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Roles of microRNAs in cancer stem cells

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1. ABSTRACT

MicroRNAs are a class of endogenous non-coding RNAs that function as important regulatory molecules via the RNA interference mechanism. Since microRNAs play a fundamental role in regulation of a variety of cellular, physiological, and developmental processes, their aberrant expression can lead to a variety of human diseases including cancer. In particular, microRNAs have been implicated in regulation of stem cells as well as cancer stem cells. Given that cancer stem cells are believed to be responsible for the cancer initiation, metastasis and chemotherapy resistance, a better understanding of how microRNAs mediate gene expression in cancer stem cells will help identify novel cancer biomarkers and therapeutic targets, and as a result, it will aid in the development of better strategy for cancer treatment. In this review, we will update recent advances in microRNAs involved in cancer stem cells and their gene regulations in these cells.

2. INTRODUCTION

The main features of stem cells (SCs) are their ability to undergo self-renewal, as well as multi-lineage differentiation (1). SCs allow the generation of embryonic tissues and the maintenance of adult tissue (adult stem cells) and thus they play a key role in embryonic development. During embryogenesis, cells are initially proliferative and pluripotent; they only gradually become restricted to different cell fates. Unlike embryonic stem cells (ESCs), adult stem cells are found in numerous tissues of the body and play a role in tissue development, replacement, and repair (2).

Once established, the pluripotent ESCs can be maintained under defined culture conditions, but can also be induced rapidly to differentiate. Maintaining this balance of stability versus plasticity is a challenge, and extensive studies in recent years have focused on the understanding

of the contributions of transcription factors and epigenetic factors to the "stemness" properties of these cells. Identification of the molecular switches that regulate ES self-renewal versus differentiation can provide insights into the nature of the pluripotent state and enhance the potential use of these cells in therapeutic applications (3).

It is well known that the pluripotent state of SCs is under strict control by various key factors such as transcription factors and microRNAs. For example, transcription factors, such as OCT4, SOX2, NANOG, KLF4 and Myc, cooperate to ensure the self-renewal and pluripotency of ESCs (4). OCT4, SOX2 and NANOG are transcriptionally interconnected and co-occupy promoters of actively transcribed genes that promote ES self-renewal, such as KLF4 (5). On the other hand, microRNAs participate in this regulatory system by targeting these transcription factors or serve as downstream targets of the transcription factors. For example, miR-145 is highly expressed in differentiated cells whereas little expression is detected in ESCs or induced pluripotent stem cells (IPSCs) (6). Importantly, suppression of miR-145 alone is able to de-differentiate somatic cells to IPSC state, suggesting the significance of microRNAs in regulation of SCs.

Although still controversial, it is proposed that tumor cells are very heterogeneous and among this mixed population of the tumor cells, there exists a special subpopulation which expresses specific markers and behaves very much like SCs. Importantly, this subpopulation seems to be much more tumorigenic than the rest of the tumor cells. Although several terms have been used to identify this population, the term cancer stem cell (CSC) has received broad acceptance. Thus, CSCs have been defined as "cells within a tumor that possess the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor" (7). These two definitive biological properties are what make the CSCs the prime candidate for initiation of relapse, thereby becoming a crucial target for the development of novel therapies (8). Understanding the genetic basis for cancer development is an important step in the development of novel therapies targeting the CSCs. Numerous genes and signaling pathways, such as Notch which is connected with stem cell biology, have been identified as important in cancer biology. Through regulation of these pathways, microRNAs play a crucial role in control of SCs and CSCs.

3. MICRORNA FUNCTION AND REGULATION

It is evident now that the human genome makes a large amount of non-protein coding RNAs, among which are microRNAs with ~22 nucleotides in length. Since their discovery in 1993 in *C. elegans* (9), and later in humans (10), the number of microRNA has been steadily growing. To date, more than 1,000 human microRNAs have been identified and registered (www.mirbase.org). Accumulating evidence suggests that microRNAs are master gene regulators through the posttranscriptional repression mechanism and thus they play a fundamental role in diverse cellular processes such as cell growth, proliferation, and apoptosis.

Like protein-coding genes, microRNAs can be transcribed by RNA polymerase II (11) to produce primary transcripts (pri-microRNAs), which are then subject to several steps of processing, leading to final mature microRNAs. MicroRNAs can be derived from independent genes or imbedded in introns of protein-coding genes (12). These pri-microRNAs contain a cap structure at the 5' end and are poly-adenylated at the 3'end, suggesting that pri-microRNAs are structurally and functionally similar to mRNAs (13). The pri-microRNAs fold into hairpins, which act as substrates for a large nuclear microprocessor complex consisting of Drosha and the essential DiGeorge syndrome critical region gene 8 (DGCR8) binding protein (14). The product of Drosha cleavage, a ~70 nucleotide hairpin intermediate (pre-microRNA), is transported out of the nucleus by exportin-5 and its cofactor Ran-GTP (15). In the cytoplasm, the pre-microRNA is further processed to produce a short imperfect microRNA duplex (16), which is then unwounded into a mature microRNA. Finally, the mature microRNA is incorporated into the RNA induced silencing complex (RISC) and exerts its gene silencing function through the complementarity of microRNA-target mRNA (17). Given the nature of microRNA targeting, they are believed to regulate a large number of coding genes involved in almost every cellular process.

Early studies have suggested the importance of microRNAs in development. For example, lin-4 and let-7 were shown to regulate expression of genes involved in development in *C. elegans* (9, 18, 19). It becomes evident now that microRNAs may have much broader roles. For instance, there is overwhelming evidence that microRNAs play an important role in cell proliferation and apoptosis. In this regard, cancer cells are often manifested by alterations of cell proliferation and apoptosis. A good example is miR-16 which specifically targets Bcl-2 and thus, downregulation or deletion of miR-16 is frequently associated with oncogenesis (20). In addition, miR-34 family is positively regulated by p53 (21). Overexpression of miR-34a promotes p53-mediated apoptosis (22, 23). On the other hand, miR-21 exerts an anti-apoptotic function because suppression of miR-21 leads to increased apoptosis (24, 25). More importantly, microRNAs are important for stem cell division and maintenance**.** Isolation of a number of stem cell-specific microRNAs implicates their role in controlling stem cell division (26). For instance, the microRNA pathway is required for proper control of germline stem cell (GSC) division in drosophila (27). Using GSC mutants for dicer-1 (dcr-1), Hatfield et al were able to show that these mutants are defective in cell cycle control (27). We will discuss these aspects in more details later.

In addition to their role in the regulation of numerous genes, recent evidence suggests that microRNAs themselves are also subject to regulation by various mechanisms. First, like many other genes, microRNAs can be regulated at the transcriptional level. For instance, c-Myc is able to transcriptionally activate several microRNAs such as the miR-17~92 cluster (28). We have shown that p53 can directly activate the expression of miR-145 through binding to a potential p53 response element

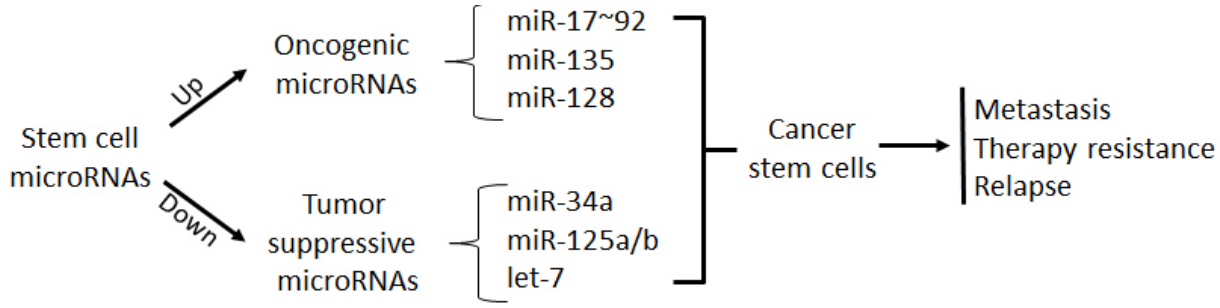

Figure 1. MicroRNA-mediated gene regulation and their role in cancer stem cells. Listed here are examples of a few of well-characterized microRNAs implicated in regulation of signaling pathways important to cancer stem cells.

(p53RE) in the miR-145 promoter (29). On the other hand, Oct4 can suppress miR-145 (6); similarly, miR-21 is repressed by REST to maintain stemness (30). Second, regulation of microRNA processing can also be critical. Due to multiple steps of microRNA processing, several important factors are required for this process. For example, accumulation of Dicer is dependent on its partner TRBP, and a decrease in TRBP leads to Dicer destabilization and pre-microRNA processing defects (31). Third, many pri-microRNAs and pre-microRNAs, such as pri-miR-142 (32) and pre-miR-151(33), are targeted by adenosine deaminases that act on RNA (ADARs) at different stages in their processing, and the modifications can affect both Drosha-mediated and Dicer-mediated cleavage, and also prevent the export of pre-microRNAs. Moreover, those effectors which are downstream of microRNA processing pathway, such as AGO and GW182, can also mediate microRNA function (34). Finally, other mechanisms may involve the regulation of microRNA intracellular localization, repression or decay (12).

Due to these regulation mechanisms, microRNAs are often aberrantly expressed in cancer. More importantly, this type of dysregulation of microRNA expression is also reflected in cancer stem cells, which may serve as a driving force for chemoresistance and the initiation of relapse. Thus, a better understanding of how these microRNAs are regulated will help identify novel biomarkers for cancer diagnosis as well as novel therapeutic targets.

4. MICRORNAS INVOLVED IN THE SELF-RENEWAL AND DIFFERENTIATION OF STEM CELLS

Disruption of genes or pathways involved in the regulation of SC self-renewal seems to be important in the SC model of cancer (35-37). It has been suggested that cancer can be initiated by a loss of polarity in SCs that may lead to impairment of asymmetric cell division, rendering the daughter cells unable to respond to normal homeostasis mechanisms that regulate cell proliferation (38). An emerging role of microRNAs in the regulation of SC self-renewal and differentiation has been revealed (39). During development, microRNA expression seems to be tissue specific, and this may imply that microRNAs are essential to establish and maintain cell type and tissue identity (40, 41). Coordinated transcription factor networks involving OCT4, SOX2, and NANOG have currently emerged as the master regulatory mechanisms of SC pluripotency and differentiation (42), and regulation of these factors by microRNAs may be a key event during ESC differentiation (6, 43). For example, miR-302 is able to convert differentiated cells to induced pluripotent stem cells through regulation of OCT4/SOX2/NANOG (44). In another case, several microRNAs associated with glioblastomas may have a normal function in regulating neural SC self-renewal and differentiation during development (45). However, their dysfunction in cancer may contribute to the maintenance of an undifferentiated proliferative phenotype by preventing the expression of differentiation targets and allowing the expression of targets promoting SC renewal (46).

However, there are great challenges with respect to identification and control of tumor initiating and propagating cells. ESCs are known to rely on polycomb group proteins to reversibly repress genes required for differentiation (47). Interestingly, it has been hypothesized that acquisition of promoter DNA methylation at these repressed genes could permanently silence them and thereby lock the cell into a perpetual state of self-renewal (47-49), predisposing to subsequent malignant transformation and cancer development. Epigenetic repressive pathways involving polycomb complexes, DNA methylation, and microRNAs seem to cooperate to reduce transcriptional noise and to prevent aberrant induction of differentiation. Given the great advances in recent year on microRNA characterization, microRNA signatures due to their dysregulation along with the corresponding key transcription factors may help identify such a cell population

5. MICRORNAS AND CANCER STEM CELLS (CSCS)

Accumulating evidence indicates that microRNAs are important regulators in CSC biology. Given that the early phases of carcinogenesis resembles embryonic development, often involving the re-expression of embryonic mesenchymal genes, regulation of these genes by microRNAs may play a critical role during this process. In this regard, microRNAs have been shown by both in vitro and in vivo models that they can function as either oncogenes or tumor suppressors. For example, many known oncogenic microRNAs are expressed in early stages of development in undifferentiated cells; however, their expression decreases in differentiated tissues. In contrast,

tumor suppressive microRNAs are increased in differentiated cells. Emerging evidence further suggests that alterations of microRNA expression cause CSC dysregulation, and lead to unlimited self-renewal and cancer progression, highlighting the significance of microRNAs in CSCs.

MicroRNAs involved in the regulation of SC can be referred to as "stem cell microRNAs" which apparently interact with "stem cell genes" in CSCs and play a vital role in the regulation of these genes and their downstream signaling pathways in cancer. Figure 1 lists some of important microRNAs and their potential links to CSCs to demonstrate that microRNAs have a pivotal function in carcinogenesis and oncogenesis by regulating self-renewal and apoptosis via CSC signaling pathways. Dysregulation of the SC genes allows them to escape the restrictions of the SC niche, resulting in unlimited self-renewal ability and potential, which further promotes cancer development and resistance to current therapies. Aberrant expression of these microRNAs is not only connected to cancers in general, but it is involved in CSC dysregulation. Functional studies of specific microRNAs within the CSCs of various cancers are crucial for the elucidation of the mechanisms behind oncogenesis in various cancers (39).

5. MICRORNA-MEDIATED "STEM CELL GENES" AND THEIR SIGNALING PATHWAYS

There is growing evidence that many pathways classically connected with cancer may also regulate normal SC development (37), implying that their connection may be important to CSCs. For example, the pivotal SC genes/signaling pathways such as Notch, Hedgehog, Wnt/β-catenin, HMGA2 (high-mobility group AT-hook 2), Bcl-2, and Bmi-1, are involved in the regulation of self-renewal, differentiation, and survival of CSCs (37, 50, 51). Recent evidence further suggests that these genes are often dysregulated in CSCs, and thus, understanding of how these genes/signaling pathways are regulated and whether they can be intervened may offer great promise for cancer therapy. We will discuss the following examples of microRNA-mediated regulation of these genes/signaling pathways to highlight the importance of microRNAs in regulation of CSCs.

5.1. Notch

The Notch is a short-range communication transducer that is involved in regulating many cellular processes during development and renewal of adult tissues. Notch signaling has been implicated as a pathway that aids in development of the breast and is frequently dysregulated in invasive breast cancer (52). Notch signaling is also important for mammary SCs to promote self-renewal and can act on early progenitor cells to promote their proliferation (53). This notion is supported by the finding that these effects can be completely inhibited by either a Notch 4 antibody or a gamma secretase inhibitor that blocks Notch processing (53). Therefore, atypical Notch signaling could lead to dysregulation of the self-renewal properties of CSCs, resulting in carcinogenesis and oncogenesis (54) (55).

MicroRNAs capable of regulating Notch signaling pathway have been well demonstrated in glioma and medulloblastoma stem cells. For example, the tumor suppressor miR-34a directly silences oncogenes c-Met, Notch-1 and Notch-2; and Notch partially mediates the inhibitory effects of miR-34a on cell proliferation and cell death (56). Furthermore, miR-34a inhibits various malignancy endpoints in glioma SCs and induces glioma SC differentiation (57). Several microRNAs such as miR-326 and miR-199b-5p have been recently shown to indirectly target the Notch signaling pathway. For example, ectopic expression of miR-326 in glioma and glioma SCs induces their apoptosis and reduces their metabolic activity (58). Further study indicates that miR-326 is downregulated in gliomas via decreased expression of its host gene. Transfection of miR-326 into both established and stem cell-like glioma lines is cytotoxic, which can be rescued by Notch restoration. Thus, miR-326/Notch axis may provide new insight into the biology of Notch, suggesting the possibility of miR-326 as a therapeutic agent (59).

In medulloblastoma cells, miR-199b-5p has been shown to down-regulate the expression of HES1, a transcription factor of the Notch signaling pathway (55). Expression of miR-199b-5p is often lost in metastatic cancer patients. Ectopic expression of miR-199b-5p is able to block Notch signaling and cause a decrease in the medulloblastoma stem cell like (CD133+) subpopulation of cells (55). Therefore, as a tumor suppressor, miR-199b-5p is important to the CSC self-regulation via the Notch signaling pathway. It is well known that miR-34a is a tumor suppressor in various types of tumors, and it also plays a suppressive role in human pancreatic cancer tumor-initiating cells by Notch 1/2 and Bcl-2. More significantly, miR-34a restoration causes an 87% reduction of the tumor-initiating cell population, accompanied by significant inhibition of tumorsphere growth in vitro and tumor formation in vivo (60).

5.2. Hedgehog

Hedgehog signaling has been implicated in the regulation of self-renewal characteristics because cell populations enriched for human hematopoietic stem cells exhibit increased self-renewal in response to Sonic Hedgehog (SHH) stimulation in vitro (37, 61). In humans, several distinctive cancers, including basal-cell carcinoma, result from mutations that aberrantly activate Hedgehog signal transduction. For example, ovarian SCs cannot proliferate in the absence of Hedgehog signaling, whereas excessive Hedgehog signaling produces excessive SCs, implying Hedgehog as an SC factor.

SHH signaling pathway has also been implicated in medulloblastomas SCs. In this regard, the miR-17~92 cluster is able to activate the SHH signaling pathway in engineered medulloblastomas (62), resulting in increased self-renewal. A similar relationship between the miR-17~92 cluster and Hedgehog was reported by Northcott et al (63) who found that high levels of miR-17~92 are a hallmark of SHH-associated medulloblastoma in humans and in mice, and that their

expression correlates with high levels of MYC family proto-oncogenes. Furthermore, in normally proliferating cerebellar granule neural precursors (CGNPs) miR-17~92 is a SHH target whose expression is regulated by N-Myc. Thus, it appears that miR-17~92 is a positive effector of SHH-mediated proliferation and aberrant expression/amplification of this microRNA cluster confers a growth advantage to medulloblastomas. However, the underlying mechanism remains to be determined.

Several other microRNAs have also been implicated in regulation of Hedgehog signaling in cerebellar granule cell progenitor development and mat play a role in neoplastic transformation into medulloblastoma. For example, miR-125b and miR-326 function as suppressors of Smoothened together with miR-324-5p, which also targets the downstream transcription factor Gli1 (64). Their expression is often downregulated in cerebellar neuronal progenitors, but it increases during differentiation, thereby causing cell maturation and growth inhibition.

5.3. Wnt/β-catenin

Another important pathway that regulates both self-renewal and oncogenesis in different tissues is the Wnt/β-catenin signaling pathway (37). Activation of the Wnt receptor promotes the nuclear translocation of β-catenin which drives the expression of genes associated with self-renewal and increases the pool of SCs (65). Dysregulation of the Wnt/β-catenin signaling pathway contributes to the onset of cancer. Gain or loss-of function mutations of several members of this pathway have been found in many types of human tumors. For instance, in chronic myelogenous leukemia, β-catenin accumulates in the nuclei of granulocyte-macrophage progenitors, and it subsequently enhances the self-renewal activity and leukemic potential of these cells.

As oncogenes, miR-135a and miR-135b have been shown to be upregulated in colorectal adenomas and carcinomas (66). In contrast, APC is downregulated in cancers with increased expression of miR-135a and miR-135b (66). A recent study demonstrated that the miR-181 family members are upregulated in $EpCAM^+AFP^+$ HCCs and in $EpCAM^+$ HCC cells isolated from AFP^+ tumors with cancer stem/progenitor cell features (67). Moreover, the miR-181 family members are highly expressed in embryonic livers and in isolated hepatic SCs (67). Importantly, inhibition of miR-181 reduces $EpCAM^+$ HCC cell quantity and tumor initiating ability, while ectopic expression of miR-181 in HCC cells results in an enrichment of $EpCAM^+$ HCC cells.

5.4. HMGA2

HMGA2 has also been implicated in survival and self-renewal of CSCs, possibly through modulating macromolecule complexes that are involved in many biological processes (68). The expression of HMGA proteins during embryogenesis suggests that they have important functions in development. Moreover, the HMGA2 gene is able to control growth, proliferation, and differentiation and thus implicated in cancer. Overexpression of HMGA2 has been reported in lung and pancreatic carcinomas (69), and is often associated with metastasis and reduced survival of the cancer patient (68). Thus, human cancers due to excessive HMGA2 signaling might result from dysregulated cell survival and self-renewal properties of CSCs.

let-7 is a very conserved microRNAs and its expression is reduced in various lung cancer cell lines and pulmonary tumors, compared to normal lung samples (70, 71). It has been shown to target several important oncogenes. In addition to its ability to suppress the Ras oncogene (72), let-7 also targets HMGA2 (73). Of interest, chromosomal translocations previously associated with human tumors disrupt repression of HMGA2 by let-7 because these translocations involve the let-7 binding site, suggesting that HMGA2 is a direct target of let-7. Furthermore, very low levels of the let-7 family are found in breast tumor-initiating cells (74). Ectopic expression let-7 in breast tumor-initiating cells reveals the key features of breast CSCs: self-renewal in vitro, multipotent differentiation, and the ability to form tumors (74).

5.5. Bmi-1

Bmi-1 has been shown to be expressed in neural SCs and proliferating progenitor cells, but not in differentiated cells (50). Loss of Bmi-1 can result in a drastic decrease in neural SC proliferation and self-renewal (50). Bmi-1 has also been implicated in cancer because Bmi-1 is able to promote the generation of lymphomas (75), highlighting its importance in both SCs and cancer. Moreover, Bmi-1 is often activated in human breast CSCs characterized as CD44+CD24-/lowLin (76). Furthermore, Bmi-1 is able to mediate the mammosphere-initiating cell number and mammosphere size, supporting a role in the regulation of self-renewal of normal and tumorigenic human mammary SCs (76). Therefore, dysregulation of this Bmi-1 pathway within CSCs may be associated with the acquisition of self-renewal properties.

miR-128 is also a tumor suppressor involved in CSCs. In high-grade gliomas, miR-128 levels are significantly reduced, suggesting tumor suppressor properties (77). Glioma cell proliferation and growth can be inhibited by introduction of exogenous miR-128 (77). This tumor suppressor activity is also demonstrated in medulloblastoma because miR-128a can increase the intracellular ROS level by targeting Bmi-1 and this activity is partially mediated by targeting Bmi-1 (78). The implication of this finding is the possibility to modulate redox states by miR-128a in CSCs to reduce therapy resistance due to their low ROS states.

miR-302 is the major microRNA found in human ESCs and IPSCs. In addition to its ability to suppress key transcription factors, miR-302 simultaneously suppresses Bmi-1as well as the cyclin E-CDK2 and cyclin D-CDK4/6 pathways to block the G_1-S transition (79). Concurrent silencing of Bmi-1 by miR-302 further promotes tumor suppressor functions of p16Ink4a and p14/p19Arf directed against CDK4/6-mediated cell proliferation.

6. CONCLUSION

We have learned in the past that as master gene regulators, microRNAs play a fundamental role in

regulation of diverse cellular pathways. In particular, these pathways are important to the maintenance and control of SCs and CSCs. However, the underlying mechanism is still not fully understood. It appears that abnormal expression of SC microRNAs is connected to the formation of CSCs, which may lead to the initiation, development and progression of cancer. A key to understand the role of microRNAs in CSCs may be to determine how these SC microRNAs are regulated and differentially expressed in SCs and CSCs. Therefore, understanding the underlying mechanism leading to the dysregulation of microRNAs would be an exciting area of future research. Thus, identification of the critical factors required for microRNA regulation and determination of how their differential regulations in SCs and CSCs may help to develop better strategies against cancer progression, therapy resistance and relapse.

7. REFERENCE

1. G. Dontu, M. Al-Hajj, W. M. Abdallah, M. F. Clarke and M. S. Wicha: Stem cells in normal breast development and breast cancer. *Cell Prolif*, 36 Suppl 1, 59-72 (2003)

2. R. I. Glazer, X. Wang, H. Yuan and Y. Yin: Mammary stem and progenitor cell regulation. *Cancer Biomark*, 3(4-5), 171-81 (2007)

3. N. S. Christophersen and K. Helin: Epigenetic control of embryonic stem cell fate. *J Exp Med*, 207(11), 2287-95 (2010)

4. Y. H. Loh, Q. Wu, J. L. Chew, V. B. Vega, W. Zhang, X. Chen, G. Bourque, J. George, B. Leong, J. Liu, K. Y. Wong, K. W. Sung, C. W. Lee, X. D. Zhao, K. P. Chiu, L. Lipovich, V. A. Kuznetsov, P. Robson, L. W. Stanton, C. L. Wei, Y. Ruan, B. Lim and H. H. Ng: The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. *Nat Genet*, 38(4), 431-40 (2006)

5. L. A. Boyer, T. I. Lee, M. F. Cole, S. E. Johnstone, S. S. Levine, J. P. Zucker, M. G. Guenther, R. M. Kumar, H. L. Murray, R. G. Jenner, D. K. Gifford, D. A. Melton, R. Jaenisch and R. A. Young: Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell*, 122(6), 947-56 (2005)

6. N. Xu, T. Papagiannakopoulos, G. Pan, J. A. Thomson and K. S. Kosik: MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells. *Cell*, 137(4), 647-58 (2009)

7. M. F. Clarke, J. E. Dick, P. B. Dirks, C. J. Eaves, C. H. Jamieson, D. L. Jones, J. Visvader, I. L. Weissman and G. M. Wahl: Cancer stem cells--perspectives on current status and future directions: AACR Workshop on cancer stem cells. *Cancer Res*, 66(19), 9339-44 (2006)

8. S. Bomken, K. Fiser, O. Heidenreich and J. Vormoor: Understanding the cancer stem cell. *Br J Cancer*, 103(4), 439-45 (2010)

9. R. C. Lee, R. L. Feinbaum and V. Ambros: The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell*, 75(5), 843-54 (1993)

10. M. Lagos-Quintana, R. Rauhut, W. Lendeckel and T. Tuschl: Identification of novel genes coding for small expressed RNAs. *Science*, 294(5543), 853-8 (2001)

11. Y. Lee, M. Kim, J. Han, K. H. Yeom, S. Lee, S. H. Baek and V. N. Kim: MicroRNA genes are transcribed by RNA polymerase II. *Embo J*, 23(20), 4051-60 (2004)

12. J. Krol, I. Loedige and W. Filipowicz: The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet*, 11(9), 597-610 (2010)

13. X. Cai, C. H. Hagedorn and B. R. Cullen: Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. *Rna*, 10(12), 1957-66 (2004)

14. J. Han, Y. Lee, K. H. Yeom, Y. K. Kim, H. Jin and V. N. Kim: The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev*, 18(24), 3016-27 (2004)

15. R. Yi, Y. Qin, I. G. Macara and B. R. Cullen: Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev*, 17(24), 3011-6 (2003)

16. A. D. Haase, L. Jaskiewicz, H. Zhang, S. Laine, R. Sack, A. Gatignol and W. Filipowicz: TRBP, a regulator of cellular PKR and HIV-1 virus expression, interacts with Dicer and functions in RNA silencing. *EMBO Rep*, 6(10), 961-7 (2005)

17. J. Liu, M. A. Valencia-Sanchez, G. J. Hannon and R. Parker: MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. *Nat Cell Biol*, 7(7), 719-23 (2005)

18. B. Wightman, I. Ha and G. Ruvkun: Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. *Cell*, 75(5), 855-62 (1993)

19. B. J. Reinhart, F. J. Slack, M. Basson, A. E. Pasquinelli, J. C. Bettinger, A. E. Rougvie, H. R. Horvitz and G. Ruvkun: The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. *Nature*, 403(6772), 901-6 (2000)

20. G. A. Calin, C. D. Dumitru, M. Shimizu, R. Bichi, S. Zupo, E. Noch, H. Aldler, S. Rattan, M. Keating, K. Rai, L. Rassenti, T. Kipps, M. Negrini, F. Bullrich and C. M. Croce: 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*, 99(24), 15524-9 (2002)

21. L. He, X. He, L. P. Lim, E. de Stanchina, Z. Xuan, Y. Liang, W. Xue, L. Zender, J. Magnus, D. Ridzon, A. L.

Jackson, P. S. Linsley, C. Chen, S. W. Lowe, M. A. Cleary and G. J. Hannon: A microRNA component of the p53 tumour suppressor network. *Nature*, 447(7148), 1130-4 (2007)

22. T. C. Chang, E. A. Wentzel, O. A. Kent, K. Ramachandran, M. Mullendore, K. H. Lee, G. Feldmann, M. Yamakuchi, M. Ferlito, C. J. Lowenstein, D. E. Arking, M. A. Beer, A. Maitra and J. T. Mendell: Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. *Mol Cell*, 26(5), 745-52 (2007)

23. N. Raver-Shapira, E. Marciano, E. Meiri, Y. Spector, N. Rosenfeld, N. Moskovits, Z. Bentwich and M. Oren: Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. *Mol Cell*, 26(5), 731-43 (2007)

24. J. A. Chan, A. M. Krichevsky and K. S. Kosik: MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res*, 65(14), 6029-33 (2005)

25. F. Meng, R. Henson, M. Lang, H. Wehbe, S. Maheshwari, J. T. Mendell, J. Jiang, T. D. Schmittgen and T. Patel: Involvement of Human Micro-RNA in Growth and Response to Chemotherapy in Human Cholangiocarcinoma Cell Lines. *Gastroenterology*, 130(7), 2113-29 (2006)

26. B. Zhang, X. Pan and T. A. Anderson: MicroRNA: A new player in stem cells. *J Cell Physiol* (2006)

27. S. D. Hatfield, H. R. Shcherbata, K. A. Fischer, K. Nakahara, R. W. Carthew and H. Ruohola-Baker: Stem cell division is regulated by the microRNA pathway. *Nature*, 435(7044), 974-8 (2005)

28. K. A. O'Donnell, E. A. Wentzel, K. I. Zeller, C. V. Dang and J. T. Mendell: c-Myc-regulated microRNAs modulate E2F1 expression. *Nature*, 435(7043), 839-43 (2005)

29. M. Sachdeva, S. Zhu, F. Wu, H. Wu, V. Walia, S. Kumar, R. Elble, K. Watabe and Y. Y. Mo: p53 represses c-Myc through induction of the tumor suppressor miR-145. *Proc Natl Acad Sci U S A*, 106(9), 3207-12 (2009)

30. S. K. Singh, M. N. Kagalwala, J. Parker-Thornburg, H. Adams and S. Majumder: REST maintains self-renewal and pluripotency of embryonic stem cells. *Nature*, 453(7192), 223-7 (2008)

31. S. A. Melo, S. Ropero, C. Moutinho, L. A. Aaltonen, H. Yamamoto, G. A. Calin, S. Rossi, A. F. Fernandez, F. Carneiro, C. Oliveira, B. Ferreira, C. G. Liu, A. Villanueva, G. Capella, S. Schwartz, Jr., R. Shiekhattar and M. Esteller: A TARBP2 mutation in human cancer impairs microRNA processing and DICER1 function. *Nat Genet*, 41(3), 365-70 (2009)

32. A. D. Scadden: The RISC subunit Tudor-SN binds to hyper-edited double-stranded RNA and promotes its cleavage. *Nat Struct Mol Biol*, 12(6), 489-96 (2005)

33. Y. Kawahara, B. Zinshteyn, T. P. Chendrimada, R. Shiekhattar and K. Nishikura: RNA editing of the microRNA-151 precursor blocks cleavage by the Dicer-TRBP complex. *EMBO Rep*, 8(8), 763-9 (2007)

34. M. Ghildiyal, J. Xu, H. Seitz, Z. Weng and P. D. Zamore: Sorting of Drosophila small silencing RNAs partitions microRNA* strands into the RNA interference pathway. *Rna*, 16(1), 43-56 (2010)

35. B. B. Tysnes and R. Bjerkvig: Cancer initiation and progression: involvement of stem cells and the microenvironment. *Biochim Biophys Acta*, 1775(2), 283-97 (2007)

36. R. Pardal, M. F. Clarke and S. J. Morrison: Applying the principles of stem-cell biology to cancer. *Nat Rev Cancer*, 3(12), 895-902 (2003)

37. T. Reya, S. J. Morrison, M. F. Clarke and I. L. Weissman: Stem cells, cancer, and cancer stem cells. *Nature*, 414(6859), 105-11 (2001)

38. D. Bilder: Epithelial polarity and proliferation control: links from the Drosophila neoplastic tumor suppressors. *Genes Dev*, 18(16), 1909-25 (2004)

39. S. Hatfield and H. Ruohola-Baker: microRNA and stem cell function. *Cell Tissue Res*, 331(1), 57-66 (2008)

40. A. J. Giraldez, R. M. Cinalli, M. E. Glasner, A. J. Enright, J. M. Thomson, S. Baskerville, S. M. Hammond, D. P. Bartel and A. F. Schier: MicroRNAs regulate brain morphogenesis in zebrafish. *Science*, 308(5723), 833-8 (2005)

41. E. Wienholds, W. P. Kloosterman, E. Miska, E. Alvarez-Saavedra, E. Berezikov, E. de Bruijn, H. R. Horvitz, S. Kauppinen and R. H. Plasterk: MicroRNA expression in zebrafish embryonic development. *Science*, 309(5732), 310-1 (2005)

42. V. Kashyap, N. C. Rezende, K. B. Scotland, S. M. Shaffer, J. L. Persson, L. J. Gudas and N. P. Mongan: Regulation of stem cell pluripotency and differentiation involves a mutual regulatory circuit of the NANOG, OCT4, and SOX2 pluripotency transcription factors with polycomb repressive complexes and stem cell microRNAs. *Stem Cells Dev*, 18(7), 1093-108 (2009)

43. Y. Tay, J. Zhang, A. M. Thomson, B. Lim and I. Rigoutsos: MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. *Nature*, 455(7216), 1124-8 (2008)

44. K. D. Wilson, S. Venkatasubrahmanyam, F. Jia, N. Sun, A. J. Butte and J. C. Wu: MicroRNA profiling of human-induced pluripotent stem cells. *Stem Cells Dev*, 18(5), 749-58 (2009)

45. J. Godlewski, H. B. Newton, E. A. Chiocca and S. E. Lawler: MicroRNAs and glioblastoma; the stem cell connection. *Cell Death Differ*, 17(2), 221-8 (2010)

46. S. Volinia, G. A. Calin, C. G. Liu, S. Ambs, A. Cimmino, F. Petrocca, R. Visone, M. Iorio, C. Roldo, M. Ferracin, R. L. Prueitt, N. Yanaihara, G. Lanza, A. Scarpa, A. Vecchione, M. Negrini, C. C. Harris and C. M. Croce: A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci U S A*, 103(7), 2257-61 (2006)

47. L. Ringrose and R. Paro: Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. *Annu Rev Genet*, 38, 413-43 (2004)

48. K. Schuebel, W. Chen and S. B. Baylin: CIMPle origin for promoter hypermethylation in colorectal cancer? *Nat Genet*, 38(7), 738-40 (2006)

49. M. Widschwendter, H. Fiegl, D. Egle, E. Mueller-Holzner, G. Spizzo, C. Marth, D. J. Weisenberger, M. Campan, J. Young, I. Jacobs and P. W. Laird: Epigenetic stem cell signature in cancer. *Nat Genet*, 39(2), 157-8 (2007)

50. D. Zencak, M. Lingbeek, C. Kostic, M. Tekaya, E. Tanger, D. Hornfeld, M. Jaquet, F. L. Munier, D. F. Schorderet, M. van Lohuizen and Y. Arsenijevic: Bmi1 loss produces an increase in astroglial cells and a decrease in neural stem cell population and proliferation. *J Neurosci*, 25(24), 5774-83 (2005)

51. C. A. Fasano, J. T. Dimos, N. B. Ivanova, N. Lowry, I. R. Lemischka and S. Temple: shRNA knockdown of Bmi-1 reveals a critical role for p21-Rb pathway in NSC self-renewal during development. *Cell Stem Cell*, 1(1), 87-99 (2007)

52. G. Farnie and R. B. Clarke: Mammary stem cells and breast cancer--role of Notch signalling. *Stem Cell Rev*, 3(2), 169-75 (2007)

53. G. Dontu, K. W. Jackson, E. McNicholas, M. J. Kawamura, W. M. Abdallah and M. S. Wicha: Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells. *Breast Cancer Res*, 6(6), R605-15 (2004)

54. D. Hambardzumyan, O. J. Becher and E. C. Holland: Cancer stem cells and survival pathways. *Cell Cycle*, 7(10), 1371-8 (2008)

55. L. Garzia, I. Andolfo, E. Cusanelli, N. Marino, G. Petrosino, D. De Martino, V. Esposito, A. Galeone, L. Navas, S. Esposito, S. Gargiulo, S. Fattet, V. Donofrio, G. Cinalli, A. Brunetti, L. D. Vecchio, P. A. Northcott, O. Delattre, M. D. Taylor, A. Iolascon and M. Zollo: MicroRNA-199b-5p impairs cancer stem cells through negative regulation of HES1 in medulloblastoma. *PLoS One*, 4(3), e4998 (2009)

56. Y. Li, F. Guessous, Y. Zhang, C. Dipierro, B. Kefas, E. Johnson, L. Marcinkiewicz, J. Jiang, Y. Yang, T. D. Schmittgen, B. Lopes, D. Schiff, B. Purow and R. Abounader: MicroRNA-34a inhibits glioblastoma growth by targeting multiple oncogenes. *Cancer Res*, 69(19), 7569-76 (2009)

57. F. Guessous, Y. Zhang, A. Kofman, A. Catania, Y. Li, D. Schiff, B. Purow and R. Abounader: microRNA-34a is tumor suppressive in brain tumors and glioma stem cells. *Cell Cycle*, 9(6) (2010)

58. B. Kefas, L. Comeau, N. Erdle, E. Montgomery, S. Amos and B. Purow: Pyruvate kinase M2 is a target of the tumor-suppressive microRNA-326 and regulates the survival of glioma cells. *Neuro Oncol*, 12(11), 1102-12 (2010)

59. B. Kefas, L. Comeau, D. H. Floyd, O. Seleverstov, J. Godlewski, T. Schmittgen, J. Jiang, C. G. diPierro, Y. Li, E. A. Chiocca, J. Lee, H. Fine, R. Abounader, S. Lawler and B. Purow: The neuronal microRNA miR-326 acts in a feedback loop with notch and has therapeutic potential against brain tumors. *J Neurosci*, 29(48), 15161-8 (2009)

60. Q. Ji, X. Hao, M. Zhang, W. Tang, M. Yang, L. Li, D. Xiang, J. T. Desano, G. T. Bommer, D. Fan, E. R. Fearon, T. S. Lawrence and L. Xu: MicroRNA miR-34 inhibits human pancreatic cancer tumor-initiating cells. *PLoS One*, 4(8), e6816 (2009)

61. G. Bhardwaj, B. Murdoch, D. Wu, D. P. Baker, K. P. Williams, K. Chadwick, L. E. Ling, F. N. Karanu and M. Bhatia: Sonic hedgehog induces the proliferation of primitive human hematopoietic cells via BMP regulation. *Nat Immunol*, 2(2), 172-80 (2001)

62. T. Uziel, F. V. Karginov, S. Xie, J. S. Parker, Y. D. Wang, A. Gajjar, L. He, D. Ellison, R. J. Gilbertson, G. Hannon and M. F. Roussel: The miR-17~92 cluster collaborates with the Sonic Hedgehog pathway in medulloblastoma. *Proc Natl Acad Sci U S A*, 106(8), 2812-7 (2009)

63. P. A. Northcott, L. A. Fernandez, J. P. Hagan, D. W. Ellison, W. Grajkowska, Y. Gillespie, R. Grundy, T. Van Meter, J. T. Rutka, C. M. Croce, A. M. Kenney and M. D. Taylor: 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*, 69(8), 3249-55 (2009)

64. E. Ferretti, E. De Smaele, E. Miele, P. Laneve, A. Po, M. Pelloni, A. Paganelli, L. Di Marcotullio, E. Caffarelli, I. Screpanti, I. Bozzoni and A. Gulino: Concerted microRNA control of Hedgehog signalling in cerebellar neuronal progenitor and tumour cells. *Embo J*, 27(19), 2616-27 (2008)

65. T. Reya, A. W. Duncan, L. Ailles, J. Domen, D. C. Scherer, K. Willert, L. Hintz, R. Nusse and I. L. Weissman: A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature*, 423(6938), 409-14 (2003)

66. R. Nagel, C. le Sage, B. Diosdado, M. van der Waal, J. A. Oude Vrielink, A. Bolijn, G. A. Meijer and R. Agami:

Regulation of the adenomatous polyposis coli gene by the miR-135 family in colorectal cancer. *Cancer Res*, 68(14), 5795-802 (2008)

67. J. Ji, T. Yamashita, A. Budhu, M. Forgues, H. L. Jia, C. Li, C. Deng, E. Wauthier, L. M. Reid, Q. H. Ye, L. X. Qin, W. Yang, H. Y. Wang, Z. Y. Tang, C. M. Croce and X. W. Wang: Identification of microRNA-181 by genome-wide screening as a critical player in EpCAM-positive hepatic cancer stem cells. *Hepatology*, 50(2), 472-80 (2009)

68. A. Fusco and M. Fedele: Roles of HMGA proteins in cancer. *Nat Rev Cancer*, 7(12), 899-910 (2007)

69. B. Meyer, S. Loeschke, A. Schultze, T. Weigel, M. Sandkamp, T. Goldmann, E. Vollmer and J. Bullerdiek: HMGA2 overexpression in non-small cell lung cancer. *Mol Carcinog*, 46(7), 503-11 (2007)

70. C. D. Johnson, A. Esquela-Kerscher, G. Stefani, M. Byrom, K. Kelnar, D. Ovcharenko, M. Wilson, X. Wang, J. Shelton, J. Shingara, L. Chin, D. Brown and F. J. Slack: The let-7 microRNA represses cell proliferation pathways in human cells. *Cancer Res*, 67(16), 7713-22 (2007)

71. A. Esquela-Kerscher, P. Trang, J. F. Wiggins, L. Patrawala, A. Cheng, L. Ford, J. B. Weidhaas, D. Brown, A. G. Bader and F. J. Slack: The let-7 microRNA reduces tumor growth in mouse models of lung cancer. *Cell Cycle*, 7(6), 759-64 (2008)

72. S. M. Johnson, H. Grosshans, J. Shingara, M. Byrom, R. Jarvis, A. Cheng, E. Labourier, K. L. Reinert, D. Brown and F. J. Slack: RAS is regulated by the let-7 microRNA family. *Cell*, 120(5), 635-47 (2005)

73. C. Mayr, M. T. Hemann and D. P. Bartel: Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation. *Science*, 315(5818), 1576-9 (2007)

74. F. Yu, H. Yao, P. Zhu, X. Zhang, Q. Pan, C. Gong, Y. Huang, X. Hu, F. Su, J. Lieberman and E. Song: let-7 regulates self renewal and tumorigenicity of breast cancer cells. *Cell*, 131(6), 1109-23 (2007)

75. A. Sparmann and M. van Lohuizen: Polycomb silencers control cell fate, development and cancer. *Nat Rev Cancer*, 6(11), 846-56 (2006)

76. S. Liu, G. Dontu, I. D. Mantle, S. Patel, N. S. Ahn, K. W. Jackson, P. Suri and M. S. Wicha: Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. *Cancer Res*, 66(12), 6063-71 (2006)

77. J. Godlewski, M. O. Nowicki, A. Bronisz, S. Williams, A. Otsuki, G. Nuovo, A. Raychaudhury, H. B. Newton, E. A. Chiocca and S. Lawler: Targeting of the Bmi-1 oncogene/stem cell renewal factor by microRNA-128 inhibits glioma proliferation and self-renewal. *Cancer Res*, 68(22), 9125-30 (2008)

78. S. Venkataraman, I. Alimova, R. Fan, P. Harris, N. Foreman and R. Vibhakar: MicroRNA 128a increases intracellular ROS level by targeting Bmi-1 and inhibits medulloblastoma cancer cell growth by promoting senescence. *PLoS One*, 5(6), e10748 (2010)

79. S. L. Lin, D. C. Chang, S. Y. Ying, D. Leu and D. T. Wu: MicroRNA miR-302 Inhibits the Tumorigenecity of Human Pluripotent Stem Cells by Coordinate Suppression of the CDK2 and CDK4/6 Cell Cycle Pathways. *Cancer Res*, 70(22), 9473-82 (2010)

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