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microRNA and stem cell function

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Abstract

The identification and characterization of stem cells for various tissues has led to a greater understanding of development, tissue maintenance, and cancer pathology. Stem cells possess the ability to divide throughout their life and to produce differentiated daughter cells while maintaining a population of undifferentiated cells that remain in the stem cell niche and that retain stem cell identity. Many cancers also have small populations of cells with stem cell characteristics. These cells have been called cancer stem cells and are a likely cause of relapse in cancer patients. Understanding the biology of stem cells and cancer stem cells offers great promise in the fields of regenerative medicine and cancer treatment. microRNAs (miRNAs) are emerging as important regulators of post-transcriptional gene expression and are considered crucial for proper stem cell maintenance and function. miRNAs have also been strongly implicated in the development and pathology of cancer. In this review, we discuss the characteristics of various stem cell types, including cancer stem cells, and the importance of miRNAs therein.

Keywords

Stem cells; microRNA; Cancer; Regulators; Self-renewal; Division; Maintenance

Stem cell biology

Stem cells are undifferentiated cells that are defined by their ability for lifelong self-renewing division that results in a daughter that can differentiate and a daughter that remains a stem cell. The two major stem cell classes are embryonic stem (ES) cells and adult stem cells. ES cells are considered pluripotent and can be isolated from an embryo at the blastocyst stage (Martin 1981). These cells are capable of differentiating into a large number of disparate cell lineages in cell culture (Keller et al. 1993; Kennedy and Keller 2003). Stem cell populations have been identified in many adult tissues. These cells are essential for maintaining tissue homeostasis in the adult. Examples of adult stem cells include hematopoietic stem cells (Goodman and Hodgson 1962), which give rise to all of the cells in the blood, and neural stem cells (Williams et al. 1991; Kilpatrick and Bartlett 1993; Morshead et al. 1994), which give rise to neurons and glial cells in the nervous system. Stem cells of different classes are likely to display similarities that can be studied in model systems (e.g., mouse ES cells or germline stem cells (GSCs) in the fruit fly *Drosophila melanogaster*) to further our knowledge of general stem cell biology.

The formation of embryonic tissues and the regeneration of adult tissues in the animal kingdom both depend on stem cells. ES cells are considered pluripotent because of their ability to become almost any cell type if placed into an appropriate context (Boiani and

Scholer 2005). Adult stem cells are undifferentiated cells that reside in microenvironments known as niches, and they possess the ability to produce an undifferentiated stem cell and a daughter cell that may differentiate (Fuchs and Whartenby 2004). Interestingly, recent data suggest that human ES cells (hESCs), in some situations, are able to generate their own niche. One such instance is the in vitro production of hESC-derived fibroblasts (hdFs) that are responsive to basic fibroblast growth factor, and that surround the hESCs from which they spontaneously differentiate. These hdFs are the apparent source of paracrine signals, such as insulin-like growth factor-II and transforming growth factor- β 1, that sustain self-renewal and pluripotency in hESCs (Bendall et al. 2007), although analysis of hESC markers suggest that hdFs alone maintain the hESCs at a lower efficiency than the mouse embryonic fibroblasts that are typically used as feeder cells (Wang et al. 2005). However, conditions also exist in which the niche is not observed or required for hESC growth, as is the case with human embryonic carcinoma cells that express hESC markers (i.e., Oct4, Sox2, and Nanog) and, like hESCs, exhibit self-renewal and pluripotency (Greber et al. 2007). Stem cells are controlled by extrinsic signals from their regulatory niche and also by intrinsic factors, including the hyperdynamic plasticity of chromatin proteins (Xi and Xie 2005; Meshorer et al. 2006). Unlike other cell types, many stem cell populations retain the capacity to divide for the entire life of the organism (e.g., for over 4,700 years in the bristlecone pine tree “Methuselah”). However, this division must be carefully regulated because too few divisions will result in a loss of tissue homeostasis, whereas too many divisions might result in cancer.

Recent data suggest that stem cells have a special bivalent chromatin state that remains silenced, yet poised for differentiation; several transcription factors influence this chromatin state in ES cells. Nanog, Oct4, and Sox2 are turned on shortly after fertilization and are required to specify inner cell mass and to derive ES cell lines (Niwa 2007). A genome-wide analysis has shown sites of co-occupancy by all three factors in key regulatory genes, including some Hox genes (Boyer et al. 2005; Bernstein et al. 2006). Additionally, four transcription factors, viz., Oct-4, Sox2, Klf4, and c-Myc, can convert mouse embryonic fibroblasts into ES cell like cells or germline competent pluripotent cells (Takahashi and Yamanaka 2006; Okita et al. 2007; Wernig et al. 2007).

Researchers have discovered characteristic combinatorial expression patterns of genes that are routinely used to confirm stem cell identity. In hESCs, the transcription factors Nanog, Oct4, and Sox2 (Nichols et al. 1998; Velkey and O'Shea 2003; Moorthy et al. 2005; Mossman et al. 2005; Tai et al. 2005; Loh et al. 2006; Babaie et al. 2007) are expressed in the hESCs but not in the differentiated daughter cells. Similarly, the expression of Sca-1 and c-Kit in the absence of Flk-2, Lin, and CD48 expression forms the signature for hematopoietic stem cells (Flk-2⁻ Sca-1⁺ Lin⁻ c-Kit⁺ CD48⁻; Wiles and Keller 1991; Li and Johnson 1995; McKinstry et al. 1997; Phillips et al. 2000; Donnelly and Krause 2001; Sitnicka et al. 2003). A detailed understanding of each stem cell population and the differentiation of its daughter cells is essential for the design of effective cell-based therapies.

microRNAs as regulators of stem cell function

Stem cell function is controlled by concerted actions of extrinsic signals and intrinsic factors (Chen and McKearin 2003, 2005; Song et al. 2004b, 2007; Szakmary et al. 2005; Xi and Xie 2005; Meshorer et al. 2006; Ward et al. 2006). Experimental evidence has suggested that small RNAs regulate the stem cell character in plants and animals (Hatfield et al. 2007). Moreover, some microRNAs (miRNAs) are differentially expressed in stem cells, suggesting a specialized role in stem cell regulation (Houbaviy et al. 2003; Suh et al. 2004). Recent studies in *Drosophila* and mouse suggest that miRNAs are important regulators for

stem cell self-renewal, differentiation and division (Hatfield et al. 2005; Kanellopoulou et al. 2005; Murchison et al. 2005).

Cellular and developmental processes in which miRNAs play a role

Evidence from multiple systems has demonstrated that the miRNA pathway is necessary for the proper development of organisms. Dicer (a ribonuclease) is essential for proper mouse development (Bernstein et al. 2003). The developmentally important *let-7* and *lin-4* genes in *Caenorhabditis elegans* are now known to encode miRNAs that are expressed in precisely defined temporal windows and repress mRNAs with complementary sequences in their 3'-untranslated regions (UTRs); *let-7* interacts with and represses *lin-41* mRNA (Slack et al. 2000; necessary for repression of *lin-29*, a gene that encodes an adult cell fate-promoting transcription factor), whereas *lin-4* represses *lin-14* (Lee et al. 1993; Olsen and Ambros 1999; an activator of *lin-28*, which is required for proper developmental timing).

Mammalian HoxB mRNA is targeted by miR-196 and subject to regulation by the miRNA pathway (Yekta et al. 2004). Moreover, in mammals, the miRNAs miR-1 and miR-133 are important for muscle development (Chen et al. 2006), and miR-124 promotes neuronal differentiation (Cao et al. 2007; Makeyev et al. 2007; Visvanathan et al. 2007).

Molecular processes in which miRNAs play a role

The miRNA pathway is a form of post-transcriptional gene silencing (PTGS) that uses genomically encoded RNAs (viz., miRNAs), which are 21–23 nucleotides in length, to silence specific genes by targeting the 3'UTR of their mRNAs (Elbashir et al. 2001; Lai 2002). PTGS is mediated by an endogenous mechanism that uses short double-stranded RNAs (dsRNAs) to direct a complex of proteins known as RISC (RNA-induced silencing complex) to target RNA molecules with complementary sequences (Hammond et al. 2000, 2001b). At the core of RISC is a member of the Argonaute branch of the Argonaute family of RNA-binding proteins (Hammond et al. 2001a; Liu et al. 2004). The endogenous machinery that implements the miRNA pathway is also activated either by the introduction of anti-sense RNA that is complementary to endogenous species of mRNA or long dsRNA that is homologous to endogenous or exogenous (e.g., viral) mRNAs.

Small dsRNA types other than miRNAs

In *Drosophila*, repeat-associated short interfering RNAs (rasiRNAs) have also been characterized as a distinct class of small RNAs that act through Argonaute-family proteins. rasiRNAs are typically 26–27 nucleotides in length, and their function depends on the Piwi-Aubergine branch of the Argonaute family, which consists of the Piwi, Aubergine, and Argonaute-3 proteins (Vagin et al. 2006), rather than the Argonaute branch, which consists of Argonaute-1 (AGO1) and Argonaute-2 (AGO2), and which is essential for the miRNA and siRNA pathways. In *Mus musculus*, RNAs of 29–31 nucleotides in length have been found in physical association with Miwi and Mili, murine homolog of Piwi, in pull-down experiments of cell extracts and have been called piRNAs because of their association with the Piwi homolog (Aravin et al. 2006, 2007; Grivna et al. 2006). Moreover, piRNAs have been identified in *Rattus norvegicus* (Lau et al. 2006). piRNAs also possess a 2'-O-methyl group on the 3' terminal nucleotide, a feature that is shared with plant, but not animal, miRNAs (Yang et al. 2006). In *Drosophila*, this methyl group is added to RISC-bound single-stranded RNAs by the *Drosophila* homolog of *Arabidopsis* HEN1 methyltransferase (Horwich et al. 2007; Saito et al. 2007). Similarities between features of *Drosophila* rasiRNAs and mammalian piRNAs, such as the dependence of both on Piwi-Aubergine proteins and the involvement of both in transposon silencing, suggest that these two recently described classes of short RNA are evolutionarily related.

miRNA biogenesis

Whereas siRNAs are derived from long dsRNA introduced by an infecting dsRNA virus or formed by the hybridization of an ectopically expressed antisense RNA to a complementary endogenous mRNA, metazoan miRNAs are encoded by genes that are transcribed by RNA polymerase II (and in some rare cases, RNA polymerase III). Many of the miRNAs are originally transcribed from the intronic region of mRNAs (Rodriguez et al. 2004) and are then excised as primary miRNAs (pri-miRNAs) that are 400–500 nucleotides long and that are processed by the nuclear type III RNase Drosha (Lee et al. 2003; Han et al. 2004) into hairpins of ~70 nucleotide-long RNAs containing hairpin structures known as pre-miRNAs. Additionally, some miRNAs, known as mirtrons (Ruby et al. 2007) bypass Drosha processing in *Drosophila* only. The premiRNAs are subsequently exported to the cytoplasm via Exportin-5. Both pre-miRNAs and long dsRNAs are processed into miRNAs and siRNAs, respectively, by the cytoplasmic type III RNase Dicer (Bernstein et al. 2001). In *Drosophila*, there are two known Dicer isozymes (Lee et al. 2004): Dicer-1, which specializes in miRNA production but also has limited siRNA-generating capacity, and Dicer-2, which specializes in siRNA production and has no demonstrated role in miRNA biogenesis. In mice and humans, only one Dicer enzyme has been characterized.

Implementation of miRNAs and other short dsRNAs

The earliest identified protein factors involved in RNA interference (RNAi) and/or their homologs have demonstrated roles in at least four important cellular processes: (1) PTGS, which is also known as quelling in fungi and in certain cases co-suppression in plants; (2) viral defense, which is especially important in plants but also demonstrated in *Drosophila*; (3) transposon silencing, insuring genomic stability; and (4) chromatin remodeling. PTGS is the process by which a cell is able to regulate specific gene expression by recruiting the Argonaute-driven RISC to target transcripts based on sequence complementarity; miRNAs and siRNAs are the short dsRNA species that can function in RNAi. Viral resistance is mediated through the RNAi machinery by cleavage of viral dsRNA species by the cytoplasmic type III RNase, Dicer to produce virus-specific siRNAs that are subsequently recruited into RISC, thereby directing the RISC-mediated destruction of viral gene transcripts based on complementarity to the bound siRNA; in organisms with RNA-dependent RNA polymerases (RdRP), such as *Arabidopsis thaliana* and *C. elegans*, this effect can be amplified through the use of siRNAs as primers by RdRP (Dalmay et al. 2000; Smardon et al. 2000). Work in *Drosophila* and *C. elegans* has demonstrated a role for the RNAi machinery in the suppression of transposable DNA elements (transposons), whereby the genome is protected from random insertional mutagenesis by resident transposons that occur in the genome and that might be mobilized and pose a threat to genomic stability. Methylation states of histones (a post-translational modification that is involved in chromatin remodeling and transcriptional control) are also affected by Argonaute-family members (Piwi and Aubergine; Pal-Bhadra et al. 2004) in *Drosophila* and in fission yeast (*Saccharomyces pombe*). A RISC-related protein complex named RNA-initiated transcriptional silencer (RITS) is guided by siRNA to regulate the transcription of genes with complementary DNA sequences (Motamedi et al. 2004; Verdel et al. 2004).

PTGS can be triggered either by the presence of long dsRNA (e.g., the hybridization product of an endogenous mRNA with its ectopically expressed antisense transcript) or by the cytoplasmic form of a genomically encoded hairpin RNA (pre-miRNA) that has partial complementarity to sequences in the 3'UTR of target mRNAs. The long dsRNA or pre-miRNA is cleaved by Dicer (Bernstein et al. 2001), a type III dsRNase, into either siRNAs of uniform length (from long dsRNA) or a single mature miRNA (from the pre-miRNA). The short dsRNA (siRNA or miRNA) is then capable of programming RISC to target mRNAs with complementarity to one of the strands in the short dsRNA. Perfect

complementarity between the short dsRNA and the target mRNA (as is the case with siRNAs) will allow RISC to cleave the mRNA and lead to a decrease in the level of transcript, whereas imperfect complementarity (as is the case with miRNAs) will not allow cleavage and leads to translational repression, not necessarily through a decrease in the level of the transcript.

In animals, the nature of the interaction between RISC and the target mRNA depends on which Argonaute protein, viz., AGO1 or AGO2, is present in the RISC (Okamura et al. 2004). AGO1 or AGO2 are functionally specialized, even though miRNAs and siRNAs can both be loaded into either AGO1 or AGO2 in *Drosophila* (Forstemann et al. 2007). AGO2 is essential for miRNA-dependent silencing of target mRNAs and/or siRNA-mediated cleavage of the target and possesses a RNase activity that cleaves the mRNA; this activity is known as “slicer” activity (Liu et al. 2004; Meister et al. 2004; Song et al. 2004a) to distinguish it from other forms of mRNA degradation that are miRNA-directed but do not involve cleavage within the dsRNA hybrid. AGO1 possesses detectable slicer activity in *Drosophila* S2 cells (Miyoshi et al. 2005), although one study has demonstrated, by using *Drosophila* embryo lysates, that AGO1 is an inefficient nuclease whose catalytic rate is limited by its reaction products (Forstemann et al. 2007). AGO1 is required for slicer-independent miRNA-directed mRNA cleavage (Behm-Ansmant et al. 2006) and also appears to be involved in miRNA biogenesis (Okamura et al. 2004), a process for which AGO2 is dispensable. The removal of the polyA tail of target transcripts is an example of miRNA-directed mRNA degradation (Giraldez et al. 2006; Wakiyama et al. 2007); in this scenario, the AGO1-containing RISC recruits proteins that remove the polyA tail, thereby destabilizing the target mRNA by making it vulnerable to exonucleases.

Although the miRNA pathway is found both in plants and animals, there are major differences in the implementation of the pathway. In plants, miRNAs are perfectly complementary to their targets and are not restricted to targeting only the 3'UTR. Furthermore, plants appear to favor translational regulation of target transcripts by miRNA-mediated destruction (Llave et al. 2002), in stark contrast to the metazoan miRNA pathway which favors translational repression combined with destabilization of the mRNA. Recent studies in the green alga *Chlamydomonas reinhardtii* have revealed that miRNAs are also present in unicellular organisms (Zhao et al. 2007b) and further indicate that the *Chlamydomonas* miRNAs direct AGO-mediated cleavage of target transcripts in a manner similar to that of miRNAs in higher plants (Molnar et al. 2007). In metazoans, most miRNAs are thought to act through translational repression of the target mRNA. However, the mRNA for HoxB is cleaved in a slicer-dependent manner that is directed by miR-196 (Yekta et al. 2004); this event has been explained by the finding that only a single mismatch occurs between miR-196 and its target site in HoxB mRNA, and that this mismatch is a G:U base pair that does not destabilize the double-stranded helical region sufficiently to prevent cleavage by the AGO2-driven RISC.

miRNAs in stem cell biology

Recent studies have provided supporting evidence for the model that miRNAs are important factors in stem cell biology and in the determination of cell fate. Algorithm-based studies have predicted interactions between the set of miRNAs expressed in CD34+ hematopoietic stem-progenitor cells (HSPCs) and mRNAs that are present in these same cells, interactions that are critical for hematopoiesis (Georgantas et al. 2007). Additionally, in mice, miR-181 expression is sufficient to establish B-lymphoid cell identity (Chen et al. 2004). miR-124 has been shown to regulate both alternative splicing and transcription network in promoting neuronal differentiation (Cao et al. 2007; Makeyev 2007; Visvanathan 2007). The post-transcriptional maturation of let-7, one of the first miRNAs to be identified, is regulated

during neural cell specification (Wulczyn et al. 2007). miR-206 is known to be highly enriched in rat myogenic cells and is thought to be necessary for the attainment and/or maintenance of the differentiated state (Politz et al. 2006). The acquisition of a characteristic miRNA expression profile in specific stem cell types would yield valuable information about the mechanisms by which stem cells normally maintain their function and identity.

Importance of miRNAs in stem cells for proper division and maintenance

The importance of Dicer and the miRNA pathway for the proper division and/or maintenance of identity has been confirmed in some stem cell systems. In *Drosophila*, the lack of Dicer-1, which is essential for miRNA synthesis, in ovarian GSCs results in: (1) a reduction of GSC division frequency and a delay at the G₁/S transition (Hatfield et al. 2005) when *dicer-1*^{-/-} GSCs are induced during development; (2) a cell-autonomous failure to be maintained in the GSC niche when GSCs lacking Dicer-1 or its accessory protein Loquacious are induced in adults (Jin and Xie 2007; Maines et al. 2007; Park et al. 2007; H.R. Shcherbata et al., in preparation). The ablation of the miRNA pathway by the removal of Dicer from mouse ES cells results in a failure to differentiate, even though ES cell markers continue to be expressed (Kanellopoulou et al. 2005). Dicer-deficient mouse ES cells also appear to experience a G₁/S delay similar to that observed in *dicer-1*^{-/-} GSCs in *Drosophila* (Murchison et al. 2005).

Specific miRNA expression patterns and functions in stem cells

Although convincing evidence has been presented that miRNAs, as a class of biomolecules, are important in stem cell biology, few studies indicate which individual miRNAs are important for the maintenance and proper function of particular stem cell types. hESCs are known to express a unique set of at least 32 miRNAs relative to their differentiated products (Suh et al. 2004); however, the roles of individual miRNAs that are critical for stem cell maintenance and function in hESCs have yet to be determined. In *A. thaliana*, miR-172 has been implicated as an important regulator of stem cell fate in floral meristems (Zhao et al. 2007a). More detailed investigations of unique miRNA expression profiles and functional studies of individual miRNAs in different stem cell types will be required before the true importance of the miRNA-mediated regulation of stem cell identity and function can be appreciated.

miRNAs in cancer

There is an increasing number of reports of cells isolated, from cancers that are distinct populations with stem cell characteristics, as well as of reports of miRNA involvement in cancer development. The small sub-populations that might function as cancer stem cells are characterized by their resistance to anti-cancer drugs and their implied ability to reconstitute the cancer cell population after treatment of the patient with chemotherapeutic drugs. The identification of these side populations of drug-resistant cancer stem cells and their subsequent characterization might reveal a distinct expression profile that can be targeted by new classes of drugs.

Examples of cancer stem cells

Cancer stem cells have been identified on the basis of being both morphologically and functionally distinct from other cells within the heterogeneous tumor mass, for many human cancers (Hirschmann-Jax et al. 2004; Yuan et al. 2004; Eisterer et al. 2005; Gu et al. 2007; Ho et al. 2007; Wang et al. 2007; Zheng et al. 2007). Each of the cancer stem cell types hitherto identified has a cell surface marker signature that differs from that of other cell types in the tumor mass (for a review, see Tang et al. 2007). Each cancer stem cell type also possesses the ability to reconstitute the cancer in the non-obese diabetic, severe combined

immunodeficient (NOD-SCID) mouse. Cancer stem cells with characteristic surface marker signatures have been isolated from acute myeloid leukemia (Bonnet and Dick 1997; Hope et al. 2004), breast tumors (Al-Hajj et al. 2003), brain tumors (Singh et al. 2004), bone sarcoma (Gibbs et al. 2005), adult glioblastoma multiforme (GBM; Yuan et al. 2004), chronic myeloid leukemia (Eisterer et al. 2005), prostate cancer (Collins et al. 2005; Patrawala et al. 2006), metastatic melanoma (Fang et al. 2005), lung cancers including adenocarcinoma (Kim et al. 2005), head and neck squamous cell carcinoma (Prince et al. 2007), colon cancer (O'Brien et al. 2007; Ricci-Vitiani et al. 2007), colorectal cancer (Dalerba et al. 2007), and pancreatic cancer (Li et al. 2007). Cancer stem cells have also been identified among established cancer cell lines (Zheng et al. 2007; Wang et al. 2007). The ability to isolate cancer stem cells allows for detailed studies of cancer stem cell maintenance and establishment.

The development of treatments that target cancer stem cells specifically depends on the elucidation of the cellular processes that establish and/or maintain the cancer stem cells. Indeed, two groups have demonstrated that cancer stem cells can be depleted by the pharmacological inhibition of the intercellular signaling pathways that support their tumorigenicity. Elevated Notch signaling is a hallmark of the CD133⁺ cancer stem cells in embryonal brain tumors, and inhibition of Notch pathway signaling by γ -secretase inhibitors causes a depletion of CD133⁺ stem-like cells in these tumors (Fan et al. 2006). The Hedgehog signaling pathway is highly active in nonneoplastic stem cells and has been suspected to act in the establishment of cancer stem cells that initiate GBM. The inhibition of Hedgehog signaling by the administration of cyclopamine to GBM neurospheres renders them unable to form new neurospheres when transplanted to cyclopamine-free media (Bar et al. 2007). Once molecular targets are identified for the various cancer stem cell types, the process of developing therapeutic with minimal side effects can begin.

Aberrant miRNA expression in cancer stem cells

Although no studies have been published, to date, describing miRNA profiles specific for cancer stem cells, there is direct evidence that specific miRNAs are instrumental in the establishment and/or progression of various cancers. In head and neck cancer cell lines, miR-21 and miR-205 are differentially up-regulated and are both predicted to act as oncogenes by targeting the transcripts of tumor suppressor genes (Tran et al. 2007). In some human cancer cell lines, miR-124a has been shown to be epigenetically silenced by CpG islands in a manner typically observed for some tumor suppressor genes (Lujambio et al. 2007). Five miRNAs are transcriptionally up-regulated in papillary thyroid carcinoma tumors (He et al. 2005a). In breast cancers, miR-21 has been implicated as an oncogene whose overexpression leads to tumorigenesis, possibly because of severe repression of tumor suppressor genes such as Tropomyosin-1 (Si et al. 2007; Zhu et al. 2007). miR-372 and miR-373 have been identified in a screen as possible oncogenes in testicular germ cell tumors (Voorhoeve et al. 2006). The human *mir-17-92* polycistron (cluster) is overexpressed in B-cell lymphoma development (He et al. 2005b; Dews et al. 2006) and lung cancers (Hayashita et al. 2005). Some cancers are possibly caused by viral oncogenic miRNAs that are introduced during infection, such as in the case of Kaposi's sarcoma (Cai et al. 2005).

Specific miRNAs are not only found to be up-regulated in cancer cells; the absence of key miRNAs has also been noted. In pituitary adenomas, miR-15 and miR-16 are down-regulated (Bottoni et al. 2005). In mice, miRNAs are frequently located at cancer susceptibility loci (Sevignani et al. 2007). In breast cancer, the *mir-17-5p* cluster has been implicated as a tumor suppressor that is expressed at decreased levels (Hossain et al. 2006). In KMCH cholangiocarcinoma, miR-29 appears to function as a tumor suppressor gene whose normal regulation of the anti-apoptotic protein Mcl-1 (Mott et al. 2007) is relieved when miR-29 function is lost, thus preventing the programmed cell death of cancerous cells.

miR-15 and miR-16 appear to function as tumor suppressor genes in chronic lymphocytic leukemia by losing their ability to regulate the anti-apoptotic protein Bcl2 when these miRNAs are down-regulated or deleted (Cimmino et al. 2005).

Abberant miRNA expression in cancer stem cells

An understanding of the biology of cancer stem cells and the involvement of aberrantly expressed miRNAs might provide therapeutic targets for next-generation anti-cancer drugs that should eliminate the cancer-initiating cells and greatly reduce the risk of patient relapse. Functional studies of specific miRNAs within the cancer stem cells of various cancers will be crucial for the elucidation of the mechanisms behind oncogenesis in various cancers. miRNAs that are identified as oncogenic, which promote cancer when expressed at reduced levels (in contrast to protein-coding oncogenes, which are over-expressed or constitutively active in cancers), could be targeted by locally administered antagomirs (Krutzfeldt et al. 2005). On the other hand, miRNAs that can be classified as tumor suppressors but that promote cancer when down-regulated might be candidates for suicide gene therapy if an appropriate viral vector system can be designed to target the particular cancer stem cell. However, much greater insight into the roles that miRNAs play in the survival and maintenance of cancer stem cells will be required before their potential as pharmacological targets can be fully realized.

References

- Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 2003;100:3983–3988. [PubMed: 12629218]
- Aravin A, Gaidatzis D, Pfeffer S, Lagos-Quintana M, Landgraf P, Iovino N, Morris P, Brownstein MJ, Kuramochi-Miyagawa S, Nakano T, Chien M, Russo JJ, Ju J, Sheridan R, Sander C, Zavolan M, Tuschl T. A novel class of small RNAs bind to MILI protein in mouse testes. *Nature* 2006;442:203–207. [PubMed: 16751777]
- Aravin AA, Sachidanandam R, Girard A, Fejes-Toth K, Hannon GJ. Developmentally regulated piRNA clusters implicate MILI in transposon control. *Science* 2007;316:744–747. [PubMed: 17446352]
- Babaie Y, Herwig R, Greber B, Brink TC, Wruck W, Groth D, Lehrach H, Burdon T, Adjaye J. Analysis of Oct4-dependent transcriptional networks regulating self-renewal and pluripotency in human embryonic stem cells. *Stem Cells* 2007;25:500–510. [PubMed: 17068183]
- Bar EE, Chaudhry A, Lin A, Fan X, Schreck K, Matsui W, Vescovi AL, Piccirillo S, Dimeco F, Olivi A, Eberhart CG. Cyclopamine-mediated Hedgehog pathway inhibition depletes stem-like cancer cells in glioblastoma. *Stem Cells* 2007;25:2524–2533. [PubMed: 17628016]
- Behm-Ansmant I, Rehwinkel J, Doerks T, Stark A, Bork P, Izaurralde E. mRNA degradation by miRNAs and GW182 requires both CCR4:NOT deadenylase and DCP1:DCP2 decapping complexes. *Genes Dev* 2006;20:1885–1898. [PubMed: 16815998]
- Bendall SC, Stewart MH, Menendez P, George D, Vijayaragavan K, Werbowetski-Ogilvie T, Ramos-Mejia V, Rouleau A, Yang J, Bosse M, Lajoie G, Bhatia M. IGF and FGF cooperatively establish the regulatory stem cell niche of pluripotent human cells in vitro. *Nature* 2007;448:1015–1021. [PubMed: 17625568]
- Bernstein E, Caudy AA, Hammond SM, Hannon GJ. Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* 2001;409:363–366. [PubMed: 11201747]
- Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ, Mills AA, Elledge SJ, Anderson KV, Hannon GJ. Dicer is essential for mouse development. *Nat Genet* 2003;35:215–217. [PubMed: 14528307]
- Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, Lander ES. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* 2006;125:315–326. [PubMed: 16630819]

- Boiani M, Scholer HR. Regulatory networks in embryo-derived pluripotent stem cells. *Nat Rev Mol Cell Biol* 2005;6:872–884. [PubMed: 16227977]
- 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]
- Bottoni A, Piccin D, Tagliati F, Luchin A, Zatelli MC, degli Uberti EC. miR-15a and miR-16-1 down-regulation in pituitary adenomas. *J Cell Physiol* 2005;204:280–285. [PubMed: 15648093]
- Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, Zucker JP, Guenther MG, Kumar RM, Murray HL, Jenner RG, Gifford DK, Melton DA, Jaenisch R, Young RA. Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* 2005;122:947–956. [PubMed: 16153702]
- Cai X, Lu S, Zhang Z, Gonzalez CM, Damania B, Cullen BR. Kaposi's sarcoma-associated Herpes virus expresses an array of viral microRNAs in latently infected cells. *Proc Natl Acad Sci USA* 2005;102:5570–5575. [PubMed: 15800047]
- Cao X, Pfaff SL, Gage FH. A functional study of miR-124 in the developing neural tube. *Genes Dev* 2007;21:531–536. [PubMed: 17344415]
- Chen D, McKearin D. Dpp signaling silences Bam transcription directly to establish asymmetric divisions of germline stem cells. *Curr Biol* 2003;13:1786–1791. [PubMed: 14561403]
- Chen D, McKearin D. Gene circuitry controlling a stem cell niche. *Curr Biol* 2005;15:179–184. [PubMed: 15668176]
- Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. *Science* 2004;303:83–86. [PubMed: 14657504]
- Chen JF, Mandel EM, Thomson JM, Wu Q, Callis TE, Hammond SM, Conlon FL, Wang DZ. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat Genet* 2006;38:228–233. [PubMed: 16380711]
- Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci USA* 2005;102:13944–13949. [PubMed: 16166262]
- Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 2005;65:10946–10951. [PubMed: 16322242]
- Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, Hoey T, Gurney A, Huang EH, Simeone DM, Shelton AA, Parmiani G, Castelli C, Clarke MF. Phenotypic characterization of human colorectal cancer stem cells. *Proc Natl Acad Sci USA* 2007;104:10158–10163. [PubMed: 17548814]
- Dalmay T, Hamilton A, Rudd S, Angell S, Baulcombe DC. An RNA-dependent RNA polymerase gene in *Arabidopsis* is required for posttranscriptional gene silencing mediated by a transgene but not by a virus. *Cell* 2000;101:543–553. [PubMed: 10850496]
- Dews M, Homayouni A, Yu D, Murphy D, Sevignani C, Wentzel E, Furth EE, Lee WM, Enders GH, Mendell JT, Thomas-Tikhonenko A. Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. *Nat Genet* 2006;38:1060–1065. [PubMed: 16878133]
- Donnelly DS, Krause DS. Hematopoietic stem cells can be CD34+ or CD34. *Leuk Lymphoma* 2001;40:221–234. [PubMed: 11426544]
- Eisterer W, Jiang X, Christ O, Glimm H, Lee KH, Pang E, Lambie K, Shaw G, Holyoake TL, Petzer AL, Auewarakul C, Barnett MJ, Eaves CJ, Eaves AC. 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]
- Elbashir SM, Lendeckel W, Tuschl T. RNA interference is mediated by 21- and 22-nucleotide RNAs. *Genes Dev* 2001;15:188–200. [PubMed: 11157775]
- Fan X, Matsui W, Khaki L, Stearns D, Chun J, Li YM, Eberhart CG. Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors. *Cancer Res* 2006;66:7445–7452. [PubMed: 16885340]
- Fang D, Nguyen TK, Leishear K, Finko R, Kulp AN, Hotz S, Van Belle PA, Xu X, Elder DE, Herlyn M. A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res* 2005;65:9328–9337. [PubMed: 16230395]

- Forstemann K, Horwich MD, Wee L, Tomari Y, Zamore PD. *Drosophila* microRNAs are sorted into functionally distinct Argonaute complexes after production by Dicer-1. *Cell* 2007;130:287–297. [PubMed: 17662943]
- Fuchs EJ, Whartenby KA. Hematopoietic stem cell transplant as a platform for tumor immunotherapy. *Curr Opin Mol Ther* 2004;6:48–53. [PubMed: 15011781]
- Georgantas RW 3rd, Hildreth R, Morisot S, Alder J, Liu CG, Heimfeld S, Calin GA, Croce CM, Civin CI. CD34+ hematopoietic stem-progenitor cell microRNA expression and function: a circuit diagram of differentiation control. *Proc Natl Acad Sci USA* 2007;104:2750–2755. [PubMed: 17293455]
- Gibbs CP, Kukekov VG, Reith JD, Tchigrinova O, Suslov ON, Scott EW, Ghivizzani SC, Ignatova TN, Steindler DA. Stem-like cells in bone sarcomas: implications for tumorigenesis. *Neoplasia* 2005;7:967–976. [PubMed: 16331882]
- Giraldez AJ, Mishima Y, Rihel J, Grocock RJ, Van Dongen S, Inoue K, Enright AJ, Schier AF. Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. *Science* 2006;312:75–79. [PubMed: 16484454]
- Goodman JW, Hodgson GS. Evidence for stem cells in the peripheral blood of mice. *Blood* 1962;19:702–714. [PubMed: 13900318]
- Greber B, Lehrach H, Adjaye J. Silencing of core transcription factors in human EC cells highlights the importance of autocrine FGF signaling for self-renewal. *BMC Dev Biol* 2007;7:46. [PubMed: 17506876]
- Grivna ST, Pyhtila B, Lin H. MIWI associates with translational machinery and PIWI-interacting RNAs (piRNAs) in regulating spermatogenesis. *Proc Natl Acad Sci USA* 2006;103:13415–13420. [PubMed: 16938833]
- Gu G, Yuan J, Wills M, Kasper S. Prostate cancer cells with stem cell characteristics reconstitute the original human tumor in vivo. *Cancer Res* 2007;67:4807–4815. [PubMed: 17510410]
- Hammond SM, Bernstein E, Beach D, Hannon GJ. An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature* 2000;404:293–296. [PubMed: 10749213]
- Hammond SM, Boettcher S, Caudy AA, Kobayashi R, Hannon GJ. Argonaute2, a link between genetic and biochemical analyses of RNAi. *Science* 2001a;293:1146–1150. [PubMed: 11498593]
- Hammond SM, Caudy AA, Hannon GJ. Post-transcriptional gene silencing by double-stranded RNA. *Nat Rev Genet* 2001b;2:110–119. [PubMed: 11253050]
- Han J, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN. The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev* 2004;18:3016–3027. [PubMed: 15574589]
- Hatfield SD, Shcherbata HR, Fischer KA, Nakahara K, Carthew RW, Ruohola-Baker H. Stem cell division is regulated by the microRNA pathway. *Nature* 2005;435:974–978. [PubMed: 15944714]
- Hatfield S, Shcherbata HR, Ward EJ, Reynolds S, Ruohola-Baker H, Clarke N, Sanseau P. MicroRNAs and their involvement in stem cell division, in microRNAs; biology, function and expression. 2007
- Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, Yatabe Y, Kawahara K, Sekido Y, Takahashi T. 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]
- He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, Calin GA, Liu CG, Franssila K, Suster S, Kloos RT, Croce CM, de la Chapelle A. The role of microRNA genes in papillary thyroid carcinoma. *Proc Natl Acad Sci USA* 2005a;102:19075–19080. [PubMed: 16365291]
- He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, Powers S, Cordon-Cardo C, Lowe SW, Hannon GJ, Hammond SM. A microRNA polycistron as a potential human oncogene. *Nature* 2005b;435:828–833. [PubMed: 15944707]
- Hirschmann-Jax C, Foster AE, Wulf GG, Nuchtern JG, Jax TW, Gobel U, Goodell MA, Brenner MK. A distinct “side population” of cells with high drug efflux capacity in human tumor cells. *Proc Natl Acad Sci USA* 2004;101:14228–14233. [PubMed: 15381773]
- Ho MM, Ng AV, Lam S, Hung JY. Side population in human lung cancer cell lines and tumors is enriched with stem-like cancer cells. *Cancer Res* 2007;67:4827–4833. [PubMed: 17510412]

- Hope KJ, Jin L, Dick JE. Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. *Nat Immunol* 2004;5:738–743. [PubMed: 15170211]
- Horwich MD, Li C, Matranga C, Vagin V, Farley G, Wang P, Zamore PD. The *Drosophila* RNA methyltransferase, DmHen1, modifies germline piRNAs and single-stranded siRNAs in RISC. *Curr Biol* 2007;17:1265–1272. [PubMed: 17604629]
- 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]
- Houbaviy HB, Murray MF, Sharp PA. Embryonic stem cell-specific microRNAs. *Dev Cell* 2003;5:351–358. [PubMed: 12919684]
- Jin Z, Xie T. Dcr-1 maintains *Drosophila* ovarian stem cells. *Curr Biol* 2007;17:539–544. [PubMed: 17306537]
- Kanellopoulou C, Muljo SA, Kung AL, Ganesan S, Drapkin R, Jenuwein T, Livingston DM, Rajewsky K. Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. *Genes Dev* 2005;19:489–501. [PubMed: 15713842]
- Keller G, Kennedy M, Papayannopoulou T, Wiles MV. Hematopoietic commitment during embryonic stem cell differentiation in culture. *Mol Cell Biol* 1993;13:473–486. [PubMed: 8417345]
- Kennedy M, Keller GM. Hematopoietic commitment of ES cells in culture. *Methods Enzymol* 2003;365:39–59. [PubMed: 14696336]
- Kilpatrick TJ, Bartlett PF. Cloning and growth of multipotential neural precursors: requirements for proliferation and differentiation. *Neuron* 1993;10:255–265. [PubMed: 8439411]
- Kim CF, Jackson EL, Woolfenden AE, Lawrence S, Babar I, Vogel S, Crowley D, Bronson RT, Jacks T. Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* 2005;121:823–835. [PubMed: 15960971]
- Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, Stoffel M. Silencing of microRNAs in vivo with “antagomirs”. *Nature* 2005;438:685–689. [PubMed: 16258535]
- Lai EC. Micro RNAs are complementary to 3'UTR sequence motifs that mediate negative post-transcriptional regulation. *Nat Genet* 2002;30:363–364. [PubMed: 11896390]
- Lau NC, Seto AG, Kim J, Kuramochi-Miyagawa S, Nakano T, Bartel DP, Kingston RE. Characterization of the piRNA complex from rat testes. *Science* 2006;313:363–367. [PubMed: 16778019]
- Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993;75:843–854. [PubMed: 8252621]
- Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Radmark O, Kim S, Kim VN. The nuclear RNase III Drosha initiates microRNA processing. *Nature* 2003;425:415–419. [PubMed: 14508493]
- Lee YS, Nakahara K, Pham JW, Kim K, He Z, Sontheimer EJ, Carthew RW. Distinct roles for *Drosophila* Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. *Cell* 2004;117:69–81. [PubMed: 15066283]
- Li CL, Johnson GR. Murine hematopoietic stem and progenitor cells. I. Enrichment and biologic characterization. *Blood* 1995;85:1472–1479. [PubMed: 7534130]
- Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, Wicha M, Clarke MF, Simeone DM. Identification of pancreatic cancer stem cells. *Cancer Res* 2007;67:1030–1037. [PubMed: 17283135]
- Liu J, Carmell MA, Rivas FV, Marsden CG, Thomson JM, Song JJ, Hammond SM, Joshua-Tor L, Hannon GJ. Argonaute2 is the catalytic engine of mammalian RNAi. *Science* 2004;305:1437–1441. [PubMed: 15284456]
- Llave C, Xie Z, Kasschau KD, Carrington JC. Cleavage of Scarecrow-like mRNA targets directed by a class of *Arabidopsis* miRNA. *Science* 2002;297:2053–2056. [PubMed: 12242443]
- Loh YH, Wu Q, Chew JL, Vega VB, Zhang W, Chen X, Bourque G, George J, Leong B, Liu J, Wong KY, Sung KW, Lee CW, Zhao XD, Chiu KP, Lipovich L, Kuznetsov VA, Robson P, Stanton LW, Wei CL, Ruan Y, Lim B, Ng HH. The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. *Nat Genet* 2006;38:431–440. [PubMed: 16518401]
- Lujambio A, Roperio S, Ballestar E, Fraga MF, Cerrato C, Setien F, Casado S, Suarez-Gauthier A, Sanchez-Cespedes M, Git A, Spiteri I, Das PP, Caldas C, Miska E, Esteller M. Genetic unmasking

- of an epigenetically silenced microRNA in human cancer cells. *Cancer Res* 2007;67:1424–1429. [PubMed: 17308079]
- Maines JZ, Park JK, Williams M, McKearin DM. Stonewalling *Drosophila* stem cell differentiation by epigenetic controls. *Development* 2007;134:1471–1479. [PubMed: 17344229]
- Makeyev EV, Zhang J, Carrasco MA, Maniatis T. The microRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. *Mol Cell* August 3;2007 27(3):435–448. [PubMed: 17679093]
- Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci USA* 1981;78:7634–7638. [PubMed: 6950406]
- McKinsty WJ, Li CL, Rasko JE, Nicola NA, Johnson GR, Metcalf D. Cytokine receptor expression on hematopoietic stem and progenitor cells. *Blood* 1997;89:65–71. [PubMed: 8978278]
- Meister G, Landthaler M, Patkaniowska A, Dorsett Y, Teng G, Tuschl T. Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. *Mol Cell* 2004;15:185–197. [PubMed: 15260970]
- Meshorer E, Yellajoshula D, George E, Scambler PJ, Brown DT, Misteli T. Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells. *Dev Cell* 2006;10:105–116. [PubMed: 16399082]
- Miyoshi K, Tsukumo H, Nagami T, Siomi H, Siomi MC. Slicer function of *Drosophila* Argonautes and its involvement in RISC formation. *Genes Dev* 2005;19:2837–2848. [PubMed: 16287716]
- Molnar A, Schwach F, Studholme DJ, Thuenemann EC, Baulcombe DC. miRNAs control gene expression in the single-cell alga *Chlamydomonas reinhardtii*. *Nature* 2007;447:1126–1129. [PubMed: 17538623]
- Moorthy PP, Kumar AA, Devaraj H. Expression of the Gas7 gene and Oct4 in embryonic stem cells of mice. *Stem Cells Dev* 2005;14:664–670. [PubMed: 16433621]
- Morshead CM, Reynolds BA, Craig CG, McBurney MW, Staines WA, Morassutti D, Weiss S, van der Kooy D. Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. *Neuron* 1994;13:1071–1082. [PubMed: 7946346]
- Mossman AK, Sourris K, Ng E, Stanley EG, Elefanty AG. Mixl1 and oct4 proteins are transiently co-expressed in differentiating mouse and human embryonic stem cells. *Stem Cells Dev* 2005;14:656–663. [PubMed: 16433620]
- Motamedi MR, Verdel A, Colmenares SU, Gerber SA, Gygi SP, Moazed D. Two RNAi complexes, RITS and RDRC, physically interact and localize to noncoding centromeric RNAs. *Cell* 2004;119:789–802. [PubMed: 15607976]
- Mott JL, Kobayashi S, Bronk SF, Gores GJ. mir-29 regulates Mcl-1 protein expression and apoptosis. *Oncogene* 2007;26:6133–6140. [PubMed: 17404574]
- Murchison EP, Partridge JF, Tam OH, Cheloufi S, Hannon GJ. Characterization of Dicer-deficient murine embryonic stem cells. *Proc Natl Acad Sci USA* 2005;102:12135–12140. [PubMed: 16099834]
- Nichols J, Zevnik B, Anastasiadis K, Niwa H, Klewe-Nebenius D, Chambers I, Scholer H, Smith A. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell* 1998;95:379–391. [PubMed: 9814708]
- Niwa H. How is pluripotency determined and maintained? *Development* 2007;134:635–646. [PubMed: 17215298]
- O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007;445:106–110. [PubMed: 17122772]
- Okamura K, Ishizuka A, Siomi H, Siomi MC. Distinct roles for Argonaute proteins in small RNA-directed RNA cleavage pathways. *Genes Dev* 2004;18:1655–1666. [PubMed: 15231716]
- Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature* 2007;448:313–317. [PubMed: 17554338]
- Olsen PH, Ambros V. The lin-4 regulatory RNA controls developmental timing in *Caenorhabditis elegans* by blocking LIN-14 protein synthesis after the initiation of translation. *Dev Biol* 1999;216:671–680. [PubMed: 10642801]

- Pal-Bhadra M, Leibovitch BA, Gandhi SG, Rao M, Bhadra U, Birchler JA, Elgin SC. Heterochromatic silencing and HP1 localization in *Drosophila* are dependent on the RNAi machinery. *Science* 2004;303:669–672. [PubMed: 14752161]
- Park JK, Liu X, Strauss TJ, McKearin DM, Liu Q. The miRNA pathway intrinsically controls self-renewal of *Drosophila* germ-line stem cells. *Curr Biol* 2007;17:533–538. [PubMed: 17320391]
- Patrawala L, Calhoun T, Schneider-Broussard R, Li H, Bhatia B, Tang S, Reilly JG, Chandra D, Zhou J, Claypool K, Coghlan L, Tang DG. 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]
- Phillips RL, Ernst RE, Brunk B, Ivanova N, Mahan MA, Deanehan JK, Moore KA, Overton GC, Lemischka IR. The genetic program of hematopoietic stem cells. *Science* 2000;288:1635–1640. [PubMed: 10834841]
- Politz JC, Zhang F, Pederson T. MicroRNA-206 colocalizes with ribosome-rich regions in both the nucleolus and cytoplasm of rat myogenic cells. *Proc Natl Acad Sci USA* 2006;103:18957–18962. [PubMed: 17135348]
- Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, Weissman IL, Clarke MF, Ailles LE. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proc Natl Acad Sci USA* 2007;104:973–978. [PubMed: 17210912]
- Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, De Maria R. Identification and expansion of human colon-cancer-initiating cells. *Nature* 2007;445:111–115. [PubMed: 17122771]
- Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A. Identification of mammalian microRNA host genes and transcription units. *Genome Res* 2004;14:1902–1910. [PubMed: 15364901]
- Ruby JG, Jan CH, Bartel DP. Intronic microRNA precursors that bypass Drosha processing. *Nature* 2007;448:83–86. [PubMed: 17589500]
- Saito K, Sakaguchi Y, Suzuki T, Suzuki T, Siomi H, Siomi MC. Pimet, the *Drosophila* homolog of HEN1, mediates 2'-O-methylation of Piwi- interacting RNAs at their 3' ends. *Genes Dev* 2007;21:1603–1608. [PubMed: 17606638]
- Sevignani C, Calin GA, Nnadi SC, Shimizu M, Davuluri RV, Hyslop T, Demant P, Croce CM, Siracusa LD. MicroRNA genes are frequently located near mouse cancer susceptibility loci. *Proc Natl Acad Sci USA* 2007;104:8017–8022. [PubMed: 17470785]
- Si ML, Zhu S, Wu H, Lu Z, Wu F, Mo YY. miR-21-mediated tumor growth. *Oncogene* 2007;26:2799–2803. [PubMed: 17072344]
- Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. *Nature* 2004;432:396–401. [PubMed: 15549107]
- Sitnicka E, Buza-Vidas N, Larsson S, Nygren JM, Liuba K, Jacobsen SE. Human CD34+ hematopoietic stem cells capable of multilineage engrafting NOD/SCID mice express flt3: distinct flt3 and c-kit expression and response patterns on mouse and candidate human hematopoietic stem cells. *Blood* 2003;102:881–886. [PubMed: 12676789]
- Slack FJ, Basson M, Liu Z, Ambros V, Horvitz HR, Ruvkun G. The lin-41 RBCC gene acts in the *C. elegans* heterochronic pathway between the let-7 regulatory RNA and the LIN-29 transcription factor. *Mol Cell* 2000;5:659–669. [PubMed: 10882102]
- Smardon A, Spoerke JM, Stacey SC, Klein ME, Mackin N, Maine EM. EGO-1 is related to RNA-directed RNA polymerase and functions in germ-line development and RNA interference in *C. elegans*. *Curr Biol* 2000;10:169–178. [PubMed: 10704412]
- Song JJ, Smith SK, Hannon GJ, Joshua-Tor L. Crystal structure of Argonaute and its implications for RISC slicer activity. *Science* 2004a;305:1434–1437. [PubMed: 15284453]
- Song X, Wong MD, Kawase E, Xi R, Ding BC, McCarthy JJ, Xie T. Bmp signals from niche cells directly repress transcription of a differentiation-promoting gene, bag of marbles, in germline stem cells in the *Drosophila* ovary. *Development* 2004b;131:1353–1364. [PubMed: 14973291]
- Song X, Call GB, Kirilly D, Xie T. Notch signaling controls germline stem cell niche formation in the *Drosophila* ovary. *Development* 2007;134:1071–1080. [PubMed: 17287246]

- Suh MR, Lee Y, Kim JY, Kim SK, Moon SH, Lee JY, Cha KY, Chung HM, Yoon HS, Moon SY, Kim VN, Kim KS. Human embryonic stem cells express a unique set of microRNAs. *Dev Biol* 2004;270:488–498. [PubMed: 15183728]
- Szakmary A, Cox DN, Wang Z, Lin H. Regulatory relationship among piwi, pumilio, and bag-of-marbles in *Drosophila* germline stem cell self-renewal and differentiation. *Curr Biol* 2005;15:171–178. [PubMed: 15668175]
- Tai MH, Chang CC, Kiupel M, Webster JD, Olson LK, Trosko JE. Oct4 expression in adult human stem cells: evidence in support of the stem cell theory of carcinogenesis. *Carcinogenesis* 2005;26:495–502. [PubMed: 15513931]
- Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;126:663–676. [PubMed: 16904174]
- Tang C, Ang BT, Pervaiz S. Cancer stem cell: target for anti-cancer therapy. *FASEB J*. 2007 in press.
- Tran N, McLean T, Zhang X, Zhao CJ, Thomson JM, O'Brien C, Rose B. MicroRNA expression profiles in head and neck cancer cell lines. *Biochem Biophys Res Commun* 2007;358:12–17. [PubMed: 17475218]
- Vagin VV, Sigova A, Li C, Seitz H, Gvozdev V, Zamore PD. A distinct small RNA pathway silences selfish genetic elements in the germline. *Science* 2006;313:320–324. [PubMed: 16809489]
- Velkey JM, O'Shea KS. Oct4 RNA interference induces trophectoderm differentiation in mouse embryonic stem cells. *Genesis* 2003;37:18–24. [PubMed: 14502573]
- Verdel A, Jia S, Gerber S, Sugiyama T, Gygi S, Grewal SI, Moazed D. RNAi-mediated targeting of heterochromatin by the RITS complex. *Science* 2004;303:672–676. [PubMed: 14704433]
- Visvanathan J, Lee S, Lee B, Lee JW, Lee SK. The microRNA miR-124 antagonizes the anti-neural REST/SCP1 pathway during embryonic CNS development. *Genes Dev* April 1;2007 21(7):744–749. [PubMed: 17403776]
- Voorhoeve PM, le Sage C, Schrier M, Gillis AJ, Stoop H, Nagel R, Liu YP, van Duijse J, Drost J, Griekspoor A, Zlotorynski E, Yabuta N, De Vita G, Nojima H, Looijenga LH, Agami R. A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. *Cell* 2006;124:1169–1181. [PubMed: 16564011]
- Wakiyama M, Takimoto K, Ohara O, Yokoyama S. Let-7 microRNA-mediated mRNA deadenylation and translational repression in a mammalian cell-free system. *Genes Dev* 2007;21:1857–1862. [PubMed: 17671087]
- Wang L, Li L, Menendez P, Cerdan C, Bhatia M. Human embryonic stem cells maintained in the absence of mouse embryonic fibroblasts or conditioned media are capable of hematopoietic development. *Blood* 2005;105:4598–4603. [PubMed: 15718421]
- Wang J, Guo LP, Chen LZ, Zeng YX, Lu SH. Identification of cancer stem cell-like side population cells in human nasopharyngeal carcinoma cell line. *Cancer Res* 2007;67:3716–3724. [PubMed: 17440084]
- Ward EJ, Shcherbata HR, Reynolds SH, Fischer KA, Hatfield SD, Ruohola-Baker H. Stem cells signal to the niche through the Notch pathway in the *Drosophila* ovary. *Curr Biol* 2006;16:2352–2358. [PubMed: 17070683]
- Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, Bernstein BE, Jaenisch R. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* 2007;448:318–324. [PubMed: 17554336]
- Wiles MV, Keller G. Multiple hematopoietic lineages develop from embryonic stem (ES) cells in culture. *Development* 1991;111:259–267. [PubMed: 1893864]
- Williams BP, Read J, Price J. The generation of neurons and oligodendrocytes from a common precursor cell. *Neuron* 1991;7:685–693. [PubMed: 1931054]
- Wulczyn FG, Smirnova L, Rybak A, Brandt C, Kwidzinski E, Ninnemann O, Strehle M, Seiler A, Schumacher S, Nitsch R. Post-transcriptional regulation of the let-7 microRNA during neural cell specification. *FASEB J* 2007;21:415–426. [PubMed: 17167072]
- Xi R, Xie T. Stem cell self-renewal controlled by chromatin remodeling factors. *Science* 2005;310:1487–1489. [PubMed: 16322456]

- Yang Z, Ebright YW, Yu B, Chen X. HEN1 recognizes 21–24 nt small RNA duplexes and deposits a methyl group onto the 2' OH of the 3' terminal nucleotide. *Nucleic Acids Res* 2006;34:667–675. [PubMed: 16449203]
- Yekta S, Shih IH, Bartel DP. MicroRNA-directed cleavage of HOXB8 mRNA. *Science* 2004;304:594–596. [PubMed: 15105502]
- Yuan X, Curtin J, Xiong Y, Liu G, Waschmann-Hogiu S, Farkas DL, Black KL, Yu JS. Isolation of cancer stem cells from adult glioblastoma multiforme. *Oncogene* 2004;23:9392–9400. [PubMed: 15558011]
- Zhao L, Kim Y, Dinh TT, Chen X. miR172 regulates stem cell fate and defines the inner boundary of APETALA3 and PISTILLATA expression domain in *Arabidopsis* floral meristems. *Plant J* 2007a;51:840–849. [PubMed: 17573799]
- Zhao T, Li G, Mi S, Li S, Hannon GJ, Wang XJ, Qi Y. A complex system of small RNAs in the unicellular green alga *Chlamydomonas reinhardtii*. *Genes Dev* 2007b;21:1190–1203. [PubMed: 17470535]
- Zheng X, Shen G, Yang X, Liu W. Most C6 cells are cancer stem cells: evidence from clonal and population analyses. *Cancer Res* 2007;67:3691–3697. [PubMed: 17440081]
- Zhu S, Si ML, Wu H, Mo YY. MicroRNA-21 targets the tumor suppressor gene tropomyosin 1 (TPM1). *J Biol Chem* 2007;282:14328–14336. [PubMed: 17363372]