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MicroRNAs, Cancer and Cancer Stem Cells

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Abstract

MicroRNAs regulate self-renewal, differentiation, and division of cells via post-transcriptional gene silencing. Aberrant microRNA levels, specifically an overall downregulation, are present in many cancers, as compared to their normal tissue counterparts. Therefore, a potential therapeutic use of microRNAs is to correct these aberrant transcript levels involved in the signaling pathways of cancer. This review focuses on the current knowledge of microRNAs and their involvement with cancer cells and cancer stem cells. The methods currently being used to develop miRNA-based cancer therapeutics are examined, and the limitations halting further progress are also discussed.

1. Introduction

Cell differentiation is regulated, in part, by a recently discovered class of molecules – microRNAs (miRNAs). These regulatory molecules can directly affect differentiation in both normal stem cells and cancer stem cells [1–3]. MiRNAs are 21–23 nucleotides long and act as regulatory molecules in eukaryotic cells by binding to a non-coding region within target messenger RNAs (mRNAs), namely the 3'-untranslated region (3'-UTR). Through this mechanism miRNAs regulate self-renewal, differentiation, and division of cells via post-transcriptional gene silencing [4]. MiRNAs play important roles in many cellular processes such as development [5], stem cell division [3,6,7], apoptosis [8,9], disease [10,11], and cancer [12,13]. MiRNAs regulate gene expression by either inhibiting translation or promoting degradation of specific mRNA transcripts. An estimated 3% of human genes code for miRNAs, yet these miRNAs may regulate around 30% of the protein-coding genes [14]. This suggests not only their importance in various regulatory pathways, but also their potential for manipulation. MiRNAs themselves have been shown to act both as tumor suppressors and as oncogenes, which promote tumor growth. In addition, aberrant miRNA levels, specifically an overall downregulation, are present in many cancers, as compared to their normal tissue counterparts [15]. Therefore, a potential therapeutic use of miRNAs is to correct these aberrant transcript levels involved in the signaling pathways of cancer cells, especially cancer stem cells (CSCs).

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Conflicts of Interest Statement

None Declared

2. Biogenesis and Functions of MicroRNAs

A canonical pathway for miRNA biogenesis begins with transcription of the encoded genes by RNA polymerase II. The majority of human miRNAs is expressed from introns, the non-coding regions of the genome [16,17]. An intron of about 400 nucleotides is excised from the primary transcript and becomes the primary miRNA (pri-miRNA). The pri-miRNA is then processed by the RNase Drosha into hairpin loops about 70 nucleotides in length, forming the pre-miRNA. The pre-miRNA is then exported to the cytoplasm by the nuclear membrane protein Exportin-5. Once in the cytoplasm, the RNase Dicer completes processing, forming mature miRNAs [4,15] (Fig. 1).

In addition to the canonical pathway described above, miRNA maturation involves much more complicated regulations. The transcribed and excised pri-miRNA has the following features: a hairpin stem (HPS) averaging 33 base pairs, a terminal loop (T loop), and two single-stranded regions (SSR) - one upstream and one downstream of the hairpin [18–20]. The pri-miRNA is then cleaved into a pre-miRNA by a complex consisting of the RNase Drosha and the DiGeorge critical region gene 8 protein (DGCR8) [21]. The DGCR8 acts as a molecular ruler for the precise site of pri-miRNA cleavage, which occurs 11 base pairs away from the junction of the single- and double-stranded RNA. Drosha's two RNase domains then cleave the 5' and 3' arms of the hairpin, forming the pre-miRNA [18] (Fig. 1). Pre-miRNA formation by Drosha occurs co-transcriptionally and before splicing, regardless of whether the primary transcript is protein-coding or non-coding [22]. While miRNA encoding introns are spliced more slowly than adjacent introns, Drosha cleavage does not inhibit splicing because the latter process does not require a continuous intron [17,22].

MiRNA biogenesis is also very specific, depending on the miRNA being processed. Variations at the transcriptional level include the following: miRNA genes can be transcribed by either RNA polymerase II or RNA polymerase III, each of which recognizes specific promoters and terminators and undergoes specific regulation [23–25]; expression can additionally be controlled by transcription factors such as c-Myc or p53 [26]. There are also many variations in pri-miRNA cleavage. In some cases, Drosha cleavage is replaced by splicing if the intron is the appropriate size to form a pre-miRNA hairpin [27,28]. In other cases, pri-miRNAs recruit heterogeneous nuclear ribonucleoproteins [29] or growth factors [30] to mediate and enhance Drosha processing. Other proteins such as RNA helicases, double-stranded RNA binding proteins, and Ewing's sarcoma proteins can also be involved, depending on the specific miRNA [31].

MiRNAs regulate gene expression by either inhibiting translation or promoting degradation of specific mRNA transcripts. Post-transcriptional gene silencing begins when an miRNA recruits RISC (RNA-induced silencing complex), which is a complex of proteins that localizes the miRNA to its complementary target mRNA [15]. Although there are exceptions, the specificity of target localization involves both the miRNA and mRNA sequences. Nucleotides 2–8 of the miRNA, called the seed region (SR), must bind contiguously to a perfectly complementary sequence on the target mRNA. The binding sites for the miRNA seed region lie in the 3'-UTR of the mRNA, and the complementary sequence usually repeats multiple times within the 3'-UTR. Whether an miRNA promotes degradation or represses translation of its target mRNA likely depends on the degree of complementary binding beyond the seed region [32]. More specifically, the silencing mechanism is likely dictated by the number, type, and position of mismatched base pairs between the miRNA and mRNA [33] (Fig. 1).

MiRNAs repress translation via different mechanisms either at the initiation or elongation step of translation, based on how they modulate the interaction between the 5' cap and the 3'

polyadenylated tail of the mRNA [34]. Although the exact mechanism is not well understood, translational repression involves cytoplasmic mRNA-processing bodies, or P bodies. These P bodies are believed to play a role in mRNA silencing because target mRNAs and RISC components are co-localized to this part of the cell [35,36]. P bodies house various proteins that control messenger ribonucleoprotein (mRNP) complexes [35–38]. These mRNP complexes consist of mRNA and repressor proteins, and they lack translation initiation factors [39]. Thus, the localization of target mRNAs to P bodies, and subsequent formation of the mRNP complexes, inhibits translation. Although translational repression seems to be the default action of silencing [32], degradation of the mRNA appears to occur when the sequence beyond the seed region of the miRNA is perfectly complementary to the remaining region in the 3'-UTR of the mRNA. This degradation involves deadenylation, decapping, and exonucleolytic cleavage of the target mRNA transcript [40–42], but the exact mechanism is still unknown.

3. Cancer and Cancer Stem Cells

A tumor consists of a heterogeneous population of cells that differ by their relative states of differentiation [43]. The outside of a tumor mass contains fully differentiated cells that are susceptible to radiation and chemotherapy because of their close vicinity to the non-tumorigenic microenvironment as well as the sufficient blood flow due to induced angiogenesis, or blood vessel growth [43]. The region closer to the center of the tumor contains progenitor cells, which can undergo a limited number of mitotic cycles to form several daughter cells [44]. These daughter cells can then differentiate into select types of cells based on their relative microenvironments [44]. Within the heart of the tumor lies the cancer stem cells (CSCs), which are both structurally and functionally distinct from the other cells within a tumor mass [4].

CSCs have the ability to undergo self-renewing mitosis, where one or both of the daughter cells retain the identity of the stem cell. The other daughter cell could become a progenitor cell, which then undergoes several mitotic cycles to become a differentiated cell. It is through this mechanism that CSCs can cause proliferation and expansion of fully differentiated tumor cells [43,44]. CSCs are challenging to study because they rapidly differentiate when cultured; it is difficult to maintain a population *in vitro* that remains enriched in the CSCs [45]. CSC-enriched populations exhibit three characteristics *in vitro*: they can be isolated with cell surface marker profiles, they form tumorspheres, which appear as spherical colonies in suspension cultures, and they have increased resistance to both chemotherapeutic agents and ionizing radiation [45]. CSCs have been isolated for hematological malignancies [46], brain tumors [47,48], and colon tumors [49]. A method for enriching breast CSCs has also been developed [45]. As is often the case in research, further progress is limited by the available technologies and known techniques.

Cancer therapeutics in current use decrease tumor size, but they are unlikely to result in long-term remission unless the CSCs are also targeted. However, CSCs are difficult to target because of their innate properties. CSCs are characterized by their resistance to anti-cancer therapeutics and their repopulating ability; they can regenerate tumorigenic cells after tumor reduction from chemotherapy and radiation [4]. The multidrug resistance trait of CSCs is associated with an overexpression of genes that code for transmembrane efflux pump proteins. The proteins themselves are regulated by reactive oxygen species within the cell, which could vary by tissue type and also among cells within a tissue [50]. Thus, any therapeutics that target CSCs would require great specificity. A rapidly developing field in cancer therapeutics is targeting miRNAs, which exhibit aberrant levels in cancer cells. Functional studies of the specific miRNAs within different cancer cells, specifically CSCs, are necessary to find a therapeutic target.

4. MicroRNAs and Cancers

Since miRNAs are critical for both stem cell development and cancer pathogenesis, they are being examined for their regulatory roles in self-renewal, proliferation, and differentiation of cancer cells [2,51,52]. MiRNAs have been shown to act as both tumor suppressors, which help control growth, and oncogenes, which promote rapid growth [15]. Global inhibition of miRNA processing increased tumorigenicity and transformation in a recent study [53], which suggests their important regulatory role. In addition, many cancer-associated regions of the genome contain miRNA genes [54]. Aberrant miRNA levels, specifically an overall downregulation, are present in many cancers, as compared to their normal tissue counterparts [15]. Specific miRNA expression patterns have been described in several cancers (Table I). Although it is not entirely clear if irregular miRNA expression is a cause or an effect of the tumorigenic state, the significance of these regulatory molecules in cancer is apparent.

As tumor suppressors, decreased expression of some miRNAs in cancer stem cells results in cell proliferation. These miRNAs normally regulate apoptosis, differentiation, and self-renewal [55]. One example of these miRNAs is the miRNA-34 (miR-34) family, consisting of miR-34-a, b and c, which is down-regulated in many cancers [56] (Table I). Restoration of miR-34 in p53 deficient gastric and pancreatic cancer cells resulted in both cell cycle arrest at G₁ and apoptosis suggesting that this miRNA is involved in p53 function [26,56,57]. Indeed, in cell lines and mouse tissue, p53 regulates the abundance of all three miRNAs of the miR-34 family, which inhibit a group of mRNAs that normally support tumor formation by inhibiting apoptosis; promoting cell cycle progression past the G₁ checkpoint; preventing cellular aging; and promoting migration (Fig. 2). Interestingly, p53 normally induces growth arrest by activating p21, a cyclin-dependent kinase (cdk) inhibitor [58]. However, miR-34 can induce G₁ arrest independently of p21 in some cell types [26]. Because of its regulation and function, the miR-34 family probably has a significant effect on p53 tumor-suppression function [59].

Because of mutant p53 in over 50% of human cancers, the miR-34 family is often down regulated in tumors [26,59]; however, its abundance can also be affected at the genomic level. In vertebrate genomes, one genomic locus encodes miR-34a and another locus encodes miR-34b and miR-34c. Even among closely related species, there is little conservation in these genes except in the miRNA-encoding sequences and in the short promoter proximal regions that contain a p53-binding site [26,57,59–61]. The miR-34a gene is commonly lost in many human tumor types, including neuroblastoma [62] and pancreatic tumors [60]. Similarly, aberrant miR-34b and miR-34c expression has been observed in a subset of non-small cell lung cancers [59]. The miR-34a targets the mRNA of an important transcription factor, E2F3, which binds specifically to retinoblastoma protein pRB in a cell-cycle dependent manner and initiates production of proteins that affect cell cycle checkpoints, DNA repair, and replication [63–65]. Reduction of E2F3 level by artificial elevation of miR-34 in primary neuroblastomas inhibited cell proliferation and activated cell death pathways [62], providing further evidence for miR-34's role as a tumor suppressor.

Besides E2F3, the miR-34 family also targets Notch, HMGA2, CDK4, CDK6, Cyclin E2, and Bcl-2 gene products, which are involved in self-renewal and survival of cancer stem cells because of their effects on cell cycle control, apoptosis, and DNA repair [26,59,66] (Fig. 2). Bcl-2 is over-expressed in a majority of human cancers [67], and this increased expression has been correlated with both chemotherapeutic and radiation resistance of the CSCs [68–71]. Bcl-2 protects cancer cells from chemotherapeutic agents by preventing apoptosis [72,73]. Restoration of miR-34 levels in pancreatic and gastric cancer cells sensitized the cells to chemotherapy, inhibited tumor growth, and inhibited tumorsphere

formation [74,75]. However, the tumor suppressing function of artificial elevation of miR-34 appears to be cell type dependent. While restoration of miR-34 expression has resulted in cell cycle arrest or senescence in some studies [26], it has resulted in apoptosis in others; apoptosis also decreased with inhibition or depletion of miR-34 [59,61]. Therefore, more studies are needed to fully understand the effects of therapeutic restoration of miR-34 on different cell types, because of the great complexity in the pathways targeted.

5. MicroRNAs, Stem Cells and Cancer Stem Cells

Both normal and cancer stem cells have the ability to divide symmetrically, creating two identical daughter cells. They also have the ability to divide asymmetrically, forming one stem cell and one progenitor cell, the latter of which will undergo differentiation [76]. This asymmetric division facilitates healthy growth in normal cells due to the polarity involved in cell division. When this polarity is lost, the stem cells multiply and form a tumor [76]. Stem cell self-renewal is controlled by both intercellular mechanisms, via signaling from neighboring cells, and intracellular mechanisms, involving differential gene expression that is under epigenetic, transcriptional, translational, and post-translational control [77]. For example, the polarity of cell division in mammary stem cells is regulated by many genes including tumor suppressor p53. Loss of p53 results in more symmetric divisions and leads to tumor growth [78]. In non-tumorigenic cells, regulatory miRNA levels are generally lower in cells that are less differentiated (*i.e.*, stem cells) [2]. In adult stem cells, miRNAs regulate hematopoiesis, myogenesis, cardiogenesis, astrocyte/neuronal differentiation, osteogenic differentiation, and skin differentiation [79]. Since miRNAs play an important regulatory role in normal differentiation, aberrant expression could cause significant changes within the cell, possibly leading to cancer.

One extensively studied model for miRNA-regulated stem cell differentiation is the maturation of blood cells. Normal hematopoietic stem cells differentiate into several cell types with myeloid and lymphoid lineages, and certain miRNAs have been shown to regulate various differential steps (Fig. 3). In normal hematopoiesis, long-term reconstituting hematopoietic stem cells divide into their short-term counterparts, which then give rise to multipotent progenitors. These multipotent progenitors differentiate into either lymphoid stem cells or myeloid stem cells; this differentiation step is inhibited by miR-128a and miR-181a, but it is activated by miR-223 [80,81]. Furthermore, the differentiation of lymphoid stem cells into common lymphoid progenitors is inhibited by miR-146 and activated by miR-181 [81]. Common lymphoid progenitors then differentiate into T cells, B cells, and natural killer cells, the first of which is activated by miR-150 [82]. On the other hand, the differentiation of myeloid stem cells into common myeloid progenitors is inhibited by miR-155, miR-24a, and miR-17 [82]. Common myeloid progenitors differentiate into either granulocyte-macrophage progenitors, the formation of which is inhibited by miR-16, miR-103, and miR-107, or megakaryotic-erythroid progenitors [80]. Granulocyte-macrophage differentiation into granulocytes is inhibited by miR-223, while differentiation into monocytes is inhibited by miR-17-5p, miR-20a, and miR-106a [80,83]. Megakaryotic-erythroid progenitors differentiate into either megakaryocyte progenitors, which develop into platelets, or erythroid progenitors, the formation of which is inhibited by miR-24 [84]. Erythroid progenitors then develop into red blood cells via activation from miR-451 and miR-16, or this process is inhibited by miR-150, miR-155, miR-221, and miR-222 [80,82,85–87] (Fig. 3).

Like non-tumorigenic stem cells, CSCs contain low miRNA levels [2], but it is still unknown if this is the cause or the effect of the differentiation state. Some miRNAs suppress proliferation in differentiated cells through tumor suppressor pathways, suggesting miRNAs are the cause of differentiation state [15]. On the other hand, specific miRNAs that are

characteristic for normal tissue stem cells are present at the same level in CSCs [15]. However, aberrant miRNA expression levels, both an upregulation of some miRNAs and a downregulation or absence of others, have been found in CSCs [2]. Certain cancers have specific miRNA profiles (Table I) as well as specific membrane proteins served as surface markers. In comparison to the profiles of other cells within the tumor, cell surface marker profiles for CSCs have been characterized for various tumor types including acute myeloid leukemia [88], adult glioblastoma multiforme [89], brain tumors [90], breast cancer [91], bone sarcoma [92], chronic myeloid leukemia [93], colorectal cancer [49,94], head and neck squamous cell carcinoma [95], lung cancers [96], metastatic melanoma [97], pancreatic cancer [98], and prostate cancer [99,100]. With this knowledge, therapies can be targeted for delivery to cancer cells, especially cancer stem cells. In order to develop therapeutic applications that target CSCs, the functional role of the miRNAs in the specific tumor would need to be established.

6. Small RNAs as Potential Therapeutics for Cancer Treatment

Therapeutic microRNAs can be introduced into tumor cells to correct the aberrant miRNA levels, which could potentially result in reversal of the some of the cells' tumorigenic properties. These therapeutics are aimed to silence tumorigenic genes post-transcriptionally via the RNA interference (RNAi) pathway, in which a small RNA causes degradation of its complementary mRNA [101]. Cells use endogenous miRNAs for RNAi, as described in previous sections. Therapeutic RNAi can be utilized with two molecules: synthetic small interfering RNA (siRNA) and vector-based short hairpin RNA (shRNA). SiRNAs, like miRNAs, are double-stranded, are 21–23 nucleotides long, contain two-nucleotide overhangs at the 3' ends, and can be incorporated directly into RISC. ShRNAs are delivered in vectors, so they are actually synthesized in the nucleus like miRNAs [102]. ShRNAs are stem loop structures, similar to pre-miRNA hairpins, and undergo the same processing mechanisms as miRNAs, including Dicer processing before RISC incorporation [21,103–108]. There are practical advantages and disadvantages to the small RNA therapies. SiRNA is easy to chemically modify, but bulk production of complex structures is more costly. On the other hand, shRNA is difficult to modify and easy to produce in bulk. However, there are methods for shRNA alterations, which involve changing the inserted sequence, altering promoter regulation, using different plasmids or viral vectors for delivery, and regulating or inducing shRNA expression [109–111]. Both siRNAs [112–114] and shRNAs [112,114,115] have demonstrated effectiveness *in vivo*.

RNAi technology has increased the potential of targeted therapies. Several animal studies have shown the therapeutic efficacy of RNAi, and some RNAi-based therapeutics are currently undergoing clinical trial testing for treating cancers [116]. The therapeutic efficacy of siRNAs and shRNAs varies, depending on several factors: immune system avoidance, silencing efficiency, and off-target effects [116]. SiRNAs longer than 29–30 nucleotides induce an interferon immune response similar to double-stranded RNA, but shortening the strand or making structural modifications virtually eliminates this effect [117]. Both siRNA and shRNA also induce Toll-like receptors (TLRs) and downstream immune responses; however, this effect can be effectively diminished by using minimal plasmid vectors or unmethylated CpG-free plasmid vectors [118]. SiRNA, even when protected with chemical modifications, is susceptible to degradation and metabolic processes. While shRNA shows desired target effects when present in fewer than five copies, siRNA requires doses in the nanomolar range to achieve the same effects. This is a result of shRNA undergoing continual synthesis, yielding less transient effects [119].

On the other hand, RNAi-based therapeutics have many off-target effects that impact gene expression, resulting in off-target phenotypes. This is due to the fact that such small RNAs

can bind some mRNA transcripts with imperfect complementarity [120]. SiRNAs, like miRNAs, have a seed region sequence of nucleotide positions 2–8 that complementarily bind to the 3'-UTR of mRNA [121]. Chemical modifications, especially at position 2, can greatly improve target specificity [122]. However, the large dose of siRNA required increases the probability of off-target effects [119]. When comparing siRNA and shRNA treatments of similar doses, shRNA had fewer off-target effects, likely due to the different mechanism of action [119]. ShRNAs face the same nuclear and cytoplasmic regulation as miRNAs, while siRNAs do not [123]. Currently, shRNA sequences are being made into artificial pri-miRNA transcripts [111].

6. Concluding Remarks

Since miRNAs are critical for both stem cell development and cancer pathogenesis, they are being examined for their regulatory roles in self-renewal, proliferation, and differentiation of cancer cells [2,51,52]. Global inhibition of miRNA processing increased tumorigenicity and transformation [53], which suggests their important regulatory role. MiRNAs have been shown to act as both tumor suppressors, which help control growth, and oncogenes, which promote rapid growth [15]. In addition, many cancer-associated regions of the genome contain miRNA genes [54]. Certain cancers have specific miRNA profiles (i.e., upregulation or downregulation of certain miRNAs) as well as specific membrane proteins called surface markers. This knowledge enables development of therapeutics targeted to cancer cells, including cancer stem cells. Functional studies of the specific miRNAs within different cancer cells, specifically CSCs, are needed to find a drug target and develop effective and safe therapeutics.

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References

1. He L, Thomson JM, Hemann MT, et al. A microRNA polycistron as a potential human oncogene. *Nature* 2005;435:828–833. [PubMed: 15944707]
2. Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. *Nature* 2005;435:834–838. [PubMed: 15944708]
3. Hatfield SD, Shcherbata HR, Fischer KA, et al. Stem cell division is regulated by the microRNA pathway. *Nature* 2005;435:974–978. [PubMed: 15944714]
4. Hatfield S, Ruohola-Baker H. microRNA and stem cell function. *Cell Tissue Res* 2008;331:57–66. [PubMed: 17987317]
5. Reinhart BJ, Slack FJ, Basson M, et al. The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. *Nature* 2000;403:901–906. [PubMed: 10706289]
6. Cheng LC, Tavazoie M, Doetsch F. Stem cells: from epigenetics to microRNAs. *Neuron* 2005;46:363–367. [PubMed: 15882632]
7. Zhang BH, Pan XP, Cox SB, et al. Evidence that miRNAs are different from other RNAs. *Cell Mol Life Sci* 2006;63:246–254. [PubMed: 16395542]
8. Cheng AM, Byrom MW, Shelton J, et al. Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. *Nucleic Acids Res* 2005;33:1290–1297. [PubMed: 15741182]
9. Cimmino A, Calin GA, Fabbri M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci U S A* 2005;102:13944–13949. [PubMed: 16166262]
10. Abelson JF, Kwan KY, O'Roak BJ, et al. Sequence variants in *SLITRK1* are associated with Tourette's syndrome. *Science* 2005;310:317–320. [PubMed: 16224024]

11. Alvarez-Garcia I, Miska EA. MicroRNA functions in animal development and human disease. *Development* 2005;132:4653–4662. [PubMed: 16224045]
12. Meltzer PS. Cancer genomics: small RNAs with big impacts. *Nature* 2005;435:745–746. [PubMed: 15944682]
13. Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci U S A* 2006;103:2257–2261. [PubMed: 16461460]
14. Wang V, Wu W. MicroRNA-based therapeutics for cancer. *BioDrugs* 2009;23:15–23. [PubMed: 19344188]
15. Hammond SM. MicroRNAs as oncogenes. *Curr Opin Genet Dev* 2006;16:4–9. [PubMed: 16361094]
16. Rodriguez A, Griffiths-Jones S, Ashurst JL, et al. Identification of mammalian microRNA host genes and transcription units. *Genome Res* 2004;14:1902–1910. [PubMed: 15364901]
17. Kim YK, Kim VN. Processing of intronic microRNAs. *EMBO J* 2007;26:775–783. [PubMed: 17255951]
18. Han J, Lee Y, Yeom KH, et al. Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. *Cell* 2006;125:887–901. [PubMed: 16751099]
19. Zeng Y, Cullen BR. Sequence requirements for micro RNA processing and function in human cells. *RNA* 2003;9:112–123. [PubMed: 12554881]
20. Zeng Y, Cullen BR. Efficient processing of primary microRNA hairpins by Drosha requires flanking nonstructured RNA sequences. *J Biol Chem* 2005;280:27595–27603. [PubMed: 15932881]
21. Lee Y, Ahn C, Han J, et al. The nuclear RNase III Drosha initiates microRNA processing. *Nature* 2003;425:415–419. [PubMed: 14508493]
22. Morlando M, Ballarino M, Gromak N, et al. Primary microRNA transcripts are processed co-transcriptionally. *Nat Struct Mol Biol* 2008;15:902–909. [PubMed: 19172742]
23. Lee Y, Kim M, Han J, et al. MicroRNA genes are transcribed by RNA polymerase II. *EMBO J* 2004;23:4051–4060. [PubMed: 15372072]
24. Cai X, Hagedorn CH, Cullen BR. Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. *RNA* 2004;10:1957–1966. [PubMed: 15525708]
25. Borchert GM, Lanier W, Davidson BL. RNA polymerase III transcribes human microRNAs. *Nat Struct Mol Biol* 2006;13:1097–1101. [PubMed: 17099701]
26. He L, He X, Lim LP, et al. A microRNA component of the p53 tumour suppressor network. *Nature* 2007;447:1130–1134. [PubMed: 17554337]
27. Okamura K, Hagen JW, Duan H, et al. The mirtron pathway generates microRNA-class regulatory RNAs in *Drosophila*. *Cell* 2007;130:89–100. [PubMed: 17599402]
28. Ruby JG, Jan CH, Bartel DP. Intronic microRNA precursors that bypass Drosha processing. *Nature* 2007;448:83–86. [PubMed: 17589500]
29. Guil S, Caceres JF. The multifunctional RNA-binding protein hnRNP A1 is required for processing of miR-18a. *Nat Struct Mol Biol* 2007;14:591–596. [PubMed: 17558416]
30. Davis BN, Hilyard AC, Lagna G, et al. SMAD proteins control DROSHA-mediated microRNA maturation. *Nature* 2008;454:56–61. [PubMed: 18548003]
31. Gregory RI, Yan KP, Amuthan G, et al. The Microprocessor complex mediates the genesis of microRNAs. *Nature* 2004;432:235–240. [PubMed: 15531877]
32. Carthew RW, Sontheimer EJ. Origins and Mechanisms of miRNAs and siRNAs. *Cell* 2009;136:642–655. [PubMed: 19239886]
33. Aleman LM, Doench J, Sharp PA. Comparison of siRNA-induced off-target RNA and protein effects. *RNA* 2007;13:385–395. [PubMed: 17237357]
34. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet* 2008;9:102–114. [PubMed: 18197166]
35. Pillai RS, Bhattacharyya SN, Artus CG, et al. Inhibition of translational initiation by Let-7 MicroRNA in human cells. *Science* 2005;309:1573–1576. [PubMed: 16081698]
36. Liu J, Valencia-Sanchez MA, Hannon GJ, et al. MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. *Nat Cell Biol* 2005;7:719–723. [PubMed: 15937477]

37. Sen GL, Blau HM. Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies. *Nat Cell Biol* 2005;7:633–636. [PubMed: 15908945]
38. Rossi JJ. RNAi and the P-body connection. *Nat Cell Biol* 2005;7:643–644. [PubMed: 15990892]
39. Collier J, Parker R. Eukaryotic mRNA decapping. *Annu Rev Biochem* 2004;73:861–890. [PubMed: 15189161]
40. Behm-Ansmant I, Rehwinkel J, Doerks T, et al. mRNA degradation by miRNAs and GW182 requires both CCR4:NOT deadenylase and DCP1:DCP2 decapping complexes. *Genes Dev* 2006;20:1885–1898. [PubMed: 16815998]
41. Giraldez AJ, Mishima Y, Rihel J, et al. Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. *Science* 2006;312:75–79. [PubMed: 16484454]
42. Wu L, Fan J, Belasco JG. MicroRNAs direct rapid deadenylation of mRNA. *Proc Natl Acad Sci U S A* 2006;103:4034–4039. [PubMed: 16495412]
43. Dalerba P, Cho RW, Clarke MF. Cancer stem cells: models and concepts. *Annu Rev Med* 2007;58:267–284. [PubMed: 17002552]
44. Clarke MF, Fuller M. Stem cells and cancer: two faces of eve. *Cell* 2006;124:1111–1115. [PubMed: 16564000]
45. Gupta PB, Onder TT, Jiang G, et al. Identification of selective inhibitors of cancer stem cells by high-throughput screening. *Cell* 2009;138:645–659. [PubMed: 19682730]
46. Huntly BJ, Gilliland DG. Leukaemia stem cells and the evolution of cancer-stem-cell research. *Nat Rev Cancer* 2005;5:311–321. [PubMed: 15803157]
47. Gilbertson RJ, Rich JN. Making a tumour's bed: glioblastoma stem cells and the vascular niche. *Nat Rev Cancer* 2007;7:733–736. [PubMed: 17882276]
48. Vescovi AL, Galli R, Reynolds BA. Brain tumour stem cells. *Nat Rev Cancer* 2006;6:425–436. [PubMed: 16723989]
49. Ricci-Vitiani L, Lombardi DG, Pilozzi E, et al. Identification and expansion of human colon-cancer-initiating cells. *Nature* 2007;445:111–115. [PubMed: 17122771]
50. Tothova Z, Gilliland DG. A radical bailout strategy for cancer stem cells. *Cell Stem Cell* 2009;4:196–197. [PubMed: 19265655]
51. Croce CM, Calin GA. miRNAs, cancer, and stem cell division. *Cell* 2005;122:6–7. [PubMed: 16009126]
52. Caldas C, Brenton JD. Sizing up miRNAs as cancer genes. *Nat Med* 2005;11:712–714. [PubMed: 16015356]
53. Kumar MS, Lu J, Mercer KL, et al. Impaired microRNA processing enhances cellular transformation and tumorigenesis. *Nat Genet* 2007;39:673–677. [PubMed: 17401365]
54. Calin GA, Sevignani C, Dumitru CD, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci U S A* 2004;101:2999–3004. [PubMed: 14973191]
55. Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. *Nat Rev Cancer* 2006;6:259–269. [PubMed: 16557279]
56. He X, He L, Hannon GJ. The guardian's little helper: microRNAs in the p53 tumor suppressor network. *Cancer Res* 2007;67:11099–11101. [PubMed: 18056431]
57. Tarasov V, Jung P, Verdoodt B, et al. Differential regulation of microRNAs by p53 revealed by massively parallel sequencing: miR-34a is a p53 target that induces apoptosis and G1-arrest. *Cell Cycle* 2007;6:1586–1593. [PubMed: 17554199]
58. Miyashita T, Reed JC. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. *Cell* 1995;80:293–299. [PubMed: 7834749]
59. Bommer GT, Gerin I, Feng Y, et al. p53-mediated activation of miRNA34 candidate tumor-suppressor genes. *Curr Biol* 2007;17:1298–1307. [PubMed: 17656095]
60. Chang TC, Wentzel EA, Kent OA, et al. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. *Mol Cell* 2007;26:745–752. [PubMed: 17540599]
61. Raver-Shapira N, Marciano E, Meiri E, et al. Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. *Mol Cell* 2007;26:731–743. [PubMed: 17540598]

62. Welch C, Chen Y, Stallings RL. MicroRNA-34a functions as a potential tumor suppressor by inducing apoptosis in neuroblastoma cells. *Oncogene* 2007;26:5017–5022. [PubMed: 17297439]
63. Leone G, DeGregori J, Yan Z, et al. E2F3 activity is regulated during the cell cycle and is required for the induction of S phase. *Genes Dev* 1998;12:2120–2130. [PubMed: 9679057]
64. Leone G, Nuckolls F, Ishida S, et al. Identification of a novel E2F3 product suggests a mechanism for determining specificity of repression by Rb proteins. *Mol Cell Biol* 2000;20:3626–3632. [PubMed: 10779352]
65. McClellan KA, Ruzhynsky VA, Douda DN, et al. Unique requirement for Rb/E2F3 in neuronal migration: evidence for cell cycle-independent functions. *Mol Cell Biol* 2007;27:4825–4843. [PubMed: 17452454]
66. Dontu G, Jackson KW, McNicholas E, et al. Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells. *Breast Cancer Res* 2004;6:R605–R615. [PubMed: 15535842]
67. Han Z, Hong L, Han Y, et al. Phospho Akt mediates multidrug resistance of gastric cancer cells through regulation of P-gp, Bcl-2 and Bax. *J Exp Clin Cancer Res* 2007;26:261–268. [PubMed: 17725107]
68. Reed JC. Bcl-2 family proteins: regulators of apoptosis and chemoresistance in hematologic malignancies. *Semin Hematol* 1997;34:9–19. [PubMed: 9408956]
69. DiPaola RS, Aisner J. Overcoming bcl-2- and p53-mediated resistance in prostate cancer. *Semin Oncol* 1999;26:112–116. [PubMed: 10190792]
70. Makin G, Dive C. Apoptosis and cancer chemotherapy. *Trends Cell Biol* 2001;11:S22–S26. [PubMed: 11684438]
71. Reed JC. Bcl-2 family proteins: strategies for overcoming chemoresistance in cancer. *Adv Pharmacol* 1997;41:501–532. [PubMed: 9204157]
72. Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. *Oncogene* 2007;26:1324–1337. [PubMed: 17322918]
73. Phillips TM, McBride WH, Pajonk F. The response of CD24(–/low)/CD44+ breast cancer-initiating cells to radiation. *J Natl Cancer Inst* 2006;98:1777–1785. [PubMed: 17179479]
74. 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]
75. Ji Q, Hao X, Meng Y, et al. Restoration of tumor suppressor miR-34 inhibits human p53-mutant gastric cancer tumorspheres. *BMC Cancer* 2008;8:266. [PubMed: 18803879]
76. Morrison SJ, Kimble J. Asymmetric and symmetric stem-cell divisions in development and cancer. *Nature* 2006;441:1068–1074. [PubMed: 16810241]
77. Lin H. The stem-cell niche theory: lessons from flies. *Nat Rev Genet* 2002;3:931–940. [PubMed: 12459723]
78. Cicalese A, Bonizzi G, Pasi CE, et al. The Tumor Suppressor p53 Regulates Polarity of Self-Renewing Divisions in Mammary Stem Cells. *Cell* 2009;138:1083–1095. [PubMed: 19766563]
79. Gangaraju VK, Lin H. MicroRNAs: key regulators of stem cells. *Nat Rev Mol Cell Biol* 2009;10:116–125. [PubMed: 19165214]
80. Georgantas RW 3rd, Hildreth R, Morisot S, et al. CD34+ hematopoietic stem-progenitor cell microRNA expression and function: a circuit diagram of differentiation control. *Proc Natl Acad Sci U S A* 2007;104:2750–2755. [PubMed: 17293455]
81. Chen CZ, Li L, Lodish HF, et al. MicroRNAs modulate hematopoietic lineage differentiation. *Science* 2004;303:83–86. [PubMed: 14657504]
82. Bruchova H, Yoon D, Agarwal AM, et al. Regulated expression of microRNAs in normal and polycythemia vera erythropoiesis. *Exp Hematol* 2007;35:1657–1667. [PubMed: 17976518]
83. Fontana L, Pelosi E, Greco P, et al. MicroRNAs 17-5p-20a-106a control monocytopenia through AML1 targeting and M-CSF receptor upregulation. *Nat Cell Biol* 2007;9:775–787. [PubMed: 17589498]
84. Wang Q, Huang Z, Xue H, et al. MicroRNA miR-24 inhibits erythropoiesis by targeting activin type I receptor ALK4. *Blood* 2008;111:588–595. [PubMed: 17906079]

85. Felli N, Fontana L, Pelosi E, et al. MicroRNAs 221 and 222 inhibit normal erythropoiesis and erythroleukemic cell growth via kit receptor down- modulation. *Proc Natl Acad Sci U S A* 2005;102:18081–18086. [PubMed: 16330772]
86. Zhou B, Wang S, Mayr C, et al. miR-150, a microRNA expressed in mature B and T cells, blocks early B cell development when expressed prematurely. *Proc Natl Acad Sci U S A* 2007;104:7080–7085. [PubMed: 17438277]
87. Dore LC, Amigo JD, Dos Santos CO, et al. A GATA-1 -regulated microRNA locus essential for erythropoiesis. *Proc Natl Acad Sci U S A* 2008;105:3333–3338. [PubMed: 18303114]
88. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 1997;3:730–737. [PubMed: 9212098]
89. Yuan X, Curtin J, Xiong Y, et al. Isolation of cancer stem cells from adult glioblastoma multiforme. *Oncogene* 2004;23:9392–9400. [PubMed: 15558011]
90. Singh SK, Hawkins C, Clarke ID, et al. Identification of human brain tumour initiating cells. *Nature* 2004;432:396–401. [PubMed: 15549107]
91. Al-Hajj M, Wicha MS, Benito-Hernandez A, et al. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci U S A* 2003;100:3983–3988. [PubMed: 12629218]
92. Gibbs CP, Kukekov VG, Reith JD, et al. Stem-like cells in bone sarcomas: implications for tumorigenesis. *Neoplasia* 2005;7:967–976. [PubMed: 16331882]
93. Eisterer W, Jiang X, Christ O, et al. Different subsets of primary chronic myeloid leukemia stem cells engraft immunodeficient mice and produce a model of the human disease. *Leukemia* 2005;19:435–441. [PubMed: 15674418]
94. O'Brien CA, Pollett A, Gallinger S, et al. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007;445:106–110. [PubMed: 17122772]
95. Prince ME, Sivanandan R, Kaczorowski A, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proc Natl Acad Sci U S A* 2007;104:973–978. [PubMed: 17210912]
96. Kim CF, Jackson EL, Woolfenden AE, et al. Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* 2005;121:823–835. [PubMed: 15960971]
97. Fang D, Nguyen TK, Leishear K, et al. A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res* 2005;65:9328–9337. [PubMed: 16230395]
98. Li C, Heidt DG, Dalerba P, et al. Identification of pancreatic cancer stem cells. *Cancer Res* 2007;67:1030–1037. [PubMed: 17283135]
99. Collins AT, Berry PA, Hyde C, et al. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 2005;65:10946–10951. [PubMed: 16322242]
100. Patrawala L, Calhoun T, Schneider-Broussard R, et al. Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. *Oncogene* 2006;25:1696–1708. [PubMed: 16449977]
101. Filipowicz W. RNAi: the nuts and bolts of the RISC machine. *Cell* 2005;122:17–20. [PubMed: 16009129]
102. Cullen BR. RNAi the natural way. *Nat Genet* 2005;37:1163–1165. [PubMed: 16254559]
103. Zhang H, Kolb FA, Brondani V, et al. Human Dicer preferentially cleaves dsRNAs at their termini without a requirement for ATP. *EMBO J* 2002;21:5875–5885. [PubMed: 12411505]
104. Lee Y, Jeon K, Lee JT, et al. MicroRNA maturation: stepwise processing and subcellular localization. *EMBO J* 2002;21:4663–4670. [PubMed: 12198168]
105. Cullen BR. Transcription and processing of human microRNA precursors. *Mol Cell* 2004;16:861–865. [PubMed: 15610730]
106. Yi R, Qin Y, Macara IG, et al. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev* 2003;17:3011–3016. [PubMed: 14681208]
107. Lund E, Guttinger S, Calado A, et al. Nuclear export of microRNA precursors. *Science* 2004;303:95–98. [PubMed: 14631048]
108. Lee YS, Nakahara K, Pham JW, et al. Distinct roles for *Drosophila* Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. *Cell* 2004;117:69–81. [PubMed: 15066283]

109. Gossen M, Bujard H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc Natl Acad Sci U S A* 1992;89:5547–5551. [PubMed: 1319065]
110. Gupta S, Schoer RA, Egan JE, et al. Inducible, reversible, and stable RNA interference in mammalian cells. *Proc Natl Acad Sci U S A* 2004;101:1927–1932. [PubMed: 14762164]
111. Dickins RA, Hemann MT, Zilfou JT, et al. Probing tumor phenotypes using stable and regulated synthetic microRNA precursors. *Nat Genet* 2005;37:1289–1295. [PubMed: 16200064]
112. Tong AW, Zhang YA, Nemunaitis J. Small interfering RNA for experimental cancer therapy. *Curr Opin Mol Ther* 2005;7:114–124. [PubMed: 15844618]
113. Aigner A. Cellular delivery in vivo of siRNA-based therapeutics. *Curr Pharm Des* 2008;14:3603–3619. [PubMed: 19075737]
114. Tong AW, Jay CM, Senzer N, et al. Systemic therapeutic gene delivery for cancer: crafting Paris' arrow. *Curr Gene Ther* 2009;9:45–60. [PubMed: 19275571]
115. Vorhies JS, Nemunaitis J. Nonviral delivery vehicles for use in short hairpin RNA-based cancer therapies. *Expert Rev Anticancer Ther* 2007;7:373–382. [PubMed: 17338656]
116. Rao DD, Senzer N, Cleary MA, et al. Comparative assessment of siRNA and shRNA off target effects: what is slowing clinical development. *Cancer Gene Ther* 2009;16:807–809. [PubMed: 19713999]
117. Bumcrot D, Manoharan M, Koteliensky V, et al. RNAi therapeutics: a potential new class of pharmaceutical drugs. *Nat Chem Biol* 2006;2:711–719. [PubMed: 17108989]
118. Yew NS, Scheule RK. Toxicity of Cationic Lipid-DNA Complexes. *Adv Genet* 2005;53PA:189–214. [PubMed: 16243064]
119. Rao DD, Vorhies JS, Senzer N, et al. siRNA vs. shRNA: similarities and differences. *Adv Drug Deliv Rev* 2009;61:746–759. [PubMed: 19389436]
120. Fedorov Y, Anderson EM, Birmingham A, et al. Off-target effects by siRNA can induce toxic phenotype. *RNA* 2006;12:1188–1196. [PubMed: 16682561]
121. Jackson AL, Burchard J, Schelter J, et al. Widespread siRNA "off-target" transcript silencing mediated by seed region sequence complementarity. *RNA* 2006;12:1179–1187. [PubMed: 16682560]
122. Hoerter JA, Walter NG. Chemical modification resolves the asymmetry of siRNA strand degradation in human blood serum. *RNA* 2007;13:1887–1893. [PubMed: 17804643]
123. Zeng Y, Cai X, Cullen BR. Use of RNA polymerase II to transcribe artificial microRNAs. *Methods Enzymol* 2005;392:371–380. [PubMed: 15644193]
124. Hayashita Y, Osada H, Tatematsu Y, et al. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. *Cancer Res* 2005;65:9628–9632. [PubMed: 16266980]
125. Tran N, McLean T, Zhang X, et al. MicroRNA expression profiles in head and neck cancer cell lines. *Biochem Biophys Res Commun* 2007;358:12–17. [PubMed: 17475218]
126. Zhu S, Si ML, Wu H, et al. MicroRNA-21 targets the tumor suppressor gene tropomyosin 1 (TPM1). *J Biol Chem* 2007;282:14328–14336. [PubMed: 17363372]
127. Si ML, Zhu S, Wu H, et al. miR-21 -mediated tumor growth. *Oncogene* 2007;26:2799–2803. [PubMed: 17072344]
128. Meng F, Henson R, Lang M, et al. Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. *Gastroenterology* 2006;130:2113–2129. [PubMed: 16762633]
129. Fulci V, Chiaretti S, Goldoni M, et al. Quantitative technologies establish a novel microRNA profile of chronic lymphocytic leukemia. *Blood* 2007;109:4944–4951. [PubMed: 17327404]
130. Roldo C, Missiaglia E, Hagan JP, et al. MicroRNA expression abnormalities in pancreatic endocrine and acinar tumors are associated with distinctive pathologic features and clinical behavior. *J Clin Oncol* 2006;24:4677–4684. [PubMed: 16966691]
131. Yu F, Yao H, Zhu P, et al. let-7 regulates self renewal and tumorigenicity of breast cancer cells. *Cell* 2007;131:1109–1123. [PubMed: 18083101]
132. Ciafre SA, Galardi S, Mangiola A, et al. Extensive modulation of a set of microRNAs in primary glioblastoma. *Biochem Biophys Res Commun* 2005;334:1351–1358. [PubMed: 16039986]

133. Voorhoeve PM, le Sage C, Schrier M, et al. A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. *Cell* 2006;124:1169–1181. [PubMed: 16564011]
134. 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;64:3753–3756. [PubMed: 15172979]
135. Bottoni A, Piccin D, Tagliati F, et al. miR-15a and miR-16-1 down-regulation in pituitary adenomas. *J Cell Physiol* 2005;204:280–285. [PubMed: 15648093]
136. Calin GA, Dumitru CD, Shimizu M, et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A* 2002;99:15524–15529. [PubMed: 12434020]
137. Bonci D, Coppola V, Musumeci M, et al. The miR-15a-miR-16-1 cluster controls prostate cancer by targeting multiple oncogenic activities. *Nat Med* 2008;14:1271–1277. [PubMed: 18931683]
138. Hossain A, Kuo MT, Saunders GF. Mir-17-5p regulates breast cancer cell proliferation by inhibiting translation of AIB1 mRNA. *Mol Cell Biol* 2006;26:8191–8201. [PubMed: 16940181]
139. Yanaihara N, Caplen N, Bowman E, et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* 2006;9:189–198. [PubMed: 16530703]
140. Mott JL, Kobayashi S, Bronk SF, et al. mir-29 regulates Mcl-1 protein expression and apoptosis. *Oncogene* 2007;26:6133–6140. [PubMed: 17404574]
141. Fabbri M, Garzon R, Cimmino A, et al. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proc Natl Acad Sci U S A* 2007;104:15805–15810. [PubMed: 17890317]
142. Akao Y, Nakagawa Y, Naoe T. MicroRNAs 143 and 145 are possible common onco-microRNAs in human cancers. *Oncol Rep* 2006;16:845–850. [PubMed: 16969504]

Figure 1. MicroRNA biogenesis and function

The introns from full-length miRNA transcripts are excised to form pri-miRNA, which then undergoes Drosha processing. The DGCR8 acts as a molecular marker for Drosha cleavage by binding to the pri-miRNA 11 base pairs from the single stranded regions (SSR). After Drosha cleavage, the pre-miRNA is exported from the nucleus into the cytoplasm by Exportin-5, and it is the processed by Dicer into the mature miRNA. After RISC incorporation, miRNAs either inhibit translation or promote degradation of their target mRNA transcripts, depending on the degree of complementarity beyond the miRNA seed region (SR). Higher complementarity results in mRNA degradation, while lower complementarity results in translocation to P bodies and translational attenuation.

Figure 2. Regulation and control of miR-34

The miR-34 family acts as a tumor suppressor downstream of p53. The three mature microRNAs are incorporated into RISC and silence the listed targets, which normally promote tumorigenesis, by either translation repression or mRNA degradation.

Figure 3. Regulation of hematopoiesis by miRNAs

The steps of normal blood cell differentiation are activated by some microRNAs and inhibited by other microRNAs (shown with $\ominus$). Misregulation of any of these microRNAs could possibly lead to cancer.

Table I

MicroRNAs in Cancer

A. Up-regulated miRNAs in cancers			
miRNA	Sub family	Cancer	References
miR-17	-92	B-Cell Lymphoma	[124]
	-92	Lung	[124]
miR-21		Head And Neck	[125]
		Breast	[126,127]
		Cholangiocarcinoma	[128]
		Chronic Lymphocytic Leukemia	[129]
		Lung	[126]
miR-103		Pancreatic	[130]
miR-107		Pancreatic	[130]
miR-155		Chronic Lymphocytic Leukemia	[129]
		Breast	[131]
miR-205		Head And Neck	[125]
miR-221		Glioblastoma	[132]
miR-372		Testicular Germ	[133]
miR-373		Testicular Germ	[133]

B. Down-regulated miRNAs in cancers			
miRNA	Sub family	Cancer	References
let-7		Breast	[131]
		Lung	[134]
miR-10	b	Breast	[131]
miR-15		Pituitary Adenoma	[135]
	a	Chronic Lymphocytic Leukemia	[9,136]
		Lung	[9,137]
miR-16		Pituitary Adenoma	[135]
	-1	Chronic Lymphocytic Leukemia	[9,136]
		Lung	[9,137]
miR-17	-5p	Breast	[138]
miR-29	a	Non Small Cell Lung Cancer	[139]
	b	Non Small Cell Lung Cancer	[13,139]
	c	Non Small Cell Lung Cancer	[13,139]
		Cholangiocarcinoma	[140]
		Lung	[140,141]
miR-34		Gastric	[75]
	a	Neuroblastoma	[62]
	a	Pancreatic	[60]

B. Down-regulated miRNAs in cancers			
miRNA	Sub family	Cancer	References
	a-c	Lung	[62]
miR-125	b	Breast	[131]
miR-128		Glioblastoma	[132]
miR-143		Colon	[142]
miR-145		Colon	[142]
		Breast	[131]
miR-155		Pancreatic	[130]
miR-181	a/b/c	Glioblastoma	[132]