RNA Biology 8:4, 557-564; July/August 2011; © 2011 Landes Bioscience

MicroRNA-9

Functional evolution of a conserved small regulatory RNA

Yeliz Yuva-Aydemir, Alfred Simkin, Eduardo Gascon and Fen-Biao Gao*
Department of Neurology; University of Massachusetts Medical School; Worcester, MA USA

The functional significance of microRNA-9 (miR-9) during evolution is evidenced by its conservation at the nucleotide level from flies to humans but not its diverse expression patterns. Recent studies in several model systems reveal that miR-9 can regulate neurogenesis through its actions in neural or non-neural cell lineages. In vertebrates, miR-9 exerts diverse cell-autonomous effects on the proliferation, migration and differentiation of neural progenitor cells by modulating different mRNA targets. In some developmental contexts, miR-9 suppresses apoptosis and is misregulated in several types of cancer cells, influencing proliferation or metastasis formation. Moreover, downregulation of miR-9 in postmitotic neurons is also implicated in some neurodegenerative diseases. Thus, miR-9 is emerging as an important regulator in development and disease through its ability to modulate different targets in a manner dependent on the developmental stage and the cellular context.

Key words: cancer cells, context-dependent, microRNA-9, neural progenitor cells, proliferation, migration, 3'UTRs

Submitted: 03/18/11

Revised: 04/18/11

Accepted: 04/30/11

DOI: 10.4161/rna.8.4.16019

*Correspondence to: Fen-Biao Gao;
Email: fen-biao.gao@umassmed.edu

Introduction

MicroRNAs (miRNAs) are endogenous, noncoding RNAs (~21–23 nucleotides) that destabilize or inhibit the translation of target mRNAs, mostly by binding to their 3' untranslated regions (3'UTRs).[1,2] Owing to their ability to fine-tune the levels of many target proteins, miRNAs are excellent candidates to regulate many complex biological processes. Indeed, recent advances in our understanding of miRNAs have firmly established the notion that this unique class of regulators of gene expression is critically important in many aspects of development and disease.[3-9] On the other hand, numerous questions remain to be addressed. For instance, many miRNAs are enriched in the brain and their expression is spatiotemporally regulated.[10-13] However, we still do not fully understand how evolutionarily conserved and species-unique miRNAs contribute to the morphogenesis and function of the diverse nervous systems in the animal kingdom.

In this review, we summarize the latest findings regarding the diverse roles of miR-9 in neuronal development and tumor formation in various model systems, including fruit fly, frog, zebrafish, chick, mouse and human embryonic stem cells. These findings illustrate the emerging theme that miR-9, and likely many other miRNAs as well, fulfills diverse functions that are dependent on the developmental stage and cellular context.

The Nucleotide Sequence of miR-9 is Highly Conserved through Evolution

The mature miR-9 sequence is identical in insects and humans (**Fig. 1A**). Of 22 vertebrate species annotated in miRbase[14] as containing miR-9, 17 have multiple copies of the gene. All vertebrate miR-9 orthologs are identical in their mature sequence, with the exception of *Xenopus tropicalis*, which possesses, in addition to three copies identical to those in the other vertebrates, a fourth miR-9 copy containing a single mutation at nucleotide 11 of the mature sequence (**Fig. 2**). However, this sequence

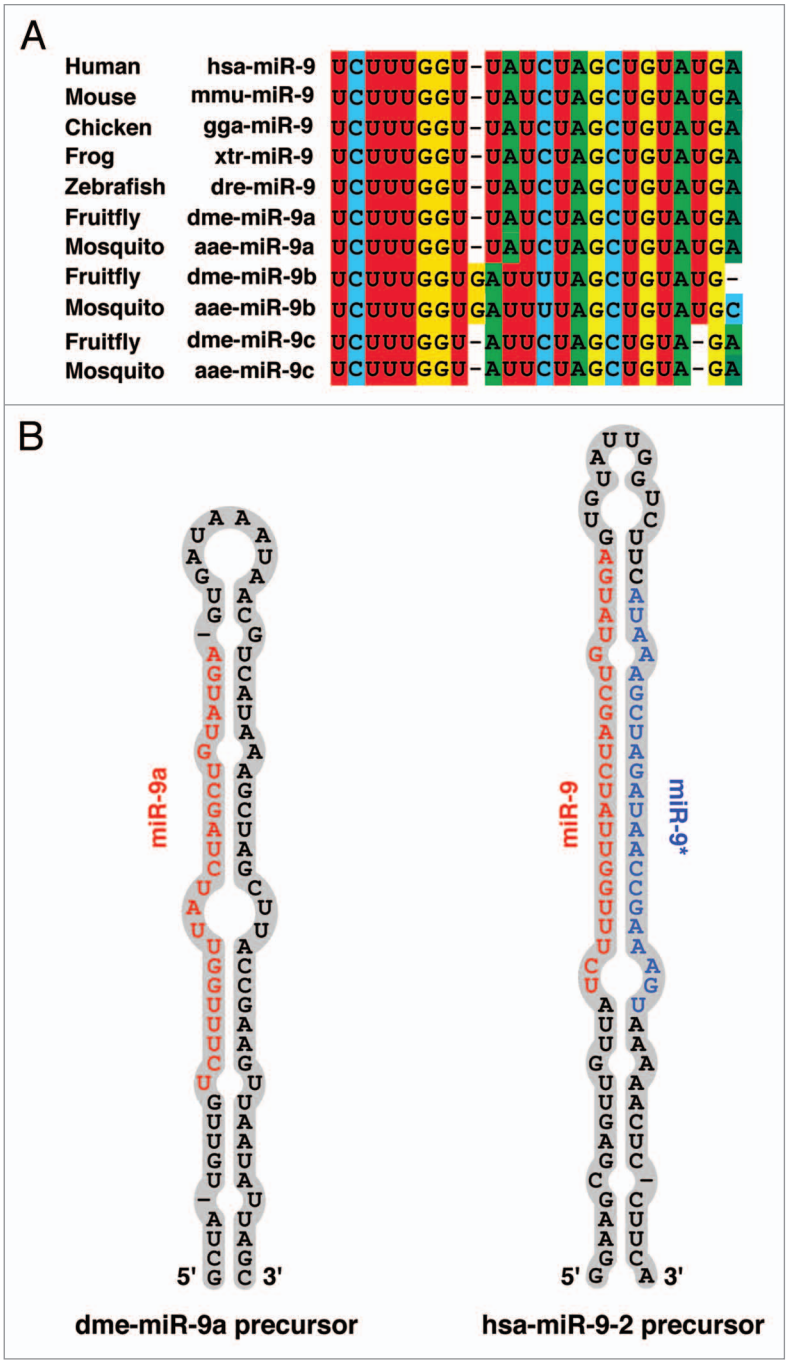

Figure 1. Sequences and conservation of miR-9 and related miRNAs through evolution. (A) All vertebrate miR-9 sequences are all identical to insect miR-9a, displaying only the small differences in length at the 3′ end that are caused by differences in 3′ processing. Mature insect miR-9 paralogs, by contrast, often differ considerably in their middle and 3′ regions while maintaining orthology across species boundaries. (B) Stem-loop structures of Drosophila miR-9a and human miR-9-2 precursors. MiR-9a and miR-9 sequences are in red. The miR-9* sequence is in blue.

annotated in miRbase needs to be confirmed and validated experimentally.

Between invertebrates, the mature miR-9 sequence is less stringently conserved. Of 30 invertebrates annotated in miRbase[14] as containing miR-9 sequences identical to those in vertebrates (named as miR-9a), 22 also possess miR-9 copies that differ in their mature sequence from human miR-9. Based on precursor sequences (**Fig. 1B**), the relative similarities of different miR-9 homologs can be analyzed using the Close-Neighbor-Interchange algorithm[16] (**Fig. 2**). Although it is difficult to construct a reliable evolutionary tree using relatively short miR precursor sequences, it does seem that different vertebrate pre-miR-9 homologs cluster into three sister groups (red, light blue and purple lines in **Fig. 2**), which likely have the same common ancestor as that of invertebrate pre-miR-9a (yellow lines in **Fig. 2**).

Vertebrate miR-9 precursors also produce a miR-9 star strand (miR-9*) that may behave as a functional miRNA (**Fig. 1B**). Interestingly, the sequence of the miR-9* seed region is identical to that of the invertebrate-specific miR-79. MiR-9/miR-9* and miR-79/miR-79* may share a common ancestor that duplicated and changed its strand expression preference multiple times during evolutionary history, while maintaining the function of both strands in vertebrates and insects.[18]

Diverse Expression Patterns of miR-9 in Different Model Systems

Despite the sequence conservation of miR-9, its expression patterns differ strikingly among species. At early embryonic stages in Drosophila, miR-9a is expressed in most epithelial cells except those in the ventral ectoderm. At stage 12, miR-9a is expressed in the ectoderm but not in the central nervous system (**Fig. 3A**).[19,20] In sharp contrast, miR-9 expression in vertebrates appears to be largely confined to the nervous system (**Fig. 3**).

In developing Xenopus embryos, miR-9 expression is restricted to the anterior nervous system and is absent from the spinal cord (**Fig. 3E and F**).[21] In zebrafish, expression begins in the telencephalon as early as 24 h after fertilization and expands to most regions of the nervous system, including the spinal cord (**Fig. 3C and D**).[22] Nonetheless, some areas such as the midbrain-hindbrain boundary (MHB) in zebrafish and Xenopus brains are devoid of miR-9.[21,22] Although information about miR-9 during chick development is incomplete, it is strongly expressed across the nervous system, including the spinal cord (**Fig. 3G and H**).[23,24]

In mice, miR-9 is readily detected in the forebrain and hindbrain starting on embryonic day (E) 9.5.[25] Initially, miR-9

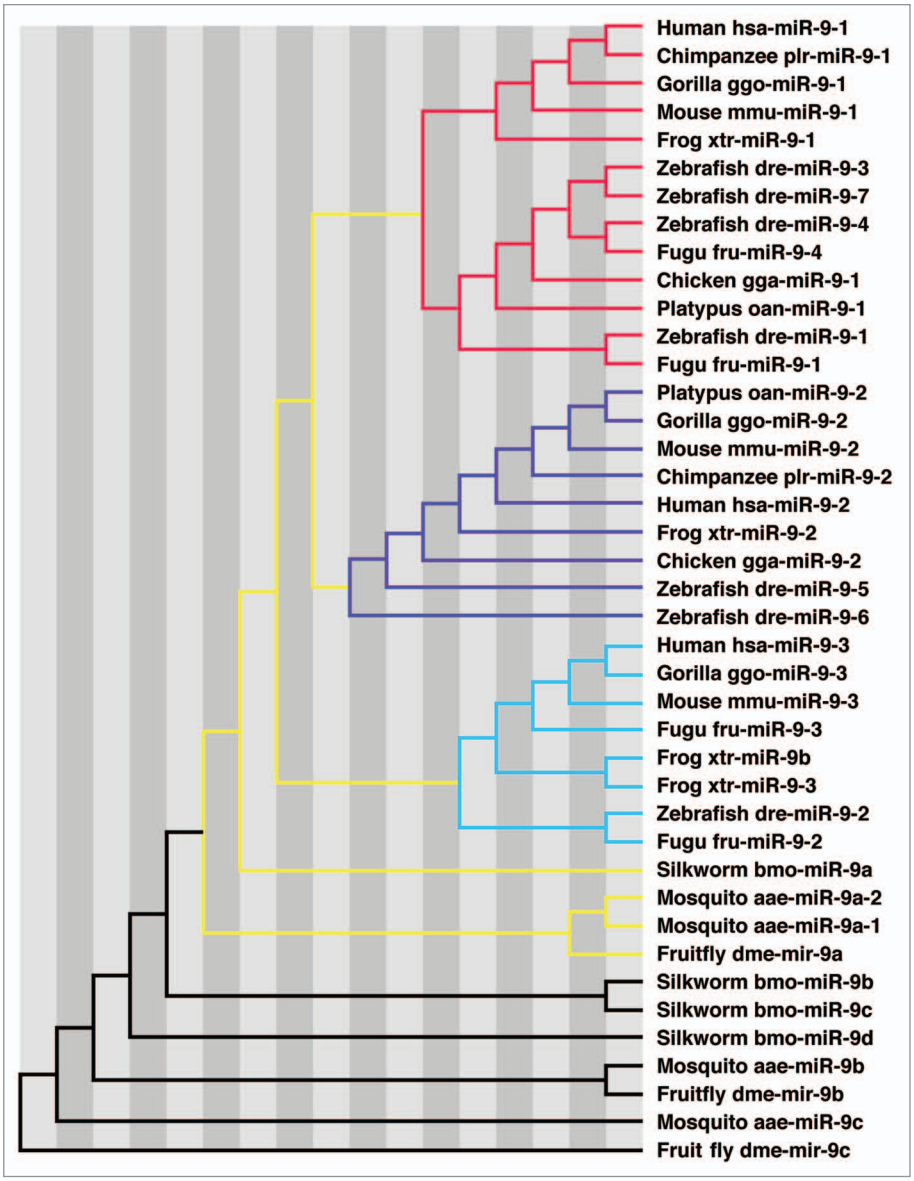

Figure 2. Alignments were generated using the PRANK alignment package[15] for miR-9 precursors in human, chimpanzee, gorilla, mouse, platypus, chicken, frog, zebrafish and fugu within vertebrates, and in fruit fly, mosquito and silkworm within invertebrates. Ggo-miR-9-1 (labeled with '#') and Ggo-miR-9-3 (labeled with '&') derive from annotations of the GorGor3 genome assembly. All other miRNAs derive directly from miRbase release 16.[14] The evolutionary history was inferred using the Maximum Parsimony method, and of several parsimonious trees, the tree most consistent with mature sequences as well as existing miR-9 nomenclature was chosen. The MP tree was obtained using the Close-Neighbor-Interchange algorithm (pg. 128 in ref. 16). Only tree topology is shown here for simplicity. Evolutionary analyses were conducted in MEGA5,[17] and the length of each line does not reflect the relative evolutionary distance.

expression is restricted to the medial pallium and caudal pallidum (E10.5) and subsequently extends to the ventricular zone of the ganglionic eminences and the cortical marginal zone (E11.5) (**Fig. 3I and J**). From E12.5, miR-9 is also expressed in the septum and in the differentiated zone corresponding to the prospective cortical plate.[25] MiR-9 expression peaks between E13.5 and E15.5 and declines afterward but is detectable in both neurons and astrocytes.[26,27] MiR-9 expression is retained in the adult subventricular zone, a neurogenic area of the mouse brain.[12]

As stated above, major spatial differences are observed across vertebrates, suggesting a high level of specialization in miR-9 functions. Thus, along the anteroposterior axis, miR-9 expression is restricted to the anterior nervous system in Xenopus[21] but extends also to the spinal cord in the zebrafish, chicken and mouse[13,23,25] (**Fig. 3**). Even in the same domain of expression, miR-9 can be differentially expressed in different cell populations. In developing zebrafish and mouse spinal cord, miR-9 is expressed in proliferating neural precursors.[12,13] In the chick, miR-9 is transiently expressed in a pool of spinal motor neurons.[24] Xenopus miR-9 seems to be confined to neural progenitor cells (NPCs) in both the forebrain and hindbrain but is expressed only in differentiated forebrain neurons.[21]

MiR-9 in Defining Neurogenic Boundaries

In Drosophila, sensory organ precursor (SOP) cells arise from proneural clusters that express proneural genes and prevent neighboring cells from adopting the SOP cell fate through a well-studied process called lateral inhibition.[28] *MiR-9a* mutant flies have subtle increases in the number of sensory bristles and sensory neurons, which are differentiated from SOP cells, indicating that miR-9a negatively regulates early neurogenesis and ensures the precise specification of SOPs in flies.[19] It does so by downregulating Senseless, a transcription factor that promotes SOP specification, in non-SOP epithelial cells.[29] Thus, in Drosophila, miR-9 ensures accurate specification of NPCs through its actions in non-neural cell lineages. Drosophila also encodes miR-9b and miR-9c, whose seed sequences resemble those of miR-9a (**Fig. 1A**). It is not known whether their functions overlap with those of miR-9a.

In zebrafish embryonic brain, the MHB is an organizing center that patterns midbrain and anterior hindbrain development. Fibroblast growth factors secreted from the MHB are involved in the patterning of the surrounding neural tissue, and their activity is tightly controlled by negative feedback inhibition.[30] MiR-9 is absent in MHB but expressed in adjacent neural tissue and targets several components of the fibroblast growth factor pathway, thereby limiting the organizing

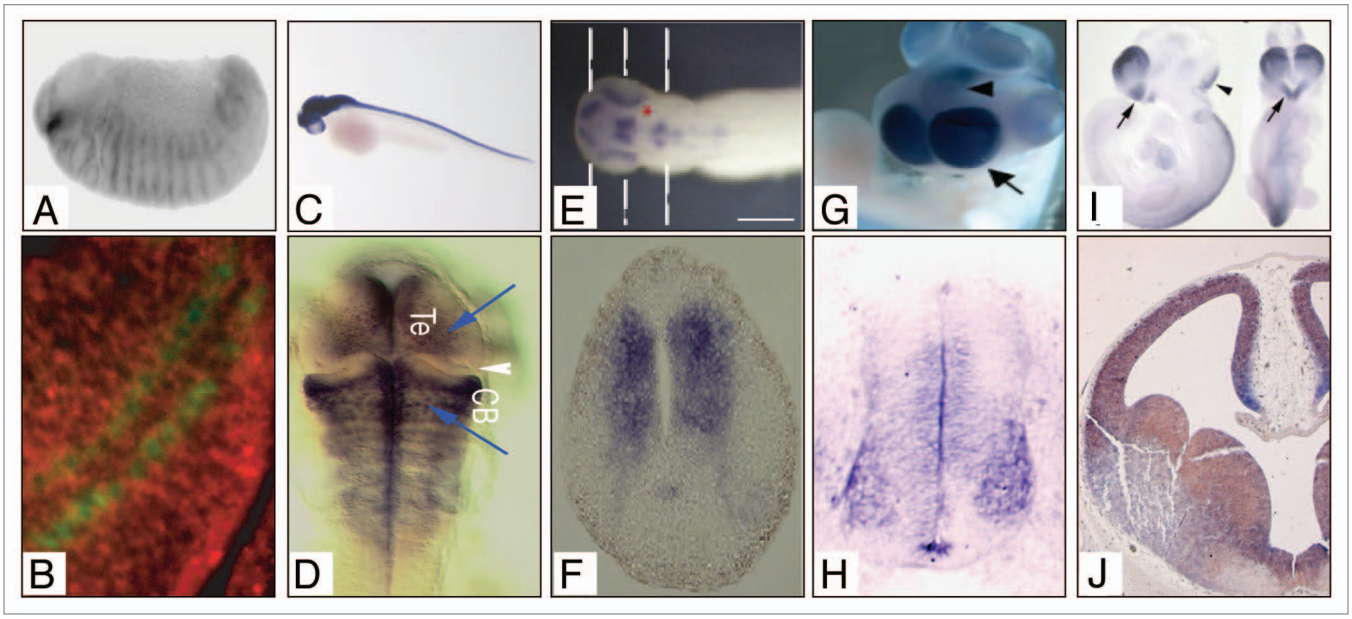

Figure 3. MiR-9 expression during development in different species, as shown by in situ hybridization. (A) MiR-9a is expressed in ectoderm but not in the central nervous system in stage 12 Drosophila embryo.[17] (B) In Drosophila wing imaginal disc, miR-9a (red) is not expressed in SOP cells (green) but is expressed in adjacent epithelial cells.[18] (C) Lateral view of whole-mount zebrafish embryo shows prominent miR-9 expression in the nervous system at 48 h after fertilization.[11] (D) Dorsal view of a zebrafish embryo (35 h after fertilization), miR-9 is detected in the midbrain and hindbrain (blue arrows) but absent in the MHB (arrowhead).[19] Te, tectum; CB, cerebellar plate. Blue arrows indicate the midbrain and the hindbrain. (E) Xenopus miR-9 is mostly expressed in the anterior neural tube but not in the spinal cord (whole mount, dorsal view, stage 30). As in zebrafish, the MHB is devoid of miR-9 (red asterisk).[20] Scale bar, 200 μm. (F) Transverse section of a stage 30 Xenopus embryo shows miR-9 expression in the proliferative ventricular zone.[20] (G) In the chick embryo (HH20), miR-9 is found in the telencephalic vesicles (arrow), diencephalon (arrowhead) and spinal cord.[21] (H) Section of developing chick spinal cord (HH24) demonstrated that miR-9 is present in both proliferating precursors in the ventricular zone and a subset of motor neurons (arrows).[22] (I) Whole-mount mouse embryo (E10) shows miR-9 expression in the telencephalon (arrow) and hindbrain (arrowhead).[23] (J) Section through the developing mouse forebrain (E12.5) shows no obvious overlap between Foxg1 (red) and miR-9 (purple) expression.[23] All images are reproduced with permission.

activity of the MHB.[22] Loss of miR-9 leads to the expansion of the MHB. Furthermore, miR-9 promotes neurogenesis in adjacent regions by suppressing her5, an antineurogenic basic helix-loop-helix Hairy/E(spl) transcription factor.[22] Thus, miR-9 prevents the brain region adjacent to the MHB from adopting the MHB fate.

MiR-9 in NPC Proliferation and Migration

In vertebrates, miR-9 is highly expressed in NPCs, and its context-dependent functions in proliferation have been extensively studied in several model systems. In zebrafish embryos, although miR-9 overexpression leads to a pronounced decrease in proliferation, miR-9 knockdown does not seem to affect the proportion of mitotic cells positive for phosphorylated histone H3 in the ventricular zone.[22] Both loss- and gain-of-function approaches reveal that miR-9 suppresses the proliferation of adult NPCs by downregulating the orphan nuclear receptor TLX, an essential regulator of neural stem cell self-renewal, by binding to its 3'UTR.[31] A consensus remains to be reached whether miR-9 regulates TLX expression at E13.5.[25,31]

In contrast, miR-9 increases the proliferation of NPCs derived from cultured human embryonic stem cells at the neurosphere stage.[32] Loss of miR-9 decreases proliferation capacity but results in precocious migration of hNPCs or rat embryonic NPCs out of neurospheres without affecting their progenitor identity.[32] When hNPCs were transplanted into medial ganglionic eminence (MGE) of brain slices from E14.5 C57BL/6 mouse embryos or the striatum of immunodeficient adult mice 1 week after induction of permanent focal ischemia, more miR-9-deficient hNPCs migrated farther away than control hNPCs from the injection site toward the neocortex or the injury site.[32] In NPC proliferation and migration, stathmin, a developmentally regulated cytosolic phosphoprotein that has catastrophe-promoting microtubule-depolymerization activity,[33] is one of the key downstream targets.[32]

During brain development in *X. tropicalis*, miR-9 exhibits region-specific expression patterns. It is expressed in both NPCs and developing neurons in the forebrain, but is expressed only in NPCs in the mid- and hindbrain.[21] Along the anteroposterior axis, endogenous miR-9 limits NPC proliferation through a single key target, hairy 1, a member of the Hes family of transcriptional regulators that is specifically expressed in neurogenic regions of the brain.[21]

All the studies described above used acute knockdown or overexpression approaches. A recent genetic deletion study in mice revealed further complexity in the context-dependent functions of miR-9 in vivo.[26] In mammals, miR-9

is encoded by three genes that also produce miR-9* (**Figs. 1 and 2**). *miR-9-2* or *miR-9-3* knockout mice are viable and grossly normal; however, *miR-9-2/3* double-knockout mice die within a week after birth.[26] From E12.5 to E13.5, the number of mitotic cells that were positive for phosphorylated histone H3 and labeled by a 30 min pulse of bromodeoxyuridine is increased in the subventricular and ventricular zones, indicating increased proliferation, which correlated with the upregulation of Foxg1, a direct target of miR-9. However, by E16.5, Foxg1 is no longer regulated by miR-9, probably because of interference by Elavl2/HuB, an AU-rich RNA-binding protein whose expression is increased at this developmental stage.[26] The absence of apoptosis and the reduced neuronal differentiation would lead one to expect that the total number of progenitor cells would be significantly higher at E16.5 in *miR-9-2/3* double-knockout mice, although this was not examined directly. Interestingly, from E15.5 to E18.5, NPC proliferation in the pallium of *miR-9-2/3* double knockout mice was significantly suppressed, which correlated with elevated expression of Nr2e1, also known as TLX.[26] The regulation of Nr2e1/TLX by miR-9 at E16.5 but not at E13.5 seems to result from a difference in the cellular context due to elevated expression of Elavl1/HuR at E16.5.[26] Thus, this genetic analysis suggests that miR-9 can either promote or suppress NPC proliferation through different targets at different stages of brain development.

In *miR-9-2/3* double-knockout mice, the tangential migration of interneurons into the pallium was compromised,[26] which is consistent with the observation that overexpression of miR-9 promoted outward migration of apparently differentiated neurons from embryonic ventricular zone.[31] It remains to be determined whether and how the migration of other NPCs or newly generated neurons is regulated by miR-9 in vivo.

MiR-9 in Neuronal Differentiation

A key function for miR-9 is to regulate the generation of postmitotic neurons from NPCs. Ectopic expression of miR-9 in mouse cerebral cortex at E11.5 induces premature differentiation of Cajal-Retzius cells. Acute miR-9 knockdown at E12.5 reduces the number of these cells but has little effect on the number of cortical NPCs.[25] This finding was largely confirmed in *miR-9-2/3* knockout mice, which have greatly reduced numbers of Cajal-Retzius cells and other early born neurons.[26] Although the total number of NPCs was not analyzed at this developmental stage in the knockout mice, the miR-9 phenotype in both studies suggests a role in neuronal differentiation, since Foxg1, which suppresses Cajal-Retzius cell differentiation, is a key direct target of miR-9 and is upregulated at E13.5.[26] Consistent with this finding, miR-9 overexpression in the ventricular zone at E13.5 causes precocious neuronal differentiation,[31] and miR-9 knockdown in mouse embryonic stem cells increases the number of glial cells at the expense of neuron production.[34]

MiR-9 is expressed not only in mouse embryonic NPCs but also in adult NPCs. Indeed, overexpression of miR-9 in adult NPCs promotes neuronal differentiation, which is mediated by the interaction between miR-9 and the mRNA encoding the nuclear receptor TLX.[31] The effect of overexpressed miR-9 on TLX expression is relevant only in adult NPCs shortly before terminal differentiation is induced with retinoic acid or forskolin, suggesting that miR-9 alone rather facilitates, but cannot induce, neural differentiation of adult NPCs. Because acute inhibition of miR-9 activity does not affect the neuronal differentiation of adult NPCs,[31] the exact physiological functions of miR-9 in adult mouse brain remain to be clarified.

The functional significance of miR-9 in neuronal differentiation has been demonstrated in other experimental systems. In zebrafish embryos, miR-9 knockdown reduces the production of HuC-positive postmitotic neurons, an action mediated in part by her5 and her9, 2 proneural transcription factors targeted by miR-9.[22] During brain development in *X. tropicalis*, miR-9 is required for neuronal differentiation in all regions along the anteroposterior axis.[21] In chick spinal cord, miR-9 has a dynamic expression pattern and regulates the differentiation of motor neuron subtypes by fine-tuning the expression of FoxP1, which defines motor neuron identity in lateral, medial and preganglionic motor columns.[24] While miR-9 plays a central role in the differentiation of motor neuron subtypes, it does not seem to affect overall neurogenesis, as the number of NPCs and differentiated neurons remains normal,[24] which is in stark contrast to its roles in the brains of several model organisms.

MiR-9 and miR-9* also seem to regulate the morphological differentiation of postmitotic neurons. Ectopic expression of miR-9a in Drosophila larval sensory neurons significantly increases dendritic branching.[35] In mice, miR-9* targets the 3'UTR of BAF53a, a key component of ATP-dependent chromatin-remodeling complexes.[36] Expression of BAF53a does not affect activity-dependent dendritic growth of cultured rodent hippocampal neurons. However, BAF53a with a mutant 3'UTR lacking miR-9* and miR-124 binding sites was not regulated simultaneously by these miRNAs and inhibited this differentiation process.[36] Thus, miR-9* together with miR-124 may contribute to activity-dependent dendritic growth by downregulating BAF53a.

MiR-9 in Apoptotic Cell Death

A potential role for miR-9 in apoptotic cell death has been suggested by studies in some but not other model systems. Acute knockdown of miR-9 does not affect the survival of hNPCs in newly formed neurospheres or embryonic rat NPCs, as shown by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) analysis and a cytotoxicity assay.[32] Similarly, the number of TUNEL-positive or active caspase3-positive cells was unchanged in the developing telencephalon of *miR-9-2/3* double knockout mice.[26] In contrast, treatment with anti-miR-9 morpholino in the forebrain but not the hindbrain of stage 30 Xenopus embryos significantly increased apoptosis of NPCs.[21] How miR-9 specifically affects apoptosis only in the forebrain is unknown, although it likely involves the p53 pathway.[21] These findings suggest that, under certain circumstances, miR-9

helps to suppress apoptotic cell death in vertebrate NPCs.

A context-dependent role in apoptosis seems to be an ancient feature of miR-9 function. A prominent phenotype in *miR-9a* knockout flies is a completely penetrant notching defect in the posterior wing margin.[19,37,38] MiR-9 is highly expressed in most, if not all, epithelial cells in the wing disc,[19] but the notching phenotype is much more pronounced at the posterior than the anterior wing margin.[19,37,38] The defect is caused by apoptotic cell death and the transcriptional regulator Drosophila LIM only (dLMO) is the key target of miR-9 in this process.[37,38] Loss of one copy of *dLMO* rescued the apoptosis and wing margin defects in *miR-9a* mutants.[37,38] It is not known why the posterior wing margin is more sensitive to miR-9a activity than the anterior wing margin.

MiR-9 in Brain Function and Neurodegeneration

Although much has been learned about the cellular functions of miR-9, we still do not understand how it contributes to brain function and dysfunction. In adult rat brain, alcohol increases miR-9 expression in supraoptic nucleus neurons and striatal neurons.[39] A key factor in the development of alcohol tolerance is the large conductance calcium- and voltage-activated potassium channel. MiR-9 contributes to drug adaptation and adult brain plasticity by downregulating specific mRNA splice variants of this channel.[39]

The potential involvement of miRNAs in age-dependent neurodegeneration is suggested by findings in conditional *dicer* knockout mice in which specific types of neurons are lost;[40-42] however, the exact mechanisms are unclear. In principle, loss of miRNAs can cause accumulation of toxic proteins and subsequent neuronal loss and might affect neuronal survival by changing the levels of trophic or prosurvival factors. Recent evidence suggests that miR-9 may play a role in neurodegenerative diseases. Increased miR-9 levels have been found in postmortem Alzheimer disease brains.[43] MiR-9 and miR-9* are downregulated in motor neurons differentiated from mouse embryonic stem cells (mESCs) carrying the SMN1 mutation. MiR-9 downregulation leads to increased expression of heavy neurofilament, which alters the intermediate filament dynamics in this model of spinal muscular atrophy.[44] Interestingly, miR-9 levels are dramatically decreased after ischemia due to middle cerebral artery occlusion in rats.[45]

In neuronal cells, RE1-silencing transcription factor (REST) is sequestered in the cytoplasm in part as a result of binding to huntingtin, the protein in which an abnormal polyglutamine expansion causes Huntington's disease. In non-neuronal cells, REST suppresses neuronal gene expression by binding to RE1 consensus sequences and recruiting CoREST and other corepressors.[46] In Huntington's disease, polyglutamine expansions in huntingtin abolish binding to REST, which then translocates to the nucleus and represses the expression of neuronal genes, including miR-9-1, miR-9-2 and miR-9-3.[46,47] Indeed, miR-9 and miR-9* levels are reduced in the early stages of Huntington's disease. Interestingly, miR-9 targets REST and miR-9* targets CoREST, providing a negative feedback loop between the REST silencing complex and miRNAs.[46,47] It remains to be determined whether the deregulated expression of miRNAs is directly involved in disease progression and contributes to neuronal cell death.

MiR-9 in Cancer Cells

Aberrant miR-9 levels have been reported in many types of cancer, suggesting that miR-9 is involved in tumor formation or progression. However, by regulating various mRNA targets, miR-9 may have opposing effects on proliferation in different types of cancer cells.[48-63] For instance, miR-9 is overexpressed in human Hodgkin's lymphoma cells,[51] primary brain tumors[53,57] and CDX2-negative gastric cancer cells.[63] MiR-9 knockdown inhibits the proliferation of human gastric cancer cells and overexpression of CDX2, a direct target of miR-9, has a similar effect.[63] In contrast, miR-9 is downregulated in human ovarian tumor cells and overexpression of miR-9 suppresses their proliferation, in part by downregulating NFkappaB1.[50,56] Overexpression of miR-9 in human neuroblastoma and medulloblastoma cells also inhibits cell growth.[47,54]

MiR-9 seems to be a useful marker for tumor metastasis, but its role in this process is also dependent on the type of cancer. For instance, miR-9-3 is downregulated in breast cancers with vascular invasion or lymph node metastases.[48] MiR-9 expression is also significantly lower in metastatic than primary brain tumors.[53] Reduced miR-9 expression due to hypermethylation is associated with metastasis of cancer cells in the lymph node[52] and metastatic recurrence in patients with clear-cell renal cell carcinoma.[61] In contrast, miR-9 expression is significantly higher in breast cancers with metastases than in those without metastases and seems to promote metastasis.[60]

MiR-9 induces epithelial mesenchymal transition in human epithelial cells and breast carcinoma cell lines by targeting the adherens junction protein E-cadherin.[60] Cells overexpressing miR-9 lose contact with each other, and their motility and migration are enhanced in vitro. MiR-9 also induces transcription of vascular endothelial growth factor, in part by downregulating E-cadherin, which leads to release of β-catenin from the junctions and translocation into the nucleus. However, additional miR-9 targets might also be responsible for increased transcription of vascular endothelial growth factor and miR-9-induced migration, since E-cadherin knockdown does not mimic the miR-9 overexpression phenotype in vitro. When breast cancer cells overexpressing miR-9 are implanted in mice, the tumors show enhanced angiogenesis and grow faster than those formed by cells with low levels of miR-9. These findings imply that miR-9 promotes metastasis of epithelial tumors. Indeed, forced expression of miR-9 in nonmetastatic breast tumors facilitates the formation of pulmonary micrometastases and miR-9 knockdown inhibits metastasis of highly metastatic cells.[60]

Concluding Remarks

There is strong evidence that miR-9 acts at different stages of neurogenesis and coordinates the complex genetic programs necessary for the proper generation

of postmitotic neurons. The *miR-9-2/3* double-knockout mice recapitulate many defects in cortical development observed in a mouse model in which cortical NPCs are devoid of all mature miRNAs,[64] confirming the prominent role of miR-9 as an intrinsic positive regulator in this process. In contrast, Drosophila miR-9a seems to negatively regulate early neurogenesis through its actions in non-neural cell lineages.

MiR-9 can exert opposite effects on NPC proliferation, migration and differentiation, depending on the developmental stage and cellular context. Although miR-9 is enriched in vertebrate brain, its expression pattern is spatially and temporally regulated. Accordingly, miR-9 uses different targets to mediate its diverse functions. The recent finding that some RNA binding proteins can affect the interaction between miR-9 and its targets might also explain in part how different cellular contexts can influence miR-9 function.

In addition to its role in neurogenesis, miR-9 seems to be important for apoptosis in some developmental contexts. Moreover, aberrant expression of miR-9 in many cancer types makes it an important candidate as a diagnostic and prognostic marker. MiR-9 has also been implicated in other human pathological conditions such as neurodegeneration. However, it remains to be seen whether miR-9 contributes significantly to pathogenic processes. Further understanding of the functions of miR-9 and its target interactions in various cellular contexts will shed light on both development and disease.

Acknowledgments

We thank S. Ordway for editorial assistance, A. Wilson for help with graphics and lab members for discussions. We also thank Drs. S. Aizawa, L. Bally-Cuif and T. Sun for sharing the original high-resolution images reproduced in Figure 3. This work was supported by grant RO1 NS066586 from the National Institutes of Health (F.B.G.).

References

1. Ambros V. microRNAs: tiny regulators with great potential. Cell 2001; 107:823-6; PMID: 11779458; DOI: 10.1016/S0092-8674(01)00616-X.
2. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136:215-33; PMID: 19167326; DOI: 10.1016/j.cell.2009.01.002.
3. Small EM, Olson EN. Pervasive roles of microRNAs in cardiovascular biology. Nature 2011; 469:336-42; PMID: 21248840; DOI: 10.1038/nature09783.
4. Pauli A, Rinn JL, Schier AF. Non-coding RNAs as regulators of embryogenesis. Nat Rev Genet 2011; 12:136-49; PMID: 21245830; DOI: 10.1038/nrg2904.
5. Herranz H, Cohen SM. MicroRNAs and gene regulatory networks: managing the impact of noise in biological systems. Genes Dev 2010; 24:1339-44; PMID: 20595229; DOI: 10.1101/gad.1937010.
6. Saba R, Schratt GM. MicroRNAs in neuronal development, function and dysfunction. Brain Res 2010; 1338:3-13; PMID: 20380818; DOI: 10.1016/j.brainres.2010.03.107.
7. Gao FB. Context-dependent functions of specific microRNAs in neuronal development. Neural Dev 2010; 5:25; PMID: 20920300; DOI: 10.1186/1749-8104-5-25.
8. Leung AK, Sharp PA. MicroRNA functions in stress responses. Mol Cell 2010; 40:205-15; PMID: 20965416; DOI: 10.1016/j.molcel.2010.09.027.
9. Eacker SM, Dawson TM, Dawson VL. Understanding microRNAs in neurodegeneration. Nat Rev Neurosci 2009; 10:837-41; PMID: 19904280.
10. Sempere LF, Freemantle S, Pitha-Rowe I, Moss E, Dmitrovsky E, Ambros V. Expression profiling of mammalian microRNAs uncovers a subset of brain-expressed microRNAs with possible roles in murine and human neuronal differentiation. Genome Biol 2004; 5:13; PMID: 15003116; DOI: 10.1186/gb-2004-5-3-r13.
11. Wienholds E, Kloosterman WP, Miska E, Alvarez-Saavedra E, Berezikov E, de Bruijn E, et al. MicroRNA expression in zebrafish embryonic development. Science 2005; 309:310-1; PMID: 15919954; DOI: 10.1126/science.1114519.
12. Deo M, Yu JY, Chung KH, Tippens M, Turner DL. Detection of mammalian microRNA expression by in situ hybridization with RNA oligonucleotides. Dev Dyn 2006; 235:2538-48; PMID: 16736490; DOI: 10.1002/dvdy.20847.
13. Kapsimali M, Kloosterman WP, de Bruijn E, Rosa F, Plasterk RH, Wilson SW. MicroRNAs show a wide diversity of expression profiles in the developing and mature central nervous system. Genome Biol 2007; 8:173; PMID: 17711588; DOI: 10.1186/gb-2007-8-8-r173.
14. Kozomara A, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res 2011; 39:152-7; PMID: 21037258; DOI: 10.1093/nar/gkq1027.
15. Löytynoja A, Goldman N. An algorithm for progressive multiple alignment of sequences with insertions. Proc Natl Acad Sci USA 2005; 102:10557-62; PMID: 16000407; DOI: 10.1073/pnas.0409137102.
16. Nei M, Kumar S. Molecular Evolution and Phylogenetics. Oxford University Press, New York 2000.
17. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance and Maximum Parsimony Methods. Mol Biol Evol 2011; 28:2731-9 PMID: 21546353; DOI: 10.1093/molbev/msr121.
18. Rajasethupathy P, Fiumara F, Sheridan R, Betel D, Puthanveettil SV, Russo JJ, et al. Characterization of small RNAs in Aplysia reveals a role for miR-124 in constraining synaptic plasticity through CREB. Neuron 2009; 63:803-17; PMID: 19778509; DOI: 10.1016/j.neuron.2009.05.029.
19. Li Y, Wang F, Lee JA, Gao FB. MicroRNA-9a ensures the precise specification of sensory organ precursors in Drosophila. Genes Dev 2006; 20:2793-805; PMID: 17015424; DOI: 10.1101/gad.1466306.
20. Stark A, Brennecke J, Bushati N, Russell RB, Cohen SM. Animal MicroRNAs confer robustness to gene expression and have a significant impact on 3'UTR evolution. Cell 2005; 123:1133-46; PMID: 16337999; DOI: 10.1016/j.cell.2005.11.023.
21. Bonev B, Pisco A, Papalopulu N. MicroRNA-9 reveals regional diversity of neural progenitors along the anterior-posterior axis. Dev Cell 2011; 20:19-32; PMID: 21238922; DOI: 10.1016/j.devcel.2010.11.018.
22. Leucht C, Stigloher C, Wizenmann A, Klafke R, Folchert A, Bally-Cuif L. MicroRNA-9 directs late organizer activity of the midbrain-hindbrain boundary. Nat Neurosci 2008; 11:641-8; PMID: 18454145; DOI: 10.1038/nn.2115.
23. Darnell DK, Kaur S, Stanislaw S, Konieczka JH, Yatskievych TA, Antin PB. MicroRNA expression during chick embryo development. Dev Dyn 2006; 235:3156-65; PMID: 17013880; DOI: 10.1002/dvdy.20956.
24. Otaegi G, Pollock A, Hong J, Sun T. MicroRNA miR-9 modifies motor neuron columns by a tuning regulation of FoxP1 levels in developing spinal cords. J Neurosci 2011; 31:809-18; PMID: 21248104; DOI: 10.1523/JNEUROSCI.4330-10.2011.
25. Shibata M, Kurokawa D, Nakao H, Ohmura T, Aizawa S. MicroRNA-9 modulates Cajal-Retzius cell differentiation by suppressing Foxg1 expression in mouse medial pallium. J Neurosci 2008; 28:10415-21; PMID: 18842901; DOI: 10.1523/JNEUROSCI.3219-08.2008.
26. Shibata M, Nakao H, Kiyonari H, Abe T, Aizawa S. MicroRNA-9 regulates neurogenesis in mouse telencephalon by targeting multiple transcription factors. J Neurosci 2011; 31:3407-22; PMID: 21368052; DOI: 10.1523/JNEUROSCI.5085-10.2011.
27. Smirnova L, Gräfe A, Seiler A, Schumacher S, Nitsch R, Wulczyn FG. Regulation of miRNA expression during neural cell specification. Eur J Neurosci 2005; 21:1469-77; PMID: 15845075; DOI: 10.1111/j.1460-9568.2005.03978.x.
28. Pi H, Chien CT. Getting the edge: neural precursor selection. J Biomed Sci 2007; 14:467-73; PMID: 17357812; DOI: 10.1007/s11373-007-9156-4.
29. Nolo R, Abbott LA, Bellen HJ. Senseless, a Zn finger transcription factor, is necessary and sufficient for sensory organ development in Drosophila. Cell 2000; 102:349-62; PMID: 10975525; DOI: 10.1016/S0092-8674(00)00040-4.
30. Rhinn M, Brand M. The midbrain-hindbrain boundary organizer. Curr Opin Neurobiol 2001; 11:34-42; PMID: 11179870; DOI: 10.1016/S0959-4388(00)00171-9.
31. Zhao C, Sun G, Li S, Shi Y. A feedback regulatory loop involving microRNA-9 and nuclear receptor TLX in neural stem cell fate determination. Nat Struct Mol Biol 2009; 16:365-71; PMID: 19330006; DOI: 10.1038/nsmb.1576.
32. Delaloy C, Liu L, Lee JA, Su H, Shen F, Yang GY, et al. MicroRNA-9 coordinates proliferation and migration of human embryonic stem cell-derived neural progenitors. Cell Stem Cell 2010; 6:323-35; PMID: 20362537; DOI: 10.1016/j.stem.2010.02.015.
33. Belmont LD, Mitchison TJ. Identification of a protein that interacts with tubulin dimers and increases the catastrophe rate of microtubules. Cell 1996; 84:623-31; PMID: 8598048; DOI: 10.1016/S0092-8674(00)81037-5.
34. Krichevsky AM, Sonntag KC, Isacson O, Kosik KS. Specific microRNAs modulate embryonic stem cell-derived neurogenesis. Stem Cells 2006; 24:857-64; PMID: 16357340; DOI: 10.1634/stemcells.2005-0441.
35. Xu XL, Li Y, Wang F, Gao FB. The steady-state level of the nervous-system-specific microRNA-124a is regulated by dFMR1 in Drosophila. J Neurosci 2008; 28:11883-9; PMID: 19005053; DOI: 10.1523/JNEUROSCI.4114-08.2008.

36. Yoo AS, Staahl BT, Chen L, Crabtree GR. MicroRNA-mediated switching of chromatin-remodelling complexes in neural development. Nature 2009; 460:642-6; PMID: 19561591.
37. Biryukova I, Asmar J, Abdesselem H, Heitzler P. Drosophila miR-9a regulates wing development via fine-tuning expression of the LIM only factor, dLMO. Dev Biol 2009; 327:487-96; PMID: 19162004; DOI: 10.1016/j.ydbio.2008.12.036.
38. Bejarano F, Smibert P, Lai EC. miR-9a prevents apoptosis during wing development by repressing Drosophila LIM-only. Dev Biol 2010; 338:63-73; PMID: 19944676; DOI: 10.1016/j.ydbio.2009.11.025.
39. Pietrzykowski AZ, Friesen RM, Martin GE, Puig SI, Nowak CL, Wynne PM, et al. Posttranscriptional regulation of BK channel splice variant stability by miR-9 underlies neuroadaptation to alcohol. Neuron 2008; 59:274-87; PMID: 18667155; DOI: 10.1016/j.neuron.2008.05.032.
40. Schaefer A, O'Carroll D, Tan CL, Hillman D, Sugimori M, Llinas R, et al. Cerebellar neurodegeneration in the absence of microRNAs. J Exp Med 2007; 204:1553-8; PMID: 17606634; DOI: 10.1084/jem.20070823.
41. Cuellar TL, Davis TH, Nelson PT, Loeb GB, Harfe BD, Ullian E, et al. Dicer loss in striatal neurons produces behavioral and neuroanatomical phenotypes in the absence of neurodegeneration. Proc Natl Acad Sci USA 2008; 105:5614-9; PMID: 18385371; DOI: 10.1073/pnas.0801689105.
42. Hébert SS, Papadopoulou AS, Smith P, Galas MC, Planel E, Silahtaroglu AN, et al. Genetic ablation of Dicer in adult forebrain neurons results in abnormal tau hyperphosphorylation and neurodegeneration. Hum Mol Genet 2010; 19:3959-69; PMID: 20660113; DOI: 10.1093/hmg/ddq311.
43. Lukiw WJ. Micro-RNA speciation in fetal, adult and Alzheimer's disease hippocampus. Neuroreport 2007; 18:297-300; PMID: 17314675; DOI: 10.1097/WNR.0b013e3280148e8b.
44. Haramati S, Chapnik E, Sztainberg Y, Eilam R, Zwang R, Gershoni N, et al. miRNA malfunction causes spinal motor neuron disease. Proc Natl Acad Sci USA 2010; 107:13111-6; PMID: 20616011; DOI: 10.1073/pnas.1006151107.
45. Jeyaseelan K, Lim KY, Armugam A. MicroRNA expression in the blood and brain of rats subjected to transient focal ischemia by middle cerebral artery occlusion. Stroke 2008; 39:959-66; PMID: 18258830; DOI: 10.1161/STROKEAHA.107.500736.
46. Packer AN, Xing Y, Harper SQ, Jones L, Davidson BL. The bifunctional microRNA miR-9/miR-9* regulates REST and CoREST and is downregulated in Huntington's disease. J Neurosci 2008; 28:14341-6; PMID: 19118166; DOI: 10.1523/JNEUROSCI.2390-08.2008.
47. Laneve P, Gioia U, Andriotto A, Moretti F, Bozzoni I, Caffarelli E. A minicircuitry involving REST and CREB controls miR-9-2 expression during human neuronal differentiation. Nucleic Acids Res 2010; 38:6895-905; PMID: 20624818; DOI: 10.1093/nar/gkq604.
48. Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, et al. MicroRNA gene expression deregulation in human breast cancer. Cancer Res 2005; 65:7065-70; PMID: 16103053; DOI: 10.1158/0008-5472.CAN-05-1783.
49. Lehmann U, Hasemeier B, Christgen M, Müller M, Römermann D, Länger F, et al. Epigenetic inactivation of microRNA gene hsa-miR-9-1 in human breast cancer. J Pathol 2008; 214:17-24; PMID: 17948228; DOI: 10.1002/path.2251.
50. Laios A, O'Toole S, Flavin R, Martin C, Kelly L, Ring M, et al. Potential role of miR-9 and miR-223 in recurrent ovarian cancer. Mol Cancer 2008; 7:35; PMID: 18442408; DOI: 10.1186/1476-4598-7-35.
51. Nie K, Gomez M, Landgraf P, Garcia JF, Liu Y, Tan LH, et al. MicroRNA-mediated downregulation of PRDM1/Blimp-1 in Hodgkin/Reed-Sternberg cells: a potential pathogenetic lesion in Hodgkin lymphomas. Am J Pathol 2008; 173:242-52; PMID: 18583325; DOI: 10.2353/ajpath.2008.080009.
52. Lujambio A, Calin GA, Villanueva A, Ropero S, Sánchez-Céspedes M, Blanco D, et al. A microRNA DNA methylation signature for human cancer metastasis. Proc Natl Acad Sci USA 2008; 105:13556-61; PMID: 18768788; DOI: 10.1073/pnas.0803055105.
53. Nass D, Rosenwald S, Meiri E, Gilad S, Tabibian-Keissar H, Schlosberg A, et al. MiR-92b and miR-9/9* are specifically expressed in brain primary tumors and can be used to differentiate primary from metastatic brain tumors. Brain Pathol 2009; 19:375-83; PMID: 18624795; DOI: 10.1111/j.1750-3639.2008.00184.x.
54. Ferretti E, De Smaele E, Po A, Di Marcotullio L, Tosi E, Espinola MS, et al. MicroRNA profiling in human medulloblastoma. Int J Cancer 2009; 124:568-77; PMID: 18973228; DOI: 10.1002/ijc.23948.
55. Luo H, Zhang H, Zhang Z, Zhang X, Ning B, Guo J, et al. Downregulated miR-9 and miR-433 in human gastric carcinoma. J Exp Clin Cancer Res 2009; 28:82; PMID: 19531230; DOI: 10.1186/1756-9966-28-82.
56. Guo LM, Pu Y, Han Z, Liu T, Li YX, Liu M, et al. MicroRNA-9 inhibits ovarian cancer cell growth through regulation of NFkappaB1. FEBS J 2009; 276:5537-46; PMID: 19702828; DOI: 10.1111/j.1742-4658.2009.07237.x.
57. Malzkorn B, Wolter M, Liesenberg F, Grzendowski M, Stühler K, Meyer HE, et al. Identification and functional characterization of microRNAs involved in the malignant progression of gliomas. Brain Pathol 2010; 20:539-50; PMID: 19775293; DOI: 10.1111/j.1750-3639.2009.00328.x.
58. Myatt SS, Wang J, Monteiro LJ, Christian M, Ho KK, Fusi L, et al. Definition of microRNAs that repress expression of the tumor suppressor gene FOXO1 in endometrial cancer. Cancer Res 2010; 70:367-77; PMID: 20028871; DOI: 10.1158/0008-5472.CAN-09-1891.
59. Hu X, Schwarz JK, Lewis JS Jr, Huettner PC, Rader JS, Deasy JO, et al. A microRNA expression signature for cervical cancer prognosis. Cancer Res 2010; 70:1441-8; PMID: 20124485; DOI: 10.1158/0008-5472.CAN-09-3289.
60. Ma L, Young J, Prabhala H, Pan E, Mestdagh P, Muth D, et al. miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. Nat Cell Biol 2010; 12:247-56; PMID: 20173740.
61. Hildebrandt MA, Gu J, Lin J, Ye Y, Tan W, Tamboli P, et al. Hsa-miR-9 methylation status is associated with cancer development and metastatic recurrence in patients with clear cell renal cell carcinoma. Oncogene 2010; 29:5724-8; PMID: 20676129; DOI: 10.1038/onc.2010.305.
62. Zhong X, Li N, Liang S, Huang Q, Coukos G, Zhang L. Identification of microRNAs regulating reprogramming factor LIN28 in embryonic stem cells and cancer cells. J Biol Chem 2010; 285:41961-71; PMID: 20947512; DOI: 10.1074/jbc.M110.169607.
63. Rotkrua P, Akiyama Y, Hashimoto Y, Otsubo T. Yuasa1 Y. MiR-9 downregulates CDX2 expression in gastric cancer cells. Int J Cancer 2011; 129:2611-20; PMID: 21225631; DOI: 10.1002/ijc.25923.
64. De Pietri Tonelli D, Pulvers JN, Haffner C, Murchison EP, Hannon GJ, Huttner WB. miRNAs are essential for survival and differentiation of newborn neurons but not for expansion of neural progenitors during early neurogenesis in the mouse embryonic neocortex. Development 2008; 135:3911-21; PMID: 18997113; DOI: 10.1242/dev.025080.