92 and induce the development of medulloblastomas through activation of sonic hedgehog signaling in cerebellar neural precursors [45, 46]. All of these findings demonstrate the oncogenic nature of miR-17-92 cluster.

Another well-known oncogenic miRNA is miR-155. The high expression of miR-155 has been found in different types of cancers [30, 47–50]. The role of miR-155 has been shown to be especially significant in pancreatic cancers. Elevated miR-155 expression has been found to be significantly correlated with low overall survival. The patients with high expression of miR-155 had much higher risk of death caused by pancreatic cancers [49]. In addition, high expression of miR-155 also predicts early development of pancreatic neo-plasia [50]. The molecular experiments have been conducted in pancreatic cancer to identify the target of miR-155. It was found that *TP53INP1* (p53-dependent damage-inducible nuclear protein 1) is an important target of miR-155 and that the expression of *TP53INP1* is significantly increased in pancreatic cancer [51]. Ongoing research in the field suggests that more miRNAs with oncogenic function may be unearthed in human malignancies.

3.2. The miRNAs Functioning as Tumor Suppressors

Some miRNAs have anti-cancer activity against cancers [3]. For example, let-7 family including let-7, miR-48, miR-84, and miR-241 is one of tumor suppressive family of miRNAs [4]. The expression of let-7 family is significantly lower in various cancers compared to normal tissues. The low expression of let-7 has been used as markers in the detection and prognosis of cancers [52–55]. In an *in vitro* and *in vivo* study on lung cancer, a significantly lower expression of let-7 has been found in lung cancer tissues and cell lines [53]. Clinical data showed that lower expression of let-7 was associated with shorter survival of patients diagnosed with lung cancer after surgery. Molecular experiments have demonstrated that forced expression of let-7 in lung cancer cells caused significant reduction of cancer cell colony formation [53], suggesting that let-7 is a tumor suppressive miRNA. The targets of let-7 include *Ras* [56] and *HMGA2* [54]. This is based on the fact that in the 3'UTR of *Ras* mRNA, there are several let-7 binding sites. Additionally, forced expression of let-7 in cancer cells decreased the expression of *Ras* while transfection of anti-sense let-7 into cancer cells decreased the level of let-7 and up-regulated the expression of *RAS* level [56].

The miR-15 and miR-16 also show their anti-cancer activity in cancers. In an *in vitro* study, forced expression of miR-16 significantly inhibited the growth of several prostate cancer cell lines [57]. The tumor suppressor effect of miR-16 is likely mediated through regulation of its targets, *CDK1* and *CDK2* [57]. CDKs are well-known molecules which promote cell cycle progression and cell proliferation. In CLL, anti-cancer effect of miR-15 and miR-16 has been suggested to be mediated through apoptotic signaling [57, 58]. Experimental studies have shown that the levels of miR-15 and miR-16 in CLL cells were low while the *bcl-2* expression was up-regulated. Forced expression of miR-15 and miR-16 resulted in an

inhibition of bcl-2 expression, leading to the apoptotic cell death [59]. The tumor suppressor activity of miR-15/miR-16 is also shown in prostate cancer cells [60]. Here the miR-15/miR-16 acts through inhibition of cyclin D1 and WNT3A, which promote cell survival, proliferation and invasion [60].

Another proposed tumor suppressor miRNA is the miR-34. This miRNA is directly stimulated and transactivated by p53 signaling [58]. Therefore, miR-34 is critically involved in p53 regulated cellular signaling [58, 61]. It has also been found that miR-34 inhibits pancreatic CSCs and restores tumor suppressive activity of p53 in pancreatic cancer [25], indicating the anti-tumor ability of miR-34. In addition, forced expression of miR-34a increased apoptotic cell death, which was found to be induced by the effects of miR-34a on the regulation of genes controlling cell proliferation, apoptotic cell death, and angiogenesis [58]. Studies from our laboratory has shown that miR-34a could target androgen receptor (AR) and that the level of expression of miR-34a is lower in prostate cancer tissue specimens compared to normal prostate epithelium [62]. We have also shown that the loss of miR-34a expression was in part due to promoter methylation of miR-34a gene [62]. However, it is worth to note that although the expression of miR-34 is down-regulated in most types of cancers, the reported levels of miR34 in renal cancer cells are controversial [63, 64], which need further investigation.

4. THE miRNAs AS TARGETS FOR CANCER THERAPY

Given the functions of miRNAs in the control of initiation, progression and metastasis of cancer, these molecules appear to be novel targets for cancer treatment. Recent literature suggests that targeting miRNAs may be a promising approach for cancer therapy. The proposed mechanisms of achieving this goal include altering the expression of miRNAs to sensitize cancer cells to chemotherapeutic drug and thereby enhance anti-cancer activity [3, 4]. Since we are aware of the tumor oncogenic and tumor suppressive effects of the miRNAs, down-regulation of oncogenic miRNAs and up-regulation of tumor suppressive miRNAs would specifically target the altered miRNAs and their target genes, which will lead to the restoration of drug sensitivity. Therefore, silencing oncogenic miRNAs represents a promising approach for cancer therapy. Chemically modified antisense oligonucleotides called antagomirs showed inhibitory effects on oncogenic miRNAs [65–67]. These antagomirs would assist in cancer therapy; however, much more in-depth investigations are warranted.

Anti-miR-21 oligonucleotides have been used to inhibit the activity of miR-21 [66]. Results showed that the down-regulation of endogenous miR-21 is specific, efficient and long-lasting. An animal study was also conducted to evaluate the effects of antagomirs by intravenous injection of antagomirs against miR-16, miR-122, miR-192 and miR-194. The results showed that the injection of these antagomirs led to a reduction in the levels of specific miRNAs in animal tissues tested [65]. This also affected the downstream genes that are impacted by the anti-sense oligonucleotide administration [68]. By administration of antagomirs which target oncogenic mRNAs, the inhibition of cancers were also observed [69], suggesting that this approach is efficient and have promise for the treatment of human malignancies.

Another mechanism of targeting miRNAs for cancer treatment is by introduction of tumor suppressive miRNAs. It was found that transfection of let-7 significantly inhibited proliferation of various cancer cells [6, 52]. Importantly, introduction of let-7 and miR-200 also reduced the resistance of cancer cells to chemotherapy and radiotherapy [6, 70]. These strategies hold great promise as novel approaches for cancer treatment by manipulation of miRNAs. In our recent study, we have shown that the administration of pre-miR-200

through tail-vein in mouse model of breast cancer lung metastasis resulted in a significant decrease of lung metastasis of breast cancer cells [71], suggesting that restoration of the expression of miRNAs that are lost in cancer could become a viable approach for the treatment of cancer.

5. REGULATION OF miRNA BY NATURAL AGENTS

As documented above that because miRNAs are critically involved in the regulation of cancer progression, EMT, CSC survival and proliferation, the regulation of deregulated miRNAs could be a promising approach for cancer therapy. Down-regulation of highly expressed miRNAs or re-expression of silenced miRNAs in cancer cells may lead to overcome chemotherapeutic resistance, and expected to inhibit cancer cell proliferation, invasion, and metastasis. To that end, “Natural agents” are very promising especially because they are non-toxic agents in humans. Use of these agents to normalize the expression level of miRNAs appears noteworthy as new opportunities for effective cure of cancers (Fig. 1). The natural agents, for example, curcumin, resveratrol, isoflavone, EGCG, I3C, and DIM could regulate miRNAs and decrease cancer cell resistance to conventional agents, leading to the elimination of CSCs and EMT phenotypic cells which are known to be resistant cells to conventional therapeutics [4].

By conducting *in vivo* animal studies, diets with natural agents in animals were found to regulate the expression levels of miRNAs [72]. In addition, the targets of specific miRNAs that mechanistically impact the signal transduction was found to be altered by natural agents, which have been investigated using miRNA expression analysis by miRNA array [72]. Importantly, cancer cells harbor multiple alterations impacting critical cell signaling pathways and due to this complexity a single therapeutic regimen targeting only one molecule often fails. This has been the story for even the extremely specific inhibitors or gene therapies in cancer treatment. Whereas natural agents could be extremely beneficial in this regard since they target multiple cellular signaling pathways which could be a good attributes of natural agents. Hence their impact on cancer treatment would be more efficacious. This is one of the reasons why several clinical trials are being conducted to evaluate the clinical utility of natural agents for the treatment of human cancer.

Recent studies emphasize the synthesis of natural agent analogs based on the structure-activity assay. The liposome or nanoparticle formulations of natural agents also received much attention in anti-cancer therapy [73–75]. Combining the available cancer chemotherapeutic regimens with natural agents with known chemopreventive effects would be able to target miRNAs and other downstream signaling pathways to overcome tumor resistance and metastasis. This indeed could become a promising approach in cancer therapy in the future due in part by inhibition of cancer progression and complete elimination of cancer cells (especially CSCs and EMT phenotypic cells) that are highly resistant to conventional therapeutics. In the following section we will summarize the results of selected natural agents in cancer therapy through targeting miRNAs.

5.1. Curcumin and miRNA Regulation

Curcumin is derived from turmeric with antioxidant and anti-inflammatory properties and is under investigation for its role in cancer chemoprevention [76, 77]. It has anti-proliferative and pro-apoptotic activities in part through the regulation of cell cycle. Curcumin acts by regulating genes that are involved in cell signaling pathways. Curcumin also regulates miRNAs to control the expression of their target genes. For example, it has been demonstrated that in human pancreatic cells, curcumin treatment led to the up-regulation of miR-22 expression and down-regulated miR-199a, and that miR-22 acted through its target genes SP1 and ESR1[9]; however, there are limitations on the use of curcumin because of its

poor bioavailability. While initial studies have been promising, more research is needed to further explore the role of curcumin in cancer progression especially those cancers that are mediated through deregulated expression of miRNAs.

5.2. Resveratrol and miRNA Regulation

Resveratrol is a natural phytoalexin derived from several plants e.g. grapes, mulberries, and peanuts. It has anticancer effects which are in part mediated by growth inhibition of cancer cells and by induction of cell death via apoptosis [78, 79]. Recent studies have emphasized their role in the expression of miRNAs. It has been found that CAY10512, a synthetic resveratrol analog, could alter the level of miR-146a [8]. High expression of miR-146a has been found in the brain of patients with Alzheimer. Further study showed that complement factor H (CFH) is one of many targets of miR-146a and that CFH is an inhibitor of the inflammation in the brain. Importantly, it was found that the inhibition of miR-146a and restoration of CFH expression could be achieved through treatment with CAY10512 or transfection with miRNA-146a antagomir in stressed human neural cells [8]. These studies suggest that resveratrol could have a role in altering physiological behavior of cells through regulation of miRNA expression and thereby altering cellular signal transduction.

5.3. Isoflavones and miRNAs

Other natural compounds include soy Isoflavones which are derived primarily from soybean. These include genistein, daidzein and glycitein. Genistein inhibits proliferation, invasion and metastasis of cancer cells [80–82]. It has been postulated that it acts through regulation of miRNAs. Our group has demonstrated the role of isoflavones in pancreatic cancer which was found to be mediated through the deregulation of miRNAs [6]. Pancreatic cancer aggressiveness is mediated by miRNAs via acquisition of the EMT phenotype, which also leads to intrinsic and extrinsic drug resistance. The levels of miR-200 and let-7 families were found to be reduced in gemcitabine-resistant pancreatic cancer cells with EMT phenotype. Additionally, it was found that upon treatment with isoflavones or upon transfection with pre-miR-200, the expression of miR-200 was increased as expected and consequently the expression of vimentin was decreased and these results were consistent with reversal of EMT phenotype. Isoflavones also caused increased expression of let-7 and thereby inhibited cancer cell growth [6]. These limited studies however indicate a potential role of isoflavones in cancer treatment, which could in part be due to deregulation in the expression of specific miRNAs and their respective targets.

5.4. EGCG and miRNA Regulation

EGCG is a polyphenol derivative of natural green tea with anticancer effects [83, 84]. It has also been demonstrated to have an effect on miRNAs in human cancer cells [85]. EGCG increased the expressions of 13 miRNAs including miR-16 and decreased the levels of 48 miRNAs in HepG2 human hepatic cancer cells. The miR-16 inhibited the anti-apoptotic protein Bcl-2 and these investigators have demonstrated that EGCG treatment reduces Bcl-2 leading to apoptosis in HepG2 cells [85]. All of these experiments have indicated that EGCG has the potential to inhibit cancer growth via miRNAs although much more studies are warranted.

5.5. I3C/DIM and miRNAs

I3C and DIM are produced from glucosinolates found in naturally occurring Cruciferae. DIM is an *in vivo* dimeric product of I3C. They inhibit cancer cell growth by controlling cell proliferation, transcription and apoptosis through regulation of gene expression [86–88]. Recently these compounds have been shown to function as miRNA regulators affecting cancer cell metastasis and growth by modulating EMT. They affect miRNAs which are

oncogenic and also those that have tumor suppressor capabilities. In lung cancer, I3C has been demonstrated to correct the miRNA abnormalities [5]. Specifically it reverses the expression level of oncogenic miRNAs by inhibiting the expression of miR-21, miR-31, miR-377, miR-146b and miR-130a in lung cancer. I3C acts by inhibiting miR-21 target genes, PTEN, PDCD4 and RECK. These findings have encouraged further research in this arena postulating the role of I3C as a novel therapeutic agent targeting miR-21.

In a previous study, we have successfully demonstrated that DIM could be effective against pancreatic cancer by reversing the EMT phenomenon [6]. In gemcitabine resistant cells, DIM treatment led to the up-regulation in the expression of miR-200 and let-7 families which typically lost not only in pancreatic cancer but in many other cancers. Conversely, DIM also down-regulated vimentin and slug along with phenotypic reversal of mesenchymal to epithelial morphology. Therefore, deregulation of miRNAs and their targets by DIM could be one of many attributes of DIM, and thus DIM can potentially serve as a natural therapeutic agent for pancreatic cancer. We have also found that DIM could increase the expression of miR-34a in prostate cancer through epigenetic regulation, and that the re-expression of miR-34a led to the down-regulation in the expression of AR [62]. These results suggest favorable effects of DIM on the induction of tumor suppressive miRNAs, which could become a novel approach for the treatment of human prostate cancer.

CONCLUSIONS

Based on our research and review of the current literature, it is quite clear that miRNAs could become a novel target for cancer therapy especially because by targeting one miRNA one could target multiple genes at the same time which is very attractive for cancer therapy because cancer is a disease of multi-gene defects [3, 20]. Altered expression of miRNAs is strongly connected to tumor development, progression and metastasis, which indeed would likely be due to the existence of CSCs and EMT phenotypic cells and that the biological behavior of these cells are regulated through deregulated expression of miRNAs. These tiny non-coding RNA molecules bind to the 3' UTR of target mRNA and down-regulate the expression of genes through mRNA degradation or translation inhibition. Due to this novel attribute, the miRNAs have potential for clinical use as diagnostic and prognostic biomarkers, markers of risk stratification and targets for cancer therapeutics. In the context of therapeutics, natural agents hold immense promise since they target multiple miRNAs at the same time, which indeed will regulate multiple mRNAs that are responsible for the heterogeneity of tumor cells within a tumor mass. Natural agents including curcumin, isoflavone, I3C, DIM, EGCG, and resveratrol can impact on the expression levels of miRNAs and thereby lead to the inhibition of cancer cell growth along with induction of apoptosis and reversal of EMT to MET. Therefore, natural agents are not merely chemopreventive agents they could be very useful as adjuncts to conventional therapeutics. The use of natural agents would likely eliminate CSCs or EMT phenotypic cells which have been implicated as causative factors for tumor recurrence and resistance to conventional therapies. Therefore, natural agents could potentially serve as novel therapeutic agents to prevent tumor recurrence and overcome resistance by targeting CSCs or EMT phenotypic cells mediated through deregulation in the expression of miRNAs and their specific targets, which is expected to improve the overall survival of patients diagnosed with cancers.

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References

1. Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell*. 1993; 75:843–54. [PubMed: 8252621]
2. Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*. *Cell*. 1993; 75:855–62. [PubMed: 8252622]
3. Iorio MV, Croce CM. MicroRNAs in cancer: small molecules with a huge impact. *J Clin Oncol*. 2009; 27:5848–56. [PubMed: 19884536]
4. 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. [PubMed: 20306121]
5. 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. [PubMed: 19748927]
6. Li Y, VandenBoom TG, Kong D, et al. 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. [PubMed: 19654291]
7. Pogribny IP, Muskhelishvili L, Tryndyak VP, Beland FA. The tumor-promoting activity of 2-acetylaminofluorene is associated with disruption of the p53 signaling pathway and the balance between apoptosis and cell proliferation. *Toxicol Appl Pharmacol*. 2009; 235:305–11. [PubMed: 19167416]
8. Lukiw WJ, Zhao Y, Cui JG. An NF-kappaB-sensitive micro RNA-146a-mediated inflammatory circuit in Alzheimer disease and in stressed human brain cells. *J Biol Chem*. 2008; 283:31315–22. [PubMed: 18801740]
9. Sun M, Estrov Z, Ji Y, et al. Curcumin (diferuloylmethane) alters the expression profiles of microRNAs in human pancreatic cancer cells. *Mol Cancer Ther*. 2008; 7:464–73. [PubMed: 18347134]
10. Lee Y, Kim M, Han J, et al. MicroRNA genes are transcribed by RNA polymerase II. *EMBO J*. 2004; 23:4051–60. [PubMed: 15372072]
11. Winter J, Jung S, Keller S, Gregory RI, Diederichs S. Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat Cell Biol*. 2009; 11:228–34. [PubMed: 19255566]
12. Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ. Processing of primary microRNAs by the Microprocessor complex. *Nature*. 2004; 432:231–5. [PubMed: 15531879]
13. Han J, Lee Y, Yeom KH, et al. The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev*. 2004; 18:3016–27. [PubMed: 15574589]
14. Gregory RI, Yan KP, Amuthan G, et al. The Microprocessor complex mediates the genesis of microRNAs. *Nature*. 2004; 432:235–40. [PubMed: 15531877]
15. Landthaler M, Yalcin A, Tuschl T. The human DiGeorge syndrome critical region gene 8 and Its *D. melanogaster* homolog are required for miRNA biogenesis. *Curr Biol*. 2004; 14:2162–7. [PubMed: 15589161]
16. Lee Y, Jeon K, Lee JT, Kim S, Kim VN. MicroRNA maturation: stepwise processing and subcellular localization. *EMBO J*. 2002; 21:4663–70. [PubMed: 12198168]
17. Lee Y, Ahn C, Han J, et al. The nuclear RNase III Drosha initiates microRNA processing. *Nature*. 2003; 425:415–9. [PubMed: 14508493]
18. Hammond SM, Bernstein E, Beach D, Hannon GJ. An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature*. 2000; 404:293–6. [PubMed: 10749213]
19. Rand TA, Petersen S, Du F, Wang X. Argonaute2 cleaves the anti-guide strand of siRNA during RISC activation. *Cell*. 2005; 123:621–9. [PubMed: 16271385]
20. Vandenboom TG II, Li Y, Philip PA, Sarkar FH. MicroRNA and Cancer: Tiny Molecules with Major Implications. *Curr Genomics*. 2008; 9:97–109. [PubMed: 19440450]
21. Nicoloso MS, Spizzo R, Shimizu M, Rossi S, Calin GA. MicroRNAs--the micro steering wheel of tumour metastases. *Nat Rev Cancer*. 2009; 9:293–302. [PubMed: 19262572]
22. Lin CH, Jackson AL, Guo J, Linsley PS, Eisenman RN. Myc-regulated microRNAs attenuate embryonic stem cell differentiation. *EMBO J*. 2009; 28:3157–70. [PubMed: 19745813]

23. Dirks PB. MicroRNAs and parallel stem cell lives. *Cell*. 2009; 138:423–4. [PubMed: 19665962]
24. Wang Y, Russell I, Chen C. MicroRNA and stem cell regulation. *Curr Opin Mol Ther*. 2009; 11:292–8. [PubMed: 19479662]
25. Ji Q, Hao X, Zhang M, et al. MicroRNA miR-34 inhibits human pancreatic cancer tumor-initiating cells. *PLoS One*. 2009; 4:e6816. [PubMed: 19714243]
26. Ji J, Yamashita T, Budhu A, et al. Identification of microRNA-181 by genome-wide screening as a critical player in EpCAM-positive hepatic cancer stem cells. *Hepatology*. 2009; 50:472–80. [PubMed: 19585654]
27. Garzia L, Andolfo I, Cusanelli E, et al. MicroRNA-199b-5p impairs cancer stem cells through negative regulation of HES1 in medulloblastoma. *PLoS One*. 2009; 4:e4998. [PubMed: 19308264]
28. Adam L, Zhong M, Choi W, et al. miR-200 expression regulates epithelial-to-mesenchymal transition in bladder cancer cells and reverses resistance to epidermal growth factor receptor therapy. *Clin Cancer Res*. 2009; 15:5060–72. [PubMed: 19671845]
29. Kong D, Li Y, Wang Z, et al. miR-200 regulates PDGF-D-mediated epithelial-mesenchymal transition, adhesion, and invasion of prostate cancer cells. *Stem Cells*. 2009; 27:1712–21. [PubMed: 19544444]
30. Iorio MV, Ferracin M, Liu CG, et al. MicroRNA gene expression deregulation in human breast cancer. *Cancer Res*. 2005; 65:7065–70. [PubMed: 16103053]
31. Papagiannakopoulos T, Shapiro A, Kosik KS. MicroRNA-21 targets a network of key tumor-suppressive pathways in glioblastoma cells. *Cancer Res*. 2008; 68:8164–72. [PubMed: 18829576]
32. Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res*. 2005; 65:6029–33. [PubMed: 16024602]
33. Si ML, Zhu S, Wu H, et al. miR-21-mediated tumor growth. *Oncogene*. 2007; 26:2799–803. [PubMed: 17072344]
34. Ribas J, Ni X, Haffner M, et al. miR-21: an androgen receptor-regulated microRNA that promotes hormone-dependent and hormone-independent prostate cancer growth. *Cancer Res*. 2009; 69:7165–9. [PubMed: 19738047]
35. Seike M, Goto A, Okano T, et al. MiR-21 is an EGFR-regulated anti-apoptotic factor in lung cancer in never-smokers. *Proc Natl Acad Sci USA*. 2009; 106:12085–90. [PubMed: 19597153]
36. Park JK, Lee EJ, Esau C, Schmittgen TD. Antisense inhibition of microRNA-21 or -221 arrests cell cycle, induces apoptosis, and sensitizes the effects of gemcitabine in pancreatic adenocarcinoma. *Pancreas*. 2009; 38:e190–e199. [PubMed: 19730150]
37. Li J, Huang H, Sun L, et al. MiR-21 indicates poor prognosis in tongue squamous cell carcinomas as an apoptosis inhibitor. *Clin Cancer Res*. 2009; 15:3998–4008. [PubMed: 19509158]
38. Asangani IA, Rasheed SA, Nikolova DA, et al. MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. *Oncogene*. 2008; 27:2128–36. [PubMed: 17968323]
39. Yao Q, Xu H, Zhang QQ, Zhou H, Qu LH. MicroRNA-21 promotes cell proliferation and down-regulates the expression of programmed cell death 4 (PDCD4) in HeLa cervical carcinoma cells. *Biochem Biophys Res Commun*. 2009; 388:539–42. [PubMed: 19682430]
40. Qin W, Zhao B, Shi Y, et al. BMPRII is a direct target of miR-21. *Acta Biochim Biophys Sin (Shanghai)*. 2009; 41:618–23. [PubMed: 19578724]
41. He L, Thomson JM, Hemann MT, et al. A microRNA polycistron as a potential human oncogene. *Nature*. 2005; 435:828–33. [PubMed: 15944707]
42. Diosdado B, van de Wiel MA, Terhaar Sive Droste JS, et al. MiR-17-92 cluster is associated with 13q gain and c-myc expression during colorectal adenoma to adenocarcinoma progression. *Br J Cancer*. 2009; 101:707–14. [PubMed: 19672269]
43. Manni I, Artuso S, Careccia S, et al. The microRNA miR-92 increases proliferation of myeloid cells and by targeting p63 modulates the abundance of its isoforms. *FASEB J*. 2009; 23:3957–66. [PubMed: 19608627]
44. Dews M, Homayouni A, Yu D, et al. Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. *Nat Genet*. 2006; 38:1060–5. [PubMed: 16878133]

45. Northcott PA, Fernandez L, Hagan JP, et al. The miR-17/92 polycistron is up-regulated in sonic hedgehog-driven medulloblastomas and induced by N-myc in sonic hedgehog-treated cerebellar neural precursors. *Cancer Res.* 2009; 69:3249–55. [PubMed: 19351822]
46. Uziel T, Karginov FV, Xie S, et al. The miR-17~92 cluster collaborates with the Sonic Hedgehog pathway in medulloblastoma. *Proc Natl Acad Sci USA.* 2009; 106:2812–7. [PubMed: 19196975]
47. Fulci V, Chiaretti S, Goldoni M, et al. Quantitative technologies establish a novel microRNA profile of chronic lymphocytic leukemia. *Blood.* 2007; 109:4944–51. [PubMed: 17327404]
48. Lawrie CH, Soneji S, Marafioti T, et al. MicroRNA expression distinguishes between germinal center B cell-like and activated B cell-like subtypes of diffuse large B cell lymphoma. *Int J Cancer.* 2007; 121:1156–61. [PubMed: 17487835]
49. Greither T, Grochola LF, Udelnow A, et al. Elevated expression of microRNAs 155, 203, 210 and 222 in pancreatic tumors is associated with poorer survival. *Int J Cancer.* 2010; 126:73–80. [PubMed: 19551852]
50. Habbe N, Koorstra JB, Mendell JT, et al. MicroRNA miR-155 is a biomarker of early pancreatic neoplasia. *Cancer Biol Ther.* 2009; 8:340–6. [PubMed: 19106647]
51. Gironella M, Seux M, Xie MJ, et al. Tumor protein 53-induced nuclear protein 1 expression is repressed by miR-155, and its restoration inhibits pancreatic tumor development. *Proc Natl Acad Sci USA.* 2007; 104:16170–5. [PubMed: 17911264]
52. Johnson CD, Esquela-Kerscher A, Stefani G, et al. The let-7 microRNA represses cell proliferation pathways in human cells. *Cancer Res.* 2007; 67:7713–22. [PubMed: 17699775]
53. Takamizawa J, Konishi H, Yanagisawa K, 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. [PubMed: 15172979]
54. Lee YS, Dutta A. The tumor suppressor microRNA let-7 represses the HMGA2 oncogene. *Genes Dev.* 2007; 21:1025–30. [PubMed: 17437991]
55. Bussing I, Slack FJ, Grosshans H. let-7 microRNAs in development, stem cells and cancer. *Trends Mol Med.* 2008; 14:400–9. [PubMed: 18674967]
56. Johnson SM, Grosshans H, Shingara J, et al. RAS is regulated by the let-7 microRNA family. *Cell.* 2005; 120:635–47. [PubMed: 15766527]
57. Takeshita F, Patrawala L, Osaki M, et al. Systemic delivery of synthetic microRNA-16 inhibits the growth of metastatic prostate tumors via downregulation of multiple cell-cycle genes. *Mol Ther.* 2010; 18:181–7. [PubMed: 19738602]
58. Chang TC, Wentzel EA, Kent OA, et al. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. *Mol Cell.* 2007; 26:745–52. [PubMed: 17540599]
59. Cimmino A, Calin GA, Fabbri M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci USA.* 2005; 102:13944–9. [PubMed: 16166262]
60. Calin GA, Cimmino A, Fabbri M, et al. MiR-15a and miR-16-1 cluster functions in human leukemia. *Proc Natl Acad Sci USA.* 2008; 105:5166–71. [PubMed: 18362358]
61. Corney DC, Flesken-Nikitin A, Godwin AK, Wang W, Nikitin AY. MicroRNA-34b and MicroRNA-34c are targets of p53 and cooperate in control of cell proliferation and adhesion-independent growth. *Cancer Res.* 2007; 67:8433–8. [PubMed: 17823410]
62. Kong D, Heath E, Chen W, et al. Epigenetic silencing of miR-34a in human prostate cancer cells and tumor tissue specimens can be reversed by BR-DIM treatment. *Am J Transl Res.* 2012; 4:14–23. [PubMed: 22347519]
63. Vogt M, Munding J, Gruner M, et al. Frequent concomitant inactivation of miR-34a and miR-34b/c by CpG methylation in colorectal, pancreatic, mammary, ovarian, urothelial, and renal cell carcinomas and soft tissue sarcomas. *Virchows Arch.* 2011; 458:313–22. [PubMed: 21225432]
64. Liu H, Brannon AR, Reddy AR, et al. Identifying mRNA targets of microRNA dysregulated in cancer: with application to clear cell Renal Cell Carcinoma. *BMC Syst Biol.* 2010; 4:51. [PubMed: 20420713]
65. Hutvagner G, Simard MJ, Mello CC, Zamore PD. Sequence-specific inhibition of small RNA function. *PLoS Biol.* 2004; 2:E98. [PubMed: 15024405]
66. Meister G, Landthaler M, Dorsett Y, Tuschl T. Sequence-specific inhibition of microRNA- and siRNA-induced RNA silencing. *RNA.* 2004; 10:544–50. [PubMed: 14970398]

67. Orom UA, Kauppinen S, Lund AH. LNA-modified oligonucleotides mediate specific inhibition of microRNA function. *Gene*. 2006; 372:137–41. [PubMed: 16503100]
68. Krutzfeldt J, Rajewsky N, Braich R, et al. Silencing of microRNAs *in vivo* with ‘antagomirs’. *Nature*. 2005; 438:685–9. [PubMed: 16258535]
69. Tsuda N, Ishiyama S, Li Y, et al. Synthetic microRNA designed to target glioma-associated antigen 1 transcription factor inhibits division and induces late apoptosis in pancreatic tumor cells. *Clin Cancer Res*. 2006; 12:6557–64. [PubMed: 17085671]
70. Weidhaas JB, Babar I, Nallur SM, et al. MicroRNAs as potential agents to alter resistance to cytotoxic anticancer therapy. *Cancer Res*. 2007; 67:11111–6. [PubMed: 18056433]
71. Ahmad A, Aboukameel A, Kong D, et al. Phosphoglucose isomerase/autocrine motility factor mediates epithelial-mesenchymal transition regulated by miR-200 in breast cancer cells. *Cancer Res*. 2011; 71(9):3400–9. [PubMed: 21389093]
72. Dolganiuc A, Petrascu J, Kodys K, et al. MicroRNA expression profile in Lieber-DeCarli diet-induced alcoholic and methionine choline deficient diet-induced nonalcoholic steatohepatitis models in mice. *Alcohol Clin Exp Res*. 2009; 33:1704–10. [PubMed: 19572984]
73. Takahashi M, Uechi S, Takara K, Asikin Y, Wada K. Evaluation of an oral carrier system in rats: bioavailability and antioxidant properties of liposome-encapsulated curcumin. *J Agric Food Chem*. 2009; 57:9141–6. [PubMed: 19757811]
74. Anand P, Nair HB, Sung B, et al. Design of curcumin-loaded PLGA nanoparticles formulation with enhanced cellular uptake, and increased bioactivity *in vitro* and superior bioavailability *in vivo*. *Biochem Pharmacol*. 2010; 79:330–8. [PubMed: 19735646]
75. Padhye S, Banerjee S, Chavan D, et al. Fluorocurcumin as cyclooxygenase-2 inhibitor: molecular docking, pharmacokinetics and tissue distribution in mice. *Pharm Res*. 2009; 26:2438–45. [PubMed: 19714451]
76. Mukhopadhyay A, Bueso-Ramos C, Chatterjee D, Pantazis P, Aggarwal BB. Curcumin downregulates cell survival mechanisms in human prostate cancer cell lines. *Oncogene*. 2001; 20:7597–609. [PubMed: 11753638]
77. Shao ZM, Shen ZZ, Liu CH, et al. Curcumin exerts multiple suppressive effects on human breast carcinoma cells. *Int J Cancer*. 2002; 98:234–40. [PubMed: 11857414]
78. Kundu JK, Surh YJ. Cancer chemopreventive and therapeutic potential of resveratrol: mechanistic perspectives. *Cancer Lett*. 2008; 269:243–61. [PubMed: 18550275]
79. Whyte L, Huang YY, Torres K, Mehta RG. Molecular mechanisms of resveratrol action in lung cancer cells using dual protein and microarray analyses. *Cancer Res*. 2007; 67:12007–17. [PubMed: 18089832]
80. Li Y, Sarkar FH. Down-regulation of invasion and angiogenesis-related genes identified by cDNA microarray analysis of PC3 prostate cancer cells treated with genistein. *Cancer Lett*. 2002; 186:157–64. [PubMed: 12213285]
81. Dixon RA, Ferreira D. Genistein. *Phytochemistry*. 2002; 60:205–11. [PubMed: 12031439]
82. Barnes S. The chemopreventive properties of soy isoflavonoids in animal models of breast cancer. *Breast Cancer Res Treat*. 1997; 46:169–79. [PubMed: 9478272]
83. Katiyar SK, Afaq F, Perez A, Mukhtar H. Green tea polyphenol (–)-epigallocatechin-3-gallate treatment of human skin inhibits ultraviolet radiation-induced oxidative stress. *Carcinogenesis*. 2001; 22:287–94. [PubMed: 11181450]
84. Mukhtar H, Ahmad N. Green tea in chemoprevention of cancer. *Toxicol Sci*. 1999; 52:111–7. [PubMed: 10630599]
85. 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. [PubMed: 19269153]
86. Garikapaty VP, Ashok BT, Tadi K, Mittelman A, Tiwari RK. 3,3'-Diindolylmethane downregulates pro-survival pathway in hormone independent prostate cancer. *Biochem Biophys Res Commun*. 2006; 340:718–25. [PubMed: 16380095]
87. Li Y, Li X, Sarkar FH. Gene expression profiles of I3C- and DIM-treated PC3 human prostate cancer cells determined by cDNA microarray analysis. *J Nutr*. 2003; 133:1011–9. [PubMed: 12672912]

88. Chinni SR, Li Y, Upadhyay S, Koppolu PK, Sarkar FH. Indole-3-carbinol (I3C) induced cell growth inhibition, G1 cell cycle arrest and apoptosis in prostate cancer cells. *Oncogene*. 2001; 20:2927–36. [PubMed: 11420705]
89. Spizzo R, Nicoloso MS, Croce CM, Calin GA. SnapShot: MicroRNAs in Cancer. *Cell*. 2009; 137:586. [PubMed: 19410551]

Fig. 1.
The effects of natural agents on miRNA regulated drug resistance, metastasis and recurrence of cancer.

Table 1

The miRNAs which Show Tumor Suppressive or Oncogenic Activity [4, 89].

miRNA	Observed alterations in tumors
<i>Tumor suppressor activity</i>	
let-7	Down-regulated in lung, ovarian cancer, breast, prostate and colorectal cancers.
miR-15/miR-16	Down-regulated in CLL *, multiple myeloma, prostate, pancreatic, gastric, and lung cancers.
miR-34	Down-regulated in Burkitt's lymphoma, prostate, breast, pancreatic, and colon cancers.
miR-29	Down-regulated in CLL, AML *, prostate, lung, colon, and breast cancers.
miR-101	Down-regulated in prostate, hepatic, and bladder cancers.
miR-122a	Down-regulated in hepatic cancer.
miR-124a	Down-regulated in ALL *, medulloblastoma, colon, lung, breast, and gastric cancers.
miR-127	Down-regulated in lymphoma and breast cancer.
miR-143	Down-regulated in gastric, colon, breast, lung, and prostate cancers
miR-145	Down-regulated in breast, colon, lung, cervical, and gastric cancers
miR-181	Down-regulated in colorectal cancer.
<i>Oncogenic activity</i>	
miR-21	Up-regulated in glioblastoma, colon, breast, pancreatic, lung, prostate, hepatic, cervical, and gastric cancers.
miR-155	Up-regulated in CLL, AML, pancreatic, colon, lung, and breast cancers,
miR-17-92	Up-regulated in medulloblastoma, lymphoma, lung, breast, colorectal, gastric, and pancreatic cancers.
miR-106a	Up-regulated in colon and gastric cancers.
miR-373	Up-regulated in testicular tumor, gastric cancer.
miR-197	Up-regulated in lung cancer.
miR-221	Up-regulated in CLL, glioblastoma, breast, thyroid, and hepatic cancers.
miR-222	Up-regulated in CLL, glioblastoma, breast, thyroid, and hepatic cancers
miR-372	Up-regulated in testicular tumor.

* CLL, chronic lymphoblastic leukemia; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia.