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miRNAs highlights in stem and cancer cells

Martignani E, Miretti S, Accornero P, Baratta M

Department of Veterinary Morphophysiology, University of Turin, Italy

Corresponding author:

Mario Baratta, Dept. of Veterinary Morphophysiology, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), University of Turin; tel +39-(0)11-6709146; fax +39-(0)11-2369146; Email: mario.baratta@unito.it

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Abstract

MicroRNAs (miRNAs) are approximately 22 nucleotide endogenous RNA molecules which exert their functions by base pairing with messenger RNAs (mRNAs), thereby regulating protein-coding gene expression. In eukaryotic cells, miRNAs play important roles in regulating biological processes such as proliferation, differentiation, apoptosis, and stem cell self-renewal. MiRNAs are encoded by the genome, and more than 1,000 human miRNAs have been identified so far. MiRNAs are predicted to target ~60% of human mRNAs and are expressed in all animal cells. Unique expression domains, targets, and gain- and loss-of-function phenotypes of particular miRNAs have important implications for directed differentiation of stem cell populations. Many cancers show variations in miRNA levels, and more specifically an overall downregulation, when compared to their normal counterparts. Therefore, miRNAs may be used as potential therapeutic agents to correct aberrant transcript levels found in the signaling pathways of cancer. This review examines the most recent acquisition on the role of miRNAs in regulating the cell cycle, with particular emphasis on their effects on cell proliferation and differentiation. The second part explores specifically the role of these factors in the physiological regulation of embryonic stem cells, of cellular reprogramming and their involvement in the activation of stem cells in adult tissues. In the third part, the article discusses some issues that relate to the role of miRNAs in the development of neoplastic diseases, focusing on aspects of the genetic and trascriptional alterations that determine the beginning and the development of tumor process and looking to emphasize their involvement in the activation of adult cancer stem cells .

Introduction

The discovery on the role of the *Lin-4* and *Lin-14* genes in temporal control of development in the model organism C*aenorhabditis elegans* rapidly rose the attention of researchers about the role played by these small molecules in development and cell differentiation in mammals [1]. In the same year, another finding increased the interest for this class of molecules . The *Lin-4* gene does not encode for any protein but gives rise to a 61-nucleotides (nt) precursor RNA that further matures to a more abundant 22-nt transcript. In addition it was reported that the LIN-14 protein synthesis is regulated post-transcriptionally and that LIN-14 levels are inversely proportional to those of *Lin-4* RNA [2]. Sequence analysis revealed that *Lin-4* RNA is complementary to the 3' untranslated region (3'-UTR) of the *Lin-14* gene, leading to the hypothesis that variations in *Lin-14* expression levels were due to a post-transcriptional interaction with *Lin-4.* This constituted the discovery of the first miRNA and target messenger RNA (mRNA) interaction [1,2]. At the beginning of the 21st century the evidence of a correlation between miRNA abundance and human disease, and specifically an association between the loss of miR-15 and -16 and the occurrence of B-cell leukemia [3], marked the beginning of a new era in cell biology, and permanently changed our view of the relationship between gene expression and protein levels. Analysis of the correlation between mRNAs and human disease is now expanding to include also those sequences that constitutes the remaining 90% of eukaryotic genomes and that generate non-coding RNAs. Recently a great interest was aroused when miRNAs were shown to act both as tumor suppressors and oncogenes, which promote tumor growth. In addition, aberrant expression of several miRNA has been shown in many cancers. More specifically an overall downregulation of this class of transcripts was demonstrated in cancer cells when compared to normal tissues [4]. Therefore, a potential therapeutic use of miRNAs may correct aberrant transcript levels usually found in the signaling pathways of cancer cells, and more importantly of cancer stem cells (CSCs).

Biology of miRNAs

miRNAs are endogenously produced, short RNAs of 21-25 nt that are important regulators of gene expression at the post-transcriptional level [5,6,7]. By binding to the 3'-UTR of the target mRNA, miRNAs can induce degradation or, more frequently, cause repression of protein translation [8]. The biogenesis of miRNAs comprises transcription, processing/maturation and degradation (Fig.1). Depending on the genomic location, miRNAs are transcribed differently: intergenic miRNAs are transcribed into pri-miRNAs by RNA polymerase II since they contain their own promoter and

regulatory units [9,10]. Intronic miRNAs are co-transcribed with their host genes from a common promoter [11,12]. Pri-miRNAs from intergenic region are capped at the 5' site (m^7G), polyadenylated at the 3' site and further cleaved into pre-miRNA by Drosha/DGCR8 microprocessor complex, which is minimally composed by the ribonuclease (RNase) III endonuclease Drosha and its binding partner DGCR8/Pasha [13,14]. Intronic miRNAs are directly cleaved by Drosha/DGCR8 complex into pre-miRNAs without affecting the splicing step of the host genes [15,16,17].

Precursor miRNAs are then exported from the nucleus to the cytoplasm by exportin 5 and Ran-GTP [18,19]. Here they are processed again to generate short (22-25 nt) duplexes (imperfect miRNA duplex) by the RNase III Dicer [20], which is part of the pre-miRNA processing complex [21,13]. The pre-miRNA, RNase III Dicer and Argonaute 2 (Ago2) form the pre-RISC (RNA-induced silencing complex) [22]. One strand of the short RNA duplex is loaded into the Ago2-containing RISC [14] to form a miRNA silencing complex, while its complementary (passenger) strand is subsequently degraded [23]. The RISC-loaded mature miRNA is protected from degradation by Argonaute proteins. However, after finishing its task, the mature single-strand miRNA will also be degraded by the 5′-3′ exoribonuclease XRN2 [24] or by 3′-5′ exoribonucleases, like human polynucleotide phosphorylase (PNPase) [25] and nuclear exosome [26]. A more complete review of the factors or mechanisms that regulate protein stability and degradation of miRNAs have been recently summed up [27,28]. In mammals, Ago2 is the only Argonaute family member with endonuclease activity [23,29]. Analysis of the mRNAs that co-immunoprecipitate with Dicer suggests that Dicer and its associated proteins are not part of the miRNA effector complexes, since the Dicer-enriched transcripts share no significant similarity with the miRNA targets immunoprecipitated with Ago [22]. Thus, the Ago proteins disengage from the RISC loading complex before targeting mRNAs [30]. Nucleotides in the mature miRNA pair with complementary sequences in the mRNA which forms a short helix. Complete complementarity between the 20-22 nt of the small RNA and the mRNA leads to degradation, hence 'silencing' of the transcript. In contrast, partial base pairing between nucleotides 2-8 of the miRNA (the so called seed sequence) and the mRNA leads primarily to suppression of protein translation and partial degradation [31,8].

It is still not clear how miRNAs physically find their target mRNAs in the cell, and how do protein-protein interactions facilitate targeting [32]. The free energy of base pairing drives association of the complementary miRNA with its target mRNA [33]; however, it is not known how these two nucleic acid strands are brought into close enough proximity to achieve helix formation.

A number of proteins that have been co-purified with Ago2 could potentially participate in this interaction. They include Ago family member (Ago1), several RNA binding proteins such as the

Fragile X mental retardation protein (FMRP), putative RNA binding proteins like Vasa intronic gene (VIG) and an RNA recognition motif-containing protein, Trinucleotide repeat-containing 6B, (TNRC6B)[34,35,36]. Mammalian FMRP and its autosomal paralogs, fragile X-related proteins 1 and 2 (FXR1P and FXR2P) are correlated with Dicer and other components of the RISC, including Ago [37]. Phosphorylation of FMRP eliminates association with Dicer and may function as a switch for association with the miRNA pathway [38]. Furthermore, FMRP has been demonstrated to directly associate with miRNAs *in vitro* and to specifically pair miRNAs to RNAs containing the correct seed sequence [39]. TNRC6B is a member of a family of proteins that are the vertebrate paralogs of GW182, a scaffolding protein. GW182 is present in the core miRNA silencing complex and it is important for localization to processing bodies, as well as for translation silencing and mRNA degradation [40,41]. TNRC6A, -B and -C associate with miRNA-complexed Ago proteins and the TNRC6 proteins contain a P-body localization domain [42]. It has been reported recently that GW182 interferes with mRNA circularization and also recruits the deadenylase complex through interaction with poly(A) binding protein C1 [43], this evidence support the hypothesis that GW182 is involved in miRNA-mediated silencing.

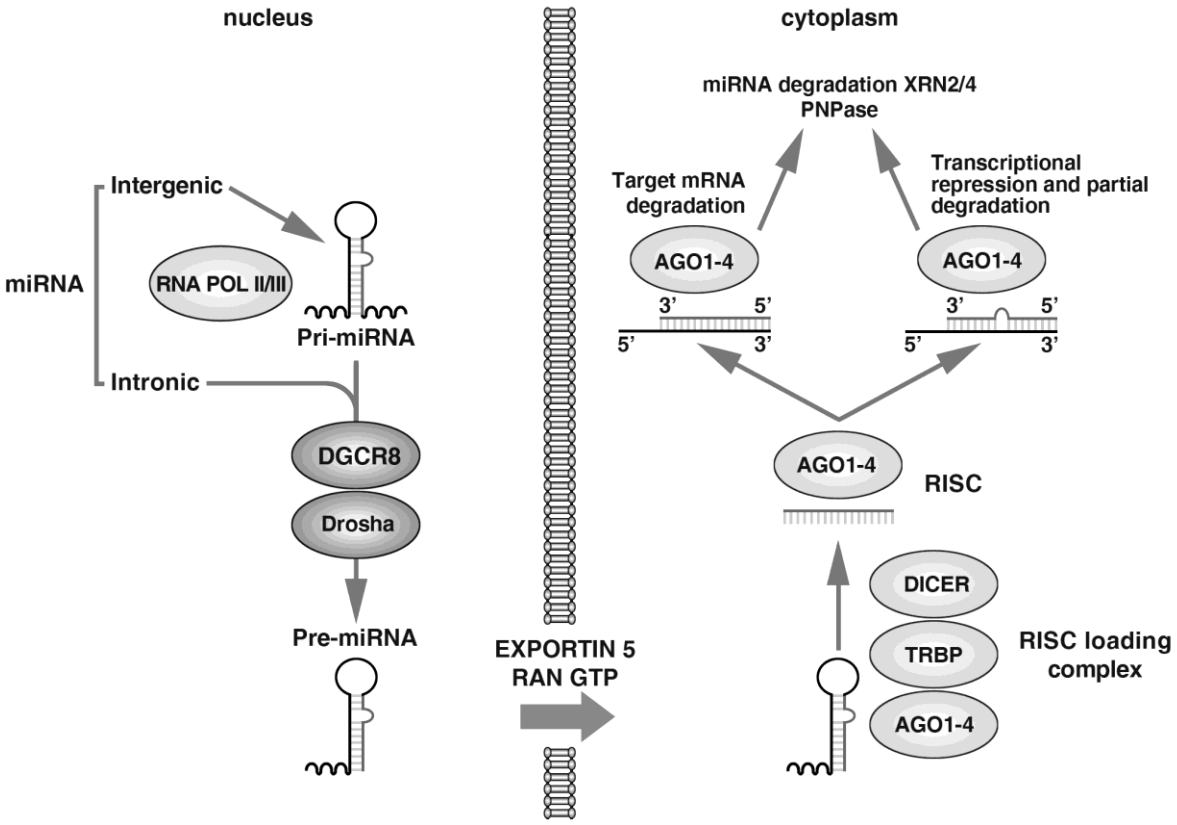

Fig.1

The miRNAs serve as meta-controllers for gene expression and are crucial for the cellular changes that are necessary for development. A few hundred miRNAs have been identified in various organisms and current estimates indicate that these miRNAs may regulate almost half of protein-coding genes [44]. Bioinformatics analysis coupled with experimental validation has lead to estimate that most animals have a few hundred miRNA coding genes [6,7]. MiRNAs are encoded by the genome, and more than 1,000 human miRNAs have been identified so far and are predicted to target ~60% of human mRNAs [45]. By regulating gene expression, miRNAs play critical roles in a variety of cellular and physiological activities [46]. In human diseases, miRNA expression is frequently altered, thus contributing to the pathogenesis [47,48,49,50,51].

miRNAs can modulate cellular response to environment, influencing the differentiation from a multipotent progenitor through progressively committed states to a more differentiated condition. These properties make miRNAs nearly ubiquitous and fundamental in all tissues.

miRNAs and cell cycle

miRNAs play an evolutionarily conserved developmental role modulating several physiological functions. Although they exhibit limited complementarity with their target mRNAs, they regulate fundamental processes during embryogenesis and in adult life. In fact, miRNAs participate in all biological processes, such as stem cell maintenance, developmental timing, cell proliferation, differentiation, apoptosis, signal pathway and pathogenesis [52,53]. A significant role of miRNA in the cell-cycle and in maintaining a balance between cell proliferation and apoptosis has been reported. This is achieved by influencing the activity of regulatory proteins, such as cyclins, that control cell proliferation. In particular, miR-221 and miR-222 promote cell-cycle progression (G1-S transition) targeting mRNAs encoding the cyclin-dependent kinase (CDK) inhibitory proteins p57 and p27 [54,55]. On the other side, miRNAs negatively regulate cell proliferation inducing growth arrest by directly decreasing the expression of cyclin/CDK complexes. Cyclin-dependent kinase 6 (CDK6) expression is inhibited by miR-129 and miR-137 respectively in normal cells [56] and in oral squamous carcinoma cells [57]. The same CDK6 and cyclin D1 are under regulation of miR-34a [58]. Moreover, miRNAs are involved in signalling pathways implicated in the cell-cycle. p53 is a tumour suppressor activated in response to cellular stress that is able to induce cell-cycle arrest, senescence, or apoptosis. miR-34 is transcriptionally activated by p53 and serves as mediator of p53 signalling pathway [59,60]. miR-192/215 and miR-194 clusters are shown to be p53-responsive and can induce cell-cycle arrest by indirectly triggering p21 expression [61,62]. The PI3K/AKT signalling pathway plays crucial roles in many cellular processes including cell proliferation, differentiation, and motility. Upon PI3K activation, Akt phosphorylates and inhibits the activity of

protein kinase GSK3b, which stimulates degradation of cyclin D, therefore activation of PI3K signalling pathway stabilizes cyclin D and enhances proliferation. This pathway is inhibited by phosphatase and tensin homolog (PTEN), which modulates cell-cycle and serves as an important tumour suppressor gene [58]. PTEN is fine-tuned by several miRNA, such as miR-17-92 family members [63].

miRNAs play a role in apoptosis modulation, as expression of several miRNAs can be induced by serum starvation, DNA damage [53], and hypoxia [64]. Cells deprived of miRNA regulation by Dicer or Ago2 silencing are more susceptible to UV [65]. miR-16 is one of the miRNAs that are down-regulated only just 4 hours after UV treatment. miR-16 and miR-21 trigger cell-cycle arrest by negatively modulating the expression of Cdc25A, a gene that is involved in both G1/S and G2/M arrest, and targeting cyclin D1 and cyclin E [53]; miR-29b is induced during the physiologically normal process of neuronal maturation and is able to inhibit apoptosis by targeting several BH3-only members of the Bcl-2 proapoptotic gene family [66].

miRNA	Cell / Tissue	Target	Function
let-7 family	Lung Fibroblast Muscle cell Neural Stem Cell	CDC25A [67,68] CDC34 [67,69] CDK4 [67] CDK6 [67] Cyclin D1 [70]	Block or delays in the G1/S transition Increase in G_2/M phase cells fraction: cell proliferation Induction of apoptosis Reduction of the G1/S transition Inhibition of proliferation and promotion of cell differentiation
miR-15 family	Colon and ovary cancer cells Embryonic Stem cell	CDC27, CDK6, Cyclin D1 [71,72] WEE1 [73]	Accumulation of cells in G0/G1, cell cycle arrest Regulation of the G2/M checkpoint
miR-17 family *miR-17-92 cluster*	Umbilical vein endothelial cells Unrestricted somatic stem cells differentiated into neuronal lineage Choriocarcinoma cell Glomerular mesangial cells	Cyclin D1 [74] E2F1 [75] p21Cip1/Waf1 [76] PTEN [63]	Arrest of cell cycle at G1/S transition Promotion of cell proliferation Cell-cycle progression Increase of PIP3 and Akt activation
miR-19a	Neural progenitor cells	CyclinD1 [77]	Neuronal differentiation, cell-cycle exit
miR-24	Lung fibroblast cell line	AURKB, CCNA2, CDC2, CDK4, E2F2, MYC [78]	Inhibition of cell-cycle progression, increase of the G1 compartment
miR-25	Gastric adenocarcinoma and corresponding normal tissue	p57Kip2 [79]	Ensure the G1/S transition
miR-34a/c	Non-small cell lung cancer A549 cells Primary keratinocytes and embryonic skin	CCND1, CDK6 [58] CDK4,Cyclin D1 [80]	G1 cell-cycle arrest G1-phase arrest
miR-124a	Medulloblastoma cells	CDK6 J [81]	Decrease cell proliferation
miR-129	Mouse lung epithelial cells and human lung adenocarcinoma cell lines	CDK6 [56]	G1 phase arrest that eventually leads to cell death
miR-137	Oral squamous cell carcinoma	CDK6 [57]	Decrease in S and G2/M phase cells and induction of accumulation of G0/G1 phase cells
miR-149*	Neuroblastoma cell line	E2F1[82]	Induction of apoptosis
miR-192/ miR-215	Normal colon tissue	p53, CDKN1A/p21[61]	Cell cycle arrest
miR-221/222	Hepatocellular carcinoma; ovarian carcinoma	CDKN1B/p27, CDKN1C/p57 [54,83]	Increase of number of cells in S-phase
miR-290	Embryonic stem cells (ESC)	WEE1, Fbxl5 [84]	Prevention of ES cells from

cluster	and embryonic carcinoma cells (ECC)		accumulating in G1 phase
miR-330	Prostate cancer cell	E2F1 [85]	Induction of apoptosis
miR-331-3p	Gastric cancer	E2F1 [86]	Block in G1/S transition
miR-322/424 miR-503	Myoblasts (C2C12) differentiating into myotubes U2OS osteosarcoma cells	CDC25A [87]	Acceleration of myogenic differentiation Promotion of G1 Arrest
miR-449a/b miR-449 and miR-34	Breast epithelial and cancer cells(MCF-10A, MCF-7); Colorectal carcinoma cell (HCT116), Osteosarcoma cell (SaOS, U2OS)	CDC25A, CDK6 [88] CDK6 [89]	Cell cycle arrest at G1 phase Cell cycle arrest and apoptosis

Table. 1 miRNA and cell cycle function

miRNAs and tissues specific functions

There are increasing evidences of roles played by miRNAs during cellular differentiation, such as targeting negative regulators of genes responsible for the epigenetic profile of precursor cells. The maintenance of stem/progenitor cells and their differentiation fate follows a well defined epigenetic program. This program is coordinately regulated by DNA methylation, histone modifications, and miRNAs [90,91,92]. Neurogenesis and myogenesis represent well-described systems in which the genetic program from committed precursors to differentiated cells has been dissected into well defined steps, in which miRNAs play important functions in modulating transcriptional regulators of specific genes.

miRNAs are dynamically regulated during neural development. Expression profiling revealed that miRNAs have spatiotemporal and cell-specific expression patterns in the nervous system [93]. The expression of some miRNAs is brain-specific, such as miR-9, miR-124 and miR-135, while other miRNAs are only enriched in the brain, such as let-7, miR-9* (* = from the opposite arm of the miRNA precursor), miR-125 and miR-128 [94]. In neural development miRNAs can be divided into cell-specific expression groups: preferentially expressed in neurons (let-7, mir-9, mir-124, mir-125, mir-128), strongly astrocytic (mir-23, mir-29), implicated in oligodendrocyte differentiation (mir-138, mir-219, mir-338) [95,96,97,98]. Segregation of miRNA populations in different cell types and the temporal disparity in the appearance of their primary transcripts and cytoplasmic precursors compared with the mature form during cell differentiation could explain which are the transcriptional control mechanisms that are essential for the shift from the pluripotent, self-

renewing state to a more committed condition. Although not restricted to the nervous system, let-7 family members are highly represented in miRNA brain libraries [70]. let-7 is a valid example to explain the importance of miRNAs for spatiotemporal and cell-specific expression patterns: mature let-7 is lacking in undifferentiated cells, but the processing activity on its precursor increases in parallel with neural differentiation. Rybak and colleagues have demonstrated that in embryonic stem cells, the pluripotency factor Lin-28 binds the pre-let-7 RNA and inhibits processing by the Dicer ribonuclease [99]. In embryonic neural stem cells, Lin-28 is downregulated by mir-125 and let-7, allowing processing of pre-let-7 to proceed. Deletion of let-7 or mir-125 activity in embryonic neural stem cells leads to upregulation of Lin-28 and loss of pre-let-7 processing activity, suggesting the presence of a feedback loop in which let-7, mir-125 and Lin-28 participate in an autoregulatory circuit that controls miRNA processing during neural stem cell commitment [99]. Detailed functions in neuronal development have been shown also for miR-9 and miR-124, two miRNAs specifically expressed in the mammalian nervous system. Similarly to let-7 and Lin-28, miR-9 and the nuclear receptor TLX are implicated in a feedback regulatory loop to control neural stem cell proliferation and differentiation [100]. TLX is highly expressed in neural stem cells but it is repressed upon differentiation; in contrast, the level of the miR-9 mature form is increased upon differentiation [100]. miR-124 is the most abundant miRNA in both the embryonic and adult central nervous system. The striking upregulation of miR-124 during neuronal differentiation suggests that it may have a unique function in this process. Ectopic expression of miR-124 in cultured non-neuronal cells increased the expression of neuronal genes and inhibited non-neuronal genes [101]. This process might be required during early embryonic neurogenesis to ensure that differentiation occurs at the correct time and also that the appropriate number of neurons is generated. Finally, miR-9* and miR-124 are both required to inhibit BAF53a subunit expression in order to switch the chromatin-remodelling complex during neural development and to promote differentiation [102]. Mutation of miR-9* and miR-124 recognition sites in the 3'-UTR region lead to persistent expression of BAF53a and defective activity-dependent dendritic outgrowth in neurons [102].

The development of cardiac and skeletal muscle is orchestrated by evolutionarily conserved networks of transcription factors that regulate the expression of genes involved in muscle growth, differentiation, and contractility. miRNAs have been shown to regulate skeletal myogenesis in developing embryos and during adult life [103]. The miRNAs participation in the muscle transcriptional program expands the precision and complexity of gene regulation in muscle cells. A subset of miRNAs are considered specific to the muscle tissue. The most widely studied are members of miR-1/206 and miR-133a/133b families. These miRNAs are either specifically or highly expressed in cardiac and skeletal muscle. Interestingly, miRNAs regulate muscle gene

expression, either in a positive or negative way, by targeting chromatin modifying enzymes. Examples of interactions between miRNAs and epigenetic regulators of gene transcription are provided by miR-1 that mediates downregulation of Histone Deacetylase 4 (HDAC4), a key inhibitor of muscle differentiation and miR-133 that represses the Serum Response Factor (SRF), a transcriptional activator that promotes differentiation [104]. Thus, miR-1 and miR-133 seem to have antagonistic effects on muscle lineages. MiR-1 promotes differentiation of cardiac progenitors and exit from the cell cycle. In contrast, miR-133 inhibits differentiation of skeletal myoblasts and maintains them in a proliferative state. The results of many studies in fact, indicate that miR-133 acts in partial opposition to miR-1, promoting muscle progenitor expansion and preventing terminal differentiation [104,105]. This effect may occur, in part, through miR-133 repression of cyclin D2 which controls differentiation and proliferation of muscle cells [106]. Interestingly, miR-1 and miR-133 are also important regulators of cardiomyocyte differentiation and heart development [105,107]. Other miRNAs participate in a regulatory circuit that facilitates gene program transition from proliferation of skeletal muscle satellite cells to primary myoblast differentiation. miR-206 is induced by MyoD and Myogenin and promotes muscle differentiation by a positive feedback loop. miR-1, miR-206 and miR-486 are up-regulated during satellite cell differentiation while markedly decreased during skeletal muscle regeneration [108,109]. All these miRNAs promote muscle differentiation by directly targeting and downregulating Pax7 protein and mRNA. Transfecting miR-206 or miR-486 independently increased the G1 phase population and decreased the S phase population of myoblast cells, indicating that these miRNAs also promote cell cycle quiescence [109].

miRNA	Cell / Tissue	Target	Function in neural development
let-7	Embryonic NSC	LIN-28 [99]	Promotion of neuronal lineage commitment
miR-9	Telencephalon NSC Midbrain-hindbrain	Gsh2, Foxg1 [110] TLX [100] fgf8-1, fgfr-1 [111]	Control of neural progenitor proliferation and differentiation Promotion of NSC differentiation Maintenance of organizer activity at the midbrain–hindbrain boundary
miR-9* miR-124	Neural progenitors	BAF53a [102]	Switch of neural specific chromatin-remodelling complex during neural development and promote differentiation
miR-17	SH-SY5Y cell (neuroblast)	BCL2, MEF2D and MAP3K12 [112]	Regulation of neuronal differentiation
miR-23	Oligodendrocyte	LMNB1 [113]	Oligodendrocyte development and myelination
miR-124	Adult SVZ Developing spinal cord neurons ES cell and P19 cells P19 cells	SOX9 [114] SOX9 [115] unknown [116] (REST/SCP1 [117] PTBP1 [101]	Increased neuron formation Regulation of neuroepithelial stem cells Suppresses dendritic branching Proneural activity in developing CNS Nervous System development
miR-125b	SH-SH5Y cell and neural progenitor ReNcell VM cell	Multiple targets [118]	Promotion of neuronal differentiation Promotion of neurite outgrowth
miR-128	SH-SH5Y cell and neuroblastoma glioma "stem-like" cells	Reelin, DCX [119] Bmi-1 [120]	Decrease in growth, motility and invasiveness, blocked self-renewal
miR-200	Olfactory progenitor cell	Notch and TGFβ signaling pathways and Foxg1 [121]	Proper differentiation of olfactory progenitor cells
miR-219	Oligodendrocyte cells	Sox6 and Hes5 [122] PDGFRalpha, Sox6, FoxJ3, ZFP238 [95]	Promotion of oligodendrocyte differentiation
miR-125b miR-324-5p/ miR-326	Medulloblastoma	GLI1 and SMO [123]	Inhibition of cell growth
miRNA	**Cell / Tissue**	**Target**	**Function in muscle development**
miR-1 miR-1-2	C2C12 cell Cardiomyocyte	HDAC4 [104] Hand2 [124] Irx5 [107]	Stimulation of myoblast differentiation Regulation of cardiac morphogenesis and electrical conduction

miR-1 and miR-206	C2C12 cell	Cx43 [125]	Promotion of differentiation of skeletal muscle
miR-24	Primary myoblast cells	Unknown [126]	Induction of cardiomyocyte hypertrophy
miR26a	C2C12 cell	Ezh2 [127]	Promotion of myogenesis
miR-27b	Satellite cell	PAX3 [128]	Promotion of myogenesis
miR-125b	C2C12 cell and primary myoblasts	IGF-II	Inhibition of myoblast differentiation and muscle regeneration
miR-133a	C2C12 cell	SRF [104]	Increase in myoblast proliferation
miR-145	Vascular smooth muscle cell	KLF4, CamkIId [129] [130]	Induction of differentiation of multipotent neural crest stem cells into vascular smooth muscle
miR-143 and miR-145	Smooth muscle cells	KLF4, myocardin and Elk-1 [130]	Promotion of differentiation and repress proliferation
miR-181	C2C12 cell and limb muscle precursors	Hox-A1[131]	Pro-differentiation effects
miR-206	C2C12 cell C2C12 cell and satellite cells	Unknown [132] TIMP3 [133]	Promotion of muscle differentiation
miR-206 and miR-29	C2C12 cell and primary myoblasts	HDAC4 [134]	Myogenic differentiation
miR-208	Cardiomyocytes	Thrap1, MSTN [135]	Hypertrophic growth
miR-214	C2C12 cell	N-ras [136]	Myogenic differentiation
miR-221/222	Primary cultures of quail myoblasts	p27 [137]	Progression from myoblasts to myocytes
miR-486	Neonatal cardiomyocytes	PTEN, Foxo1[138]	Regulation of PI3-kinase/Akt signaling
miR-206 and miR-486	C2C12 cell	PAX7 [109]	Stimulation of myogenic differentiation

Table. 2

miRNA role in the development and function of neuronal and muscle tissues. NSC: Neural Stem Cell, NCS Neural Central System; SVZ: sub-ventricular zone.

miRNA and stem cell biology

The path that stem cells take towards differentiation involves extensive changes in gene expression. Genes that are responsible for self-renewal and pluri/multi-potency need to be downregulated, while new transcripts are required to specialize in tissue-specific functions. Different epigenetic processes act at multiple stages to dramatically change the proteome of a stem cell: chromatin remodeling, promoter methylation and post-transcriptional regulations represent tightly controlled checkpoints in gene expression along with changes in the transcription factors pool. Such a complex network of factors results in the ability of the cell to fine tune the expression of hundreds of genes in a coordinated and predetermined manner.

miRNA and embryonic stem cells: from pluripotency to lineage commitment

Embryonic stem cells (ESCs) are defined by two main characteristics: the ability to self-renew and to differentiate in vivo in any cell type of the developing organism. Several transcription factors (TFs) have been identified as key regulators in maintaining ESCs in an undifferentiated state. Among these TFs a major role is played by Oct4, Sox2 and Nanog, which even possess the potential to revert a fully differentiated cell to a pluripotent state [139,140]. Whatever, important their role might be, they alone cannot account for the complex and extensive modulations in gene expression that are required to maintain pluripotency.

In order to understand the role that miRNAs play in this context different approaches have been adopted. Mouse ESCs in which either *Dicer* or *DGCR8* alleles have been deleted are unable to correctly process miRNAs precursors and therefore lack most of the mature miRs [141,142]. Both cell lines are viable and show phenotypes that are mostly overlapping. Cell proliferation is slower and when cultured in conditions that induce differentiation they do not express differentiation markers, downregulate pluripotency related genes or lose the ability to form colonies compared to wild type ESCs . A similar behavior has been described for human ESCs in which *Drosha* or *Dicer* where silenced: interestingly introduction of exogenous miR-195 and miR-372 family members partially rescued the phenotype of *Dicer* knockdown cells [73]. In mouse ESCs a comparable phenotype rescue of *DGCR8* knockdown was shown when miRNAs belonging to the miR-290, miR-302 and miR17-92 clusters where introduced [142]. Collectively members of these families are called ESCC (ESC-specific cell cycle-regulating) miRs and noticeably they share a very similar seed sequence, suggesting that they regulate a common set of genes.

Mouse and human ESCs show a peculiar cell cycle in which the G1-S transition occurs very quickly due to a permanent inactivation of pRb protein by hyperphosphorylation [143]. Moreover high

levels of cell cycle activators (like cyclin E/A and cyclin D3) and absence of cell cycle inhibitors (like INK4 family members) contribute to a very rapid proliferation of ESCs [144]. Quite interestingly both the *Dicer* and the *DGCR8* knockdown ESCs show an increased proportion of cells in the G0-G1 phase indicating that the G1-S transition in cell cycle might be a key target for specific pluripotency-associated miRNAs. The lack of ESCC miRs relieves the repression on Cdkn1a, Rb1, Rbl2 and Lats2. These proteins are inhibitor of the cyclin E-Cdk2 complex and therefore of the G1-S transition [145,145]. As these data suggest, these miRNAs are able to promote a quicker G1-S transition and represent key players in regulating the particular cell cycle that ESCs undergo. However other papers demonstrate that while ESCC miRNAs are involved in proliferation control, other families of miRNAs are required to control the differentiation process. Lack of specific miRNAs is necessary for maintenance of pluripotency as in the case of Let-7 family. Introduction of exogenous Let-7 in *DGCR8* knockdown cells recovers the ability of ESCs to differentiate by targeting several transcription factors that are known to be associated with stemness, like c-Myc, LIN-28 and SALL-4 [146]. Another group adopted an elegant approach to determine the function of a family of miRNAs which is expressed in mESCs: the miR-290-295 cluster is highly expressed in mouse stem cells and share a similar seed sequence. This group generated a *Dicer1*-null ES cell line where endogenous Ago2 was replaced by a myc-epitope tagged Ago2 that was catalytically inactivated through point mutations. Introduction of miR-294 in this cell line allowed for target mRNA to be trapped by miR294/Ago2-myc complexes: subsequent immunoprecipitation and RNA sequencing allowed to identify targets of this miRNA. Moreover the same authors show how miR-294 introduction resulted in upregulation of LIN-28 (a pluripotency factor) and of several targets of c-Myc [147]. In hESCs the miR-302-367 family is similarly involved in the control of differentiation: among their targets are *lefty1* and *lefty2*, which along with *Nodal* are required for differentiation and germ layer specification during embryogenesis [148]. Overexpression of miR-302s causes an unbalance between Nodal and lefties protein levels that ultimately results in a delayed ESCs differentiation. miR-302 and the transcriptional factors OCT4 and NR2F2 (COUP-TFII) are linked in a regulatory circuitry that critically regulate both pluripotency and differentiation in hESCs. In the undifferentiated state, both OCT4 and the OCT4-induced miR-302 directly repress NR2F2 at the transcriptional and post-transcriptional level, respectively [149].

While in the latter case miRNAs were responsible for maintenance of pluripotency, other families are upregulated when the cell starts differentiating: this is the case of miR-134, miR-296 and miR-470 which inhibit the expression of the pluripotency factors Nanog, Oct4 and Sox2 [150] and of miR-200c, miR-203 and miR-183 that negatively regulate Sox2 and Klf4 [151]. The same

mechanism is operating in hESCs, where miR-145 has been demonstrated to be able to block self-renewal by targeting Oct4, Sox2 and Klf4 [152].

miRNAs and cell reprogramming

Reprogramming of a differentiated cell to a pluripotent state has been achieved by introduction of a group of exogenous transcription factors [139,140]. As previously described these factors have important connections with specific families of miRNAs: not only miRs control expression of TFs, but at the same time these TFs have been shown to bind the promoters of several miRNAs families [153,154]. These data point to a complex network of both transcription factors and miRNAs that regulates pluripotency and hence suggest that several miRNAs may be involved in the reprogramming process of differentiated cells.

Notably introduction in mouse embryonic fibroblasts (MEFs) of *Oct4*, *Sox2*, *Klf4* and miR-294 (as a replacement for *c-Myc*) resulted in iPSCs colony frequency similar when *Oct4*, *Sox2*, *Klf4* and *c-Myc* were used and 10-fold higher than *Oct4*, *Sox2* and *Klf4* alone [155]. Similarly an increase in reprogramming efficiency was seen when down-regulation of Let-7 miRNA was obtained by use of an antisense inhibitor [146]. More recently, three different clusters of miRNAs (miR-17-92, miR-106b-25, miR-106a-363) have been described as highly induced during the early phase of reprogramming in mouse fibroblasts [156]. In addition miR-93 and miR-106b are able to increase the iPS generation efficiency in MEFs when used along with the four reprogramming factors, probably by promoting a mesenchymal-to-epithelial transition which has been described as the first step in reprogramming [156]. It was also shown that miR-93 and miR-106b target p21 (involved in G1/S transition) and Tgfbr2 (a member of the TGF-β signaling pathway): both genes are known to decrease reprogramming efficiency and hence their down-regulation by miRNAs has a positive effect on iPS generation [157]. Finally it has been reported that mir-302 expression in hES led to reprogramming of human hair follicle cells to iPS cells. This reprogramming mechanism acts through mir-302-targeted co-suppression of four epigenetic regulators, AOF2 (also known as KDM1 or LSD1), AOF1, MECP1-p66 and MECP2 [158]. It has been also reported that expression of the miR302/367 cluster rapidly and efficiently reprograms mouse and human somatic cells to an iPS state without the requirement for exogenous transcription factors [159].

Another interesting study reports as mir-302 is expressed most abundantly in slow-growing hES cells and quickly decreases after cell differentiation and proliferation. mir-302 not only function to reprogram cancer cells into an ES-like pluripotent state but also to maintain this state under a feeder-free culture condition, which may offer a great opportunity for therapeutic intervention since this reprogramming method does not require vector-based gene transfer. [160]. Furthermore, miR-

302b and miR-372 repress multiple target genes, with downregulation of individual targets only partially recapitulating the total miRNA effects. These targets regulate various cellular processes, including cell cycle, epithelial-mesenchymal transition, epigenetic regulation and vesicular transport. They also increase the kinetics of mesenchymal-epithelial transition during reprogramming and block TGFbeta-induced EMT of human epithelial cells [161]. A very recent study shows that it is possible to reprogram mouse and human cells to pluripotency by direct transfection of a combination of mir-200c plus mir-302 and mir-369 family miRNAs [162].

We may conclude that transcription factor-based cellular reprogramming has opened the way to converting somatic cells to a pluripotent state. However this approached has faced so far limitations resulting from the requirement for exogenous transcription factors and the relative inefficiency of the process. On the contrary, miRNAs may be used as powerful tools to discover novel pathways that are involved in cell fate transitions using dedifferentiation of somatic cells to induced pluripotent stem cells as a case study [163].

The role of miRNAs in skeletal muscle proliferation and differentiation

Many miRNAs show a tissue-restricted pattern of expression, where specific subsets are found in different tissues with little or no overlap. This might suggest a role of miRNAs in the specification of various cell types and therefore in the differentiation process.

For example, miR-9 and miR-124a have been shown to play a key role in differentiation of neural progenitors into neurons and astrocytes [164,165]. Quite interestingly expression of these miRNAs is restricted to the neurogenic areas of the mouse brain, suggesting that their expression may promote differentiation [166]. *In vitro* experiments showed how inhibition of miR-9 resulted in increased phosphorylation of STAT3 and in an impaired differentiation. On the other hand, overexpression of miR-9 or of miR-124a caused a decreased STAT3 phosphorylation associated with a limited astrocyte lineage differentiation.

The skeletal muscle is a very well studied tissue where for the first time the role of miRNAs as regulators of gene expression has been described. In this context miRNAs have been shown to be involved in lineage determination by promoting the differentiation of myoblast precursors into skeletal muscle. Specifically miR-1 and miR-133 seem to have an opposite effect: while miR-1 promotes very efficiently differentiation of myoblasts, miR-133 causes a myoblast expansion by enhancing their proliferation [104]. Interestingly introduction of exogenous miR-1 alone is able to shift the gene expression profile of HeLa cells towards skeletal muscle cells [167] and thus demonstrate the primary role that this miRNA plays in differentiation. In smooth muscle a similar

control seem to take place: reports indicate that miR-145 might play a role close to miR-1 in skeletal muscle, by inducing differentiation in smooth muscle cells and preventing their proliferation [130].

Even in the hematopoietic system miRNAs are involved in stem cell homeostasis: miR-125b is highly expressed in mouse HSC and downregulated in committed progenitors. Its overexpression in mouse HSCs resulted in reduced apoptotic rates that led to an expansion of different subsets of hematopoietic stem cells (notably the lymphoid-balanced and the lymphoid-biased subsets) [168].

miRNA and cancer

Cancer is characterized by the presence of rapid, uncoordinated cell growth. Deregulated genes, usually activated oncogenes and inactivated tumor suppressor genes act as major players in the development and spread of this malignancy. miRNAs can target multiple genes, alterations in the amount or sequence of the cell's miRNome can modify the expression of all target genes. Therefore miRNA modulation can lead to enhanced rates of proliferation and/or decreased rates of apoptosis and these changes can increase the risk of cancer development over a lifetime. This has led to the hypothesis that, similar to the coding genes involved in cancer, upregulated miRNAs (oncomiRs) may act as oncogenes and downregulated miRNAs (anti-oncomiRs) may act as tumour suppressors [169]. In support to this hypothesis a general decrease in mature miRNAs was observed in different human malignancies [170,171]. Moreover, miRNA profiles obtained from tumoral tissues reflected the developmental lineage and differentiation state of the tumours, whereas messenger RNA profiles were inaccurate [172]. The current knowledge is that miRNAs engage complex interactions with the machinery that controls the transcriptome and concurrently target multiple mRNAs. Estimates report that miRNAs can potentially regulate up to 60% of the human genome. Thus it is difficult to attribute mis-expression of a miRNA to a particular phenotype [173,167]. Another difficulty is that some miRNAs function as oncogenes in some cell types and as tumor suppressors in others [174]. Thus the definition of miRNAs as oncogenes or as tumour suppressor genes requires an indication of the type of cells in which they act. In order to understand the role of miRNAs in the initiation and in the development of cancer gain-of-function and loss-of-function experiments are adopted in combination with target prediction analyses. These studies, performed through overexpression or knockdown of specific miRNAs in cancer cell lines or mouse models of various malignancies, have supported a role for some of these miRNAs in tumorigenesis. Some of the oncogenic miRNAs that have been described are: Let-7, the first identified conserved miRNA, is an anti-oncomiR that functions as a post-transcriptional gatekeeper of cell proliferation genes, such as RAS [175]; the mir-15a-mir-16-1 cluster that has been implicated in leukaemiagenesis [176]; miR-10a, which is highly expressed in metastatic breast cancer and positively regulates cell migration and invasion [177]; mir-155 that by acting alone in the lymphoid compartment is sufficient to cause cancer [178,179]; miR-206, whose expression in human rhabdomyosarcoma cell lines blocks tumor growth in mouse xenograft models [180]. A more comprehensive list of miRNAs implicated in tumorigenesis is presented in table 3.

The mechanisms by which miRNAs express their oncogenic potential are similar to those of oncogenes and tumor suppressor genes: genomic abnormalities, transcriptional deregulation and dysregulation of the processing steps and/or the factors that control them.

Genomic Abnormalities

Chromosomal abnormalities induce oncogenic actions of miRNAs by altering their expression [181]. As mentioned, approximately 50% of all annotated human miRNA genes are located inside or close to fragile sites, like regions of loss of heterozygosity, regions of amplification and breakpoints associated with cancers [3,182]. Analogously, mice genome shows a strong association between the chromosomal location of miRNAs and those of mouse cancer susceptibility loci that influence the development of solid tumors [183]. Chromosomal translocation of the miR-17-92 cluster increases miR-19 expression and drives Notch1 signalling to promote acute T-cell lymphoblastic leukemia[184], while amplification of miR-26a has been described in glioblastoma [185]. Single nucleotide polimorphisms (SNPs) in miRNA genes may also affect the transcription of the primary transcripts, the processing of pri-miRNAs and pre-miRNAs, the stability of the mature miRNAs and the miRNAs–mRNAs interactions [186] even in the absence of apparent effects on its secondary structure [187]. A germline mutation in pri-miR-16-1 results in low levels of mature miRNA expression and has been found in a kindred with familial chronic lymphocytic leukaemia (CLL) [182] and in New Zealand black mice that naturally develop a CLL-like disease [188]. Another functional miRNA SNP located in the 3' strand of miR-146a leads to altered processing, lower expression of the mature sequence and predisposition to papillary thyroid carcinoma [189].

mRNAs binding sites for miRNAs can be altered by different mechanisms, such as point mutations, translocations or shortening of the 3′-UTR. Point mutations in miRNA targets can both create or destroy miRNA binding sites [190,191,192]. Among the 120,000 SNPs in 3′-UTR mRNAs, about 17% destroy putative conserved or non-conserved miRNA binding sites, and 8.6% create new predicted target sites [62]. Abelson *et al.* provided the demonstration that a mutation in the miR-189 binding site of SLITRK1 was associated with Tourette's syndrome [193]. miRNA modifications with 3′ deletion/addition, 5′ deletion/addition and internal modifications have recently been identified. These altered miRNAs, termed isomiRs, arise from variable cleavage sites for Drosha and Dicer1 in the hairpin. Their role in post-transcriptional regulation remains to be elucidated but it is postulated that these changes could affect miRNA half-life, subcellular localization and target specificity [186].

Transcriptional deregulation

A deregulation of miRNA expression can be a result of increased or decreased transcription from their respective miRNA genes. miRNAs activity can be altered either by aberrant transcription factor activity, by repositioning of other genes close to miRNAs promoters or regulatory regions or by relocalization of a miRNA near other regulatory elements [194]. p53 was found to directly induce miR-34a/b/c miRNAs and to facilitate pri-miRNA to pre-miRNA processing through interaction with p68 and Drosha [195]. The mir-142 gene was found at the breakpoint junction of a translocation, which causes an aggressive B cell leukaemia due to strong up-regulation of *MYC* gene. miR-10b miRNA is specifically highly expressed in breast cancer cells regulated by the transcription factor Twist [196,177].

miRNA expression can also be affected by epigenetic silencing. Silencing of miRNA genes by DNA promoter mutation, hypermethylation and/or histone hypoacetylation has also been described in solid tumours and in haematological malignancies [197,198,199]. Saito and colleagues first showed that miR-127 is downregulated due to promoter hypermethylation in human bladder cancer [200] but epigenetic silencing of several miRNAs is a frequent and early event in breast cancer [201,202].

miRNA	Cancer type	Target or Regulator	Function
Let-7 fam	Breast , colon, gastric, hepatocellular carcinoma, leiomyoma, lung, lymphoma, melanoma, ovarian, prostate	Caspase 3 [203], CDK6, CDC25A [204], HMGA2 [205], KRAS, NRAS [68], MYC [206], SOC-1 [203]	TS
miR-9	Breast, medulloblastoma, ovarian	MYC [207]	TS/OG
miR-10a/b	Breast		OG
miR-15a/16-1 cluster	Lymphoma, multiple myeloma, pituitary adenoma, prostate, pancreatic	BCL-2 [176], MCL1 [176], p53 [60]	TS
miR-17-92 fam	Breast, colon, gastric, lung 122, lymphoma, myeloma, medulloblastoma, prostate	AIB1[208], BIM, CDKN1A [209,210], E2F [209], MYC [211,209], PTEN [210]	OG
miR-21	Breast, cholangiocarcinoma, gastric, glioblastoma, lymphoma, lung, myelom, prostat, colon	PDCD4 [212], GF1alfa [213], IL-6 [214], MASPIN [215], PTEN [216], TPM1 [217],	OG
miR-22/25	Prostate	PTEN [218]	OG
miR-26a	Glioblastoma, liver, lymphoma, thyroid	EZH2 [219], MYC [194]	TS/OG
miR-29a/b/c	Breast, cholangiocarcinoma, epatocarcinoma, lung, lymphoma rhabdomyosarcoma	CDK6 [181]; MCL1 [220]; TCL1, DNMT1 [181] DNMT3α/β [221]; MYC [194]	TS
miR-34a/b/c	Breast , bladder cancer, colon, gastric, hepatocellular carcinoma, kidney, lymphoma; lung, melanoma, neuroblastoma, pancreatic, prastate	BCL2 [222]; CCND1, CDK6 [58]; CD44 [223]; CREB [224]; FOXP1 [225]; MET [226], MYC [222,225];	TS

		p53 [59]; SIRT1 [227]; ZAP70 [228]	
miR-103/107	Pancreatic		OG
miR-126	Breast		TS
miR-128	Glioblastoma		TS
miR-130	Hepatocellular carcinoma		
miR-141/200 fam	Bladder , breast, gastric, lung, ovarian, renal	E-CADHERIN [229], SUZ12 [230]	TS/OG
miR-143/145	Colon, osteosarcoma, pancreatic	MMP [231]; RAS [232]	TS
miR-155	Breast, colon, lung, lymphoma, pancreatic	NF-kB [233], CEBPB and SHIP1 [234]	OG/TS
miR-181a/b/c	Glioblastoma, hepatocellular carcinoma	RASSF1A, TIMP3 [203]	TS
miR-205	Prostate, bladder, breast, oesophageal, ovarian, lung, melanoma	E2F1 [235]	TS/OG
miR-206	Breast, rhabdomyosarcoma	TP53INP1 [236]	TS
miR-221-222	Glioblastoma, liver	P27(KIP1) [237]	OG
miR-302	Prostate	PTEN [218]	OG
miR-340	Breast	MET [238]	TS
miR-335	Breast	SOX4, TNC [239]	TS
miR-371/373	Breast, testicular , thyroid	LATS2 [240]	OG

Table 3. A list of miRNAs implicated in tumourigenesis**.** TS = tumor suppressor, OG = oncogene

Dysregulation of the miRNAs processing steps

Aberrant miRNA expression in cancer may also result from downstream miRNA processing with a decrease in pri- to pre-miRNA conversion, which may affect the composition and total pool of mature miRNAs. The global repression of miRNA maturation promotes cellular transformation and tumorigenesis. Cancer cells expressing short hairpin RNAs targeting three different components of the miRNA processing machinery (Drosha, DGCR8 and Dicer1) showed a substantial decrease in steady-state miRNA levels and a more pronounced transformed phenotype [241]. p53 mutants have been shown to affect the interaction between p68/p72 and Drosha required for efficient maturation of a subset of miRNAs [195] while RNA helicases DDX5 and DDX17 act as a scaffold and recruiting factors to the Drosha complex and promote pri-miRNA processing [242]. Also oncogenic SMADs, signal transducers of the TGF-β pathway, control Drosha-mediated miRNA maturation through interaction with DDX5 [243]. Finally, Dicer1 and transactivation-responsive RNA binding protein (TRBP) mediate pre-miRNA processing. A recent study has found that TRBP is a phosphoprotein whose phosphorylation is dependent on kinases of the MAPK/Erk pathway. Stimulation/inhibition of MAPK-mediated activation of TRBP enhances/reduces pre-miRNA processing and leads to an increase/decrease in cell proliferation [244].

A novel paradigm in tumor biology suggests that cancer growth is driven by stem-like cells within a tumor, called tumor-initiating cells (TICs) or cancer stem cells (CSCs) that are structurally and functionally distinct from the other cells of the tumor mass [245]. Although CSCs are challenging to study because they represent a small fraction of the tumor and they rapidly differentiate when cultured in vitro, these cells can be isolated based on expression of specific cell surface markers [246]. Isolation of CSCs coupled with miRNAs expression analysis shows that some miRNAs are upregulated or downregulated in TICs. Neveu and colleagues showed that a subset of 11 miRNAs can be used to classify cancer, stem cells and differentiated cells [247]. TICs within specific tumors have an altered miRNA expression profile able to modify tumor growth, spread or resistance to chemotherapeutic agents. For example miR-200b expression suppresses tumor growth and prolongs remission in mouse xenografts and its transcriptional signature is observed in metastatic breast tumors [230] while Let-7 and miR-181 family members are up-regulated in hepatocellular cancer stem cells and inhibition of Let-7 and silencing of miR-181 leads to an increase in chemosensitivity and reduction in motility and invasion [203]. In hepatocellular carcinomas TICs overexpression of miR-130b increases resistance to chemotherapeutic agents, enhances tumorigenicity in vivo, and gives greater potential for self renewal [236]. Finally, miR-34a, is underexpressed in prostate cancer stem cells and its forced expression inhibits clonogenic expansion, tumor regeneration, and metastasis while miR-34a antagomirs promote tumor development and metastasis [223].

Several difficulties arise when trying to connect altered miRNA network data to tumor initiation, growth and spread. First, patient samples are usually collected at a time in which a tumour is already well established, therefore analysis of these samples cannot unravel early changes important for tumour development. Second, tissues are composed of sub-populations made by different cell types, each expressing a distinct gene/miRNA expression signature [169]. Cancer alters both the expression programme of the affected tissue but also its cell type composition. In order to understand if an altered expression profile is important for various tumor characteristics, it will be useful to profile individual tumor cell types that may be present in a tumor sample, as well as define miRNAs cellular localization. Third, the currently used target prediction databases [5] do not always produce identical reports and necessitate the selection of a few targets for further study and validation to be prioritized from a list of hundreds. This choice is made by the researcher and it is based on a knowledge of target molecules potentially involved in the malignancy. Finally, although abundant miRNAs that regulate oncogene or tumor suppressor genes have the most significant oncogenic potential [31], miRNAs that have lower expression can, when acting in combination, fine-tune target expression of a given mRNA [248,76]. Therefore, the interplay between miRNAs expressed in a particular cell, the expression levels of their targets, and post-transcriptional gene regulatory mechanisms must all be taken into account to understand the complex regulatory mechanism of miRNA.

Conclusions

miRNAs regulate the expression of more than half of all genes. They are being examined for their regulatory roles in self-renewal, proliferation, and differentiation of normal and cancer cells. Dysregulation of miRNAs and cancer diseases have been put in evidence. The marked enthusiasm for miRNAs as a novel class of functional regulators of tissue maintenance and stress response has possibly further increased since miRNAs have been shown to be critical for both stem cell development and cancer pathogenesis. They may provide new approaches to facilitate the knockdown of expression of genes in different ES cell populations and provide new insights in our understanding of “stemness”. Many researchers think that the power of RNAi-based approaches can be applied for understanding ES cell gene function and may serve to establish foundations in ES cell-based therapies. On the other hand, global inhibition of miRNA processing increased tumorigenicity and transformation, which suggests their important regulatory role in maintaining homeostasis. miRNAs have been shown to act both as tumor suppressors, which help regulate

growth, and oncogenes, which promote rapid and uncontrolled growth. In addition, many cancer-associated regions of the genome contain miRNA genes. Certain cancers have specific miRNA profiles (i.e., upregulation or downregulation of certain miRNAs) as well as specific surface markers that can be used to associate a particular phenotype to miRNAs dysregulation. This knowledge might enable development of novel therapeutic approaches targeted to cancer cells, including cancer stem cells. However more functional studies about the role played by specific miRNAs within different cancer cells, and particularly CSCs, are needed to identify targets and develop effective and safe therapeutic agents.

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References

[1] Lee, R.C.; Feinbaum, R.L.; Ambros, V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell,* **1993**, *75,* 843-854.

[2] Wightman, B.; Ha, I.; Ruvkun, G. Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. *Cell,* **1993**, *75,* 855-862.

[3] Calin, G.A.; Dumitru, C.D.; Shimizu, M.; Bichi, R.; Zupo, S.; Noch, E.; Aldler, H.; Rattan, S.; Keating, M.; Rai, K.; Rassenti, L.; Kipps, T.; Negrini, M.; Bullrich, F.; Croce, C.M. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A,* **2002**, *99,* 15524-15529.

[4] Hammond, S.M. MicroRNAs as oncogenes. *Curr. Opin Genet. Dev,* **2006**, *16,* 4-9.

[5] Bartel, D.P. MicroRNAs: target recognition and regulatory functions. *Cell,* **2009**, *136,* 215-233.

[6] Carthew, R.W. and Sontheimer, E.J. Origins and Mechanisms of miRNAs and siRNAs. *Cell,* **2009**, *136,* 642-655.

[7] Kim, V.N.; Han, J.; Siomi, M.C. Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol,* **2009**, *10,* 126-139.

[8] Chua, J.H.; Armugam, A.; Jeyaseelan, K. MicroRNAs: biogenesis, function and applications. *Curr. Opin Mol Ther,* **2009**, *11,* 189-199.

[9] Lagos-Quintana, M.; Rauhut, R.; Lendeckel, W.; Tuschl, T. Identification of novel genes coding for small expressed RNAs. *Science,* **2001**, *294,* 853-858.

[10] Lau, N.C.; Lim, L.P.; Weinstein, E.G.; Bartel, D.P. An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. *Science,* **2001**, *294,* 858-862.

[11] Baskerville, S. and Bartel, D.P. Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes. *RNA.,* **2005**, *11,* 241-247.

[12] Rodriguez, A.; Griffiths-Jones, S.; Ashurst, J.L.; Bradley, A. Identification of mammalian microRNA host genes and transcription units. *Genome Res,* **2004**, *14,* 1902-1910.

[13] Lee, Y.; Ahn, C.; Han, J.; Choi, H.; Kim, J.; Yim, J.; Lee, J.; Provost, P.; Radmark, O.; Kim, S.; Kim, V.N. The nuclear RNase III Drosha initiates microRNA processing. *Nature,* **2003**, *425,* 415-419.

[14] Newman, M.A. and Hammond, S.M. Emerging paradigms of regulated microRNA processing. *Genes Dev,* **2010**, *24,* 1086-1092.

[15] Han, J.; Lee, Y.; Yeom, K.H.; Kim, Y.K.; Jin, H.; Kim, V.N. The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev,* **2004**, *18,* 3016-3027.

[16] Kim, Y.K. and Kim, V.N. Processing of intronic microRNAs. *EMBO J,* **2007**, *26,* 775-783.

[17] Morlando, M.; Ballarino, M.; Gromak, N.; Pagano, F.; Bozzoni, I.; Proudfoot, N.J. Primary microRNA transcripts are processed co-transcriptionally. *Nat Struct. Mol Biol,* **2008**, *15,* 902-909.

[18] Lund, E.; Guttinger, S.; Calado, A.; Dahlberg, J.E.; Kutay, U. Nuclear export of microRNA precursors. *Science,* **2004**, *303,* 95-98.

[19] Yi, R.; Qin, Y.; Macara, I.G.; Cullen, B.R. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev,* **2003**, *17,* 3011-3016.

[20] Chendrimada, T.P.; Gregory, R.I.; Kumaraswamy, E.; Norman, J.; Cooch, N.; Nishikura, K.; Shiekhattar, R. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature,* **2005**, *436,* 740-744.

[21] Bartel, D.P. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell,* **2004**, *116,* 281-297.

[22] Landthaler, M.; Gaidatzis, D.; Rothballer, A.; Chen, P.Y.; Soll, S.J.; Dinic, L.; Ojo, T.; Hafner, M.; Zavolan, M.; Tuschl, T. Molecular characterization of human Argonaute-containing ribonucleoprotein complexes and their bound target mRNAs. *RNA.,* **2008**, *14,* 2580-2596.

[23] Filipowicz, W.; Jaskiewicz, L.; Kolb, F.A.; Pillai, R.S. Post-transcriptional gene silencing by siRNAs and miRNAs. *Curr. Opin Struct. Biol,* **2005**, *15,* 331-341.

[24] Chatterjee, S. and Grosshans, H. Active turnover modulates mature microRNA activity in Caenorhabditis elegans. *Nature,* **2009**, *461,* 546-549.

[25] Das, S.K.; Sokhi, U.K.; Bhutia, S.K.; Azab, B.; Su, Z.Z.; Sarkar, D.; Fisher, P.B. Human polynucleotide phosphorylase selectively and preferentially degrades microRNA-221 in human melanoma cells. *Proc Natl Acad Sci U S A,* **2010**, *107,* 11948-11953.

[26] Pawlicki, J.M. and Steitz, J.A. Nuclear networking fashions pre-messenger RNA and primary microRNA transcripts for function. *Trends Cell Biol,* **2010**, *20,* 52-61.

[27] Kai, Z.S. and Pasquinelli, A.E. MicroRNA assassins: factors that regulate the disappearance of miRNAs. *Nat Struct. Mol Biol,* **2010**, *17,* 5-10.

[28] Siomi, H. and Siomi, M.C. Posttranscriptional regulation of microRNA biogenesis in animals. *Mol Cell,* **2010**, *38,* 323-332.

[29] Hock, J. and Meister, G. The Argonaute protein family. *Genome Biol,* **2008**, *9,* 210.

[30] MacRae, I.J.; Ma, E.; Zhou, M.; Robinson, C.V.; Doudna, J.A. In vitro reconstitution of the human RISC-loading complex. *Proc Natl Acad Sci U S A,* **2008**, *105,* 512-517.

[31] Baek, D.; Villen, J.; Shin, C.; Camargo, F.D.; Gygi, S.P.; Bartel, D.P. The impact of microRNAs on protein output. *Nature,* **2008**, *455,* 64-71.

[32] Nelson, P.T.; Hatzigeorgiou, A.G.; Mourelatos, Z. miRNP:mRNA association in polyribosomes in a human neuronal cell line. *RNA.,* **2004**, *10,* 387-394.

[33] Hofacker, I.L. How microRNAs choose their targets. *Nat Genet.,* **2007**, *39,* 1191-1192.

[34] Caudy, A.A.; Myers, M.; Hannon, G.J.; Hammond, S.M. Fragile X-related protein and VIG associate with the RNA interference machinery. *Genes Dev,* **2002**, *16,* 2491-2496.

[35] Ishizuka, A.; Siomi, M.C.; Siomi, H. A Drosophila fragile X protein interacts with components of RNAi and ribosomal proteins. *Genes Dev,* **2002**, *16,* 2497-2508.

[36] Meister, G.; Landthaler, M.; Peters, L.; Chen, P.Y.; Urlaub, H.; Luhrmann, R.; Tuschl, T. Identification of novel argonaute-associated proteins. *Curr. Biol,* **2005**, *15,* 2149-2155.

[37] Jin, P.; Zarnescu, D.C.; Ceman, S.; Nakamoto, M.; Mowrey, J.; Jongens, T.A.; Nelson, D.L.; Moses, K.; Warren, S.T. Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway. *Nat Neurosci.,* **2004**, *7,* 113-117.

[38] Cheever, A. and Ceman, S. Phosphorylation of FMRP inhibits association with Dicer. *RNA.,* **2009**, *15,* 362-366.

[39] Plante, I.; Davidovic, L.; Ouellet, D.L.; Gobeil, L.A.; Tremblay, S.; Khandjian, E.W.; Provost, P. Dicer-derived microRNAs are utilized by the fragile X mental retardation protein for assembly on target RNAs. *J Biomed. Biotechnol.,* **2006**, *2006,* 64347.

[40] 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.

[41] Eulalio, A.; Huntzinger, E.; Izaurralde, E. GW182 interaction with Argonaute is essential for miRNA-mediated translational repression and mRNA decay. *Nat Struct. Mol Biol,* **2008**, *15,* 346-353.

[42] Baillat, D. and Shiekhattar, R. Functional dissection of the human TNRC6 (GW182-related) family of proteins. *Mol Cell Biol,* **2009**, *29,* 4144-4155.

[43] Zekri, L.; Huntzinger, E.; Heimstadt, S.; Izaurralde, E. The silencing domain of GW182 interacts with PABPC1 to promote translational repression and degradation of microRNA targets and is required for target release. *Mol Cell Biol,* **2009**, *29,* 6220-6231.

[44] Brodersen, P. and Voinnet, O. Revisiting the principles of microRNA target recognition and mode of action. *Nat Rev Mol Cell Biol,* **2009**, *10,* 141-148.

[45] Yokoi, T. and Nakajima, M. Toxicological implications of modulation of gene expression by microRNAs. *Toxicol. Sci.,* **2011**.

[46] Flynt, A.S. and Lai, E.C. Biological principles of microRNA-mediated regulation: shared themes amid diversity. *Nat Rev Genet.,* **2008**, *9,* 831-842.

[47] Chang, S.; Wen, S.; Chen, D.; Jin, P. Small regulatory RNAs in neurodevelopmental disorders. *Hum. Mol Genet.,* **2009**, *18,* R18-R26.

[48] Chen, J.F.; Callis, T.E.; Wang, D.Z. microRNAs and muscle disorders. *J Cell Sci,* **2009**, *122,* 13-20.

[49] Erson, A.E. and Petty, E.M. MicroRNAs in development and disease. *Clin Genet.,* **2008**, *74,* 296-306.

[50] O'Connell, R.M.; Rao, D.S.; Chaudhuri, A.A.; Baltimore, D. Physiological and pathological roles for microRNAs in the immune system. *Nat Rev Immunol.,* **2010**, *10,* 111-122.

[51] Shenouda, S.K. and Alahari, S.K. MicroRNA function in cancer: oncogene or a tumor suppressor? *Cancer Metastasis Rev,* **2009**, *28,* 369-378.

[52] Bueno, M.J. and Malumbres, M. MicroRNAs and the cell cycle. *Biochim. Biophys. Acta,* **2011**.

[53] Wang, Y. and Blelloch, R. Cell cycle regulation by MicroRNAs in embryonic stem cells. *Cancer Res.,* **2009**, *69,* 4093-4096.

[54] Fornari, F.; Gramantieri, L.; Ferracin, M.; Veronese, A.; Sabbioni, S.; Calin, G.A.; Grazi, G.L.; Giovannini, C.; Croce, C.M.; Bolondi, L.; Negrini, M. MiR-221 controls CDKN1C/p57 and CDKN1B/p27 expression in human hepatocellular carcinoma. *Oncogene,* **2008**, *27,* 5651-5661.

[55] Medina, R.; Zaidi, S.K.; Liu, C.G.; Stein, J.L.; van Wijnen, A.J.; Croce, C.M.; Stein, G.S. MicroRNAs 221 and 222 bypass quiescence and compromise cell survival. *Cancer Res.,* **2008**, *68,* 2773-2780.

[56] Wu, J.; Qian, J.; Li, C.; Kwok, L.; Cheng, F.; Liu, P.; Perdomo, C.; Kotton, D.; Vaziri, C.; Anderlind, C.; Spira, A.; Cardoso, W.V.; Lu, J. miR-129 regulates cell proliferation by downregulating Cdk6 expression. *Cell Cycle,* **2010**, *9,* 1809-1818.

[57] Kozaki, K.; Imoto, I.; Mogi, S.; Omura, K.; Inazawa, J. Exploration of tumor-suppressive microRNAs silenced by DNA hypermethylation in oral cancer. *Cancer Res.,* **2008**, *68,* 2094-2105.

[58] Sun, F.; Fu, H.; Liu, Q.; Tie, Y.; Zhu, J.; Xing, R.; Sun, Z.; Zheng, X. Downregulation of CCND1 and CDK6 by miR-34a induces cell cycle arrest. *FEBS Lett.,* **2008**, *582,* 1564-1568.

[59] Bommer, G.T.; Gerin, I.; Feng, Y.; Kaczorowski, A.J.; Kuick, R.; Love, R.E.; Zhai, Y.; Giordano, T.J.; Qin, Z.S.; Moore, B.B.; MacDougald, O.A.; Cho, K.R.; Fearon, E.R. p53-mediated activation of miRNA34 candidate tumor-suppressor genes. *Curr. Biol.,* **2007**, *17,* 1298-1307.

[60] He, L.; He, X.; Lim, L.P.; de, S.E.; Xuan, Z.; Liang, Y.; Xue, W.; Zender, L.; Magnus, J.; Ridzon, D.; Jackson, A.L.; Linsley, P.S.; Chen, C.; Lowe, S.W.; Cleary, M.A.; Hannon, G.J. A microRNA component of the p53 tumour suppressor network. *Nature,* **2007**, *447,* 1130-1134.

[61] Braun, C.J.; Zhang, X.; Savelyeva, I.; Wolff, S.; Moll, U.M.; Schepeler, T.; Orntoft, T.F.; Andersen, C.L.; Dobbelstein, M. p53-Responsive micrornas 192 and 215 are capable of inducing cell cycle arrest. *Cancer Res.,* **2008**, *68,* 10094-10104.

[62] Georges, S.A.; Biery, M.C.; Kim, S.Y.; Schelter, J.M.; Guo, J.; Chang, A.N.; Jackson, A.L.; Carleton, M.O.; Linsley, P.S.; Cleary, M.A.; Chau, B.N. Coordinated regulation of cell cycle transcripts by p53-Inducible microRNAs, miR-192 and miR-215. *Cancer Res.,* **2008**, *68,* 10105-10112.

[63] Kato, M.; Putta, S.; Wang, M.; Yuan, H.; Lanting, L.; Nair, I.; Gunn, A.; Nakagawa, Y.; Shimano, H.; Todorov, I.; Rossi, J.J.; Natarajan, R. TGF-beta activates Akt kinase through a microRNA-dependent amplifying circuit targeting PTEN. *Nat. Cell Biol.,* **2009**, *11,* 881-889.

[64] de Oliveira, P.E.; Zhang, L.; Wang, Z.; Lazo, J.S. Hypoxia-mediated regulation of Cdc25A phosphatase by p21 and miR-21. *Cell Cycle,* **2009**, *8,* 3157-3164.

[65] Pothof, J.; Verkaik, N.S.; van, I.W.; Wiemer, E.A.; Ta, V.T.; van der Horst, G.T.; Jaspers, N.G.; van, G.; Hoeijmakers, J.H.; Persengiev, S.P. MicroRNA-mediated gene silencing modulates the UV-induced DNA-damage response. *EMBO J.,* **2009**, *28,* 2090-2099.

[66] Kole, A.J.; Swahari, V.; Hammond, S.M.; Deshmukh, M. miR-29b is activated during neuronal maturation and targets BH3-only genes to restrict apoptosis. *Genes Dev.,* **2011**, *25,* 125-130.

[67] Drummond, M.J.; McCarthy, J.J.; Sinha, M.; Spratt, H.M.; Volpi, E.; Esser, K.A.; Rasmussen, B.B. Aging and MicroRNA Expression in Human Skeletal Muscle: A Microarray and Bioinformatics Analysis. *Physiol Genomics,* **2010**.

[68] Johnson, C.D.; Esquela-Kerscher, A.; Stefani, G.; Byrom, M.; Kelnar, K.; Ovcharenko, D.; Wilson, M.; Wang, X.; Shelton, J.; Shingara, J.; Chin, L.; Brown, D.; Slack, F.J. The let-7 microRNA represses cell proliferation pathways in human cells. *Cancer Res,* **2007**, *67,* 7713-7722.

[69] Legesse-Miller, A.; Elemento, O.; Pfau, S.J.; Forman, J.J.; Tavazoie, S.; Coller, H.A. let-7 Overexpression leads to an increased fraction of cells in G2/M, direct down-regulation of Cdc34, and stabilization of Wee1 kinase in primary fibroblasts. *J Biol Chem,* **2009**, *284,* 6605-6609.

[70] Zhao, C.; Sun, G.; Li, S.; Lang, M.F.; Yang, S.; Li, W.; Shi, Y. MicroRNA let-7b regulates neural stem cell proliferation and differentiation by targeting nuclear receptor TLX signaling. *Proc. Natl. Acad. Sci. U. S. A,* **2010**, *107,* 1876-1881.

[71] Linsley, P.S.; Schelter, J.; Burchard, J.; Kibukawa, M.; Martin, M.M.; Bartz, S.R.; Johnson, J.M.; Cummins, J.M.; Raymond, C.K.; Dai, H.; Chau, N.; Cleary, M.; Jackson, A.L.; Carleton, M.; Lim, L. Transcripts targeted by the microRNA-16 family cooperatively regulate cell cycle progression. *Mol Cell Biol,* **2007**, *27,* 2240-2252.

[72] Liu, Q.; Fu, H.; Sun, F.; Zhang, H.; Tie, Y.; Zhu, J.; Xing, R.; Sun, Z.; Zheng, X. miR-16 family induces cell cycle arrest by regulating multiple cell cycle genes. *Nucleic Acids Res,* **2008**, *36,* 5391-5404.

[73] Qi, J.; Yu, J.Y.; Shcherbata, H.R.; Mathieu, J.; Wang, A.J.; Seal, S.; Zhou, W.; Stadler, B.M.; Bourgin, D.; Wang, L.; Nelson, A.; Ware, C.; Raymond, C.; Lim, L.P.; Magnus, J.; Ivanovska, I.; Diaz, R.; Ball, A.; Cleary, M.A.; Ruohola-Baker, H. microRNAs regulate human embryonic stem cell division. *Cell Cycle,* **2009**, *8,* 3729-3741.

[74] Qin, X.; Wang, X.; Wang, Y.; Tang, Z.; Cui, Q.; Xi, J.; Li, Y.S.; Chien, S.; Wang, N. MicroRNA-19a mediates the suppressive effect of laminar flow on cyclin D1 expression in human umbilical vein endothelial cells. *Proc Natl Acad Sci U S A,* **2010**, *107,* 3240-3244.

[75] Trompeter, H.I.; Abbad, H.; Iwaniuk, K.M.; Hafner, M.; Renwick, N.; Tuschl, T.; Schira, J.; Muller, H.W.; Wernet, P. MicroRNAs MiR-17, MiR-20a, and MiR-106b act in concert to modulate E2F activity on cell cycle arrest during neuronal lineage differentiation of USSC. *PLoS. One.,* **2011**, *6,* e16138.

[76] Wu, S.; Huang, S.; Ding, J.; Zhao, Y.; Liang, L.; Liu, T.; Zhan, R.; He, X. Multiple microRNAs modulate p21Cip1/Waf1 expression by directly targeting its 3' untranslated region. *Oncogene,* **2010**, *29,* 2302-2308.

[77] 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.

[78] Lal, A.; Navarro, F.; Maher, C.A.; Maliszewski, L.E.; Yan, N.; O'Day, E.; Chowdhury, D.; Dykxhoorn, D.M.; Tsai, P.; Hofmann, O.; Becker, K.G.; Gorospe, M.; Hide, W.; Lieberman, J. miR-24 Inhibits cell proliferation by targeting E2F2, MYC, and other cell-cycle genes via binding to "seedless" 3'UTR microRNA recognition elements. *Mol Cell,* **2009**, *35,* 610-625.

[79] Kim, Y.K.; Yu, J.; Han, T.S.; Park, S.Y.; Namkoong, B.; Kim, D.H.; Hur, K.; Yoo, M.W.; Lee, H.J.; Yang, H.K.; Kim, V.N. Functional links between clustered microRNAs:

suppression of cell-cycle inhibitors by microRNA clusters in gastric cancer. *Nucleic Acids Res,* **2009**, *37,* 1672-1681.

[80] Antonini, D.; Russo, M.T.; De, R.L.; Gorrese, M.; Del, V.L.; Missero, C. Transcriptional repression of miR-34 family contributes to p63-mediated cell cycle progression in epidermal cells. *J Invest Dermatol.,* **2010**, *130,* 1249-1257.

[81] Pierson, J.; Hostager, B.; Fan, R.; Vibhakar, R. Regulation of cyclin dependent kinase 6 by microRNA 124 in medulloblastoma. *J. Neurooncol.,* **2008**, *90,* 1-7.

[82] Lin, R.J.; Lin, Y.C.; Yu, A.L. miR-149* induces apoptosis by inhibiting Akt1 and E2F1 in human cancer cells. *Mol Carcinog.,* **2010**, *49,* 719-727.

[83] Wurz, K.; Garcia, R.L.; Goff, B.A.; Mitchell, P.S.; Lee, J.H.; Tewari, M.; Swisher, E.M. MiR-221 and MiR-222 alterations in sporadic ovarian carcinoma: Relationship to CDKN1B, CDKNIC and overall survival. *Genes Chromosomes. Cancer,* **2010**, *49,* 577-584.

[84] Lichner, Z.; Pall, E.; Kerekes, A.; Pallinger, E.; Maraghechi, P.; Bosze, Z.; Gocza, E. The miR-290-295 cluster promotes pluripotency maintenance by regulating cell cycle phase distribution in mouse embryonic stem cells. *Differentiation,* **2011**, *81,* 11-24.

[85] Lee, K.H.; Chen, Y.L.; Yeh, S.D.; Hsiao, M.; Lin, J.T.; Goan, Y.G.; Lu, P.J. MicroRNA-330 acts as tumor suppressor and induces apoptosis of prostate cancer cells through E2F1-mediated suppression of Akt phosphorylation. *Oncogene,* **2009**, *28,* 3360-3370.

[86] Guo, X.; Guo, L.; Ji, J.; Zhang, J.; Zhang, J.; Chen, X.; Cai, Q.; Li, J.; Gu, Q.; Liu, B.; Zhu, Z.; Yu, Y. miRNA-331-3p directly targets E2F1 and induces growth arrest in human gastric cancer. *Biochem. Biophys. Res Commun.,* **2010**, *398,* 1-6.

[87] Sarkar, S.; Dey, B.K.; Dutta, A. MiR-322/424 and -503 are induced during muscle differentiation and promote cell cycle quiescence and differentiation by down-regulation of Cdc25A. *Mol Biol Cell,* **2010**, *21,* 2138-2149.

[88] Yang, X.; Feng, M.; Jiang, X.; Wu, Z.; Li, Z.; Aau, M.; Yu, Q. miR-449a and miR-449b are direct transcriptional targets of E2F1 and negatively regulate pRb-E2F1 activity through a feedback loop by targeting CDK6 and CDC25A. *Genes Dev,* **2009**, *23,* 2388-2393.

[89] Lize, M.; Pilarski, S.; Dobbelstein, M. E2F1-inducible microRNA 449a/b suppresses cell proliferation and promotes apoptosis. *Cell Death. Differ.,* **2010**, *17,* 452-458.

[90] Cedar, H. and Bergman, Y. Linking DNA methylation and histone modification: patterns and paradigms. *Nat. Rev. Genet.,* **2009**, *10,* 295-304.

[91] Guil, S. and Esteller, M. DNA methylomes, histone codes and miRNAs: tying it all together. *Int. J. Biochem. Cell Biol.,* **2009**, *41,* 87-95.

[92] Jaenisch, R. and Bird, A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat. Genet.,* **2003**, *33 Suppl,* 245-254.

[93] Kapsimali, M.; Kloosterman, W.P.; de, B.E.; Rosa, F.; Plasterk, R.H.; Wilson, S.W. MicroRNAs show a wide diversity of expression profiles in the developing and mature central nervous system. *Genome Biol.,* **2007**, *8,* R173.

[94] Wulczyn, F.G.; 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.

[95] Dugas, J.C.; Cuellar, T.L.; Scholze, A.; Ason, B.; Ibrahim, A.; Emery, B.; Zamanian, J.L.; Foo, L.C.; McManus, M.T.; Barres, B.A. Dicer1 and miR-219 Are required for normal oligodendrocyte differentiation and myelination. *Neuron,* **2010**, *65,* 597-611.

[96] Lagos-Quintana, M.; Rauhut, R.; Yalcin, A.; Meyer, J.; Lendeckel, W.; Tuschl, T. Identification of tissue-specific microRNAs from mouse. *Curr. Biol.,* **2002**, *12,* 735-739.

[97] Sempere, L.F.; 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,* R13.

[98] Smirnova, L.; Grafe, A.; Seiler, A.; Schumacher, S.; Nitsch, R.; Wulczyn, F.G. Regulation of miRNA expression during neural cell specification. *Eur. J. Neurosci.,* **2005**, *21,* 1469-1477.

[99] Rybak, A.; Fuchs, H.; Smirnova, L.; Brandt, C.; Pohl, E.E.; Nitsch, R.; Wulczyn, F.G. A feedback loop comprising lin-28 and let-7 controls pre-let-7 maturation during neural stem-cell commitment. *Nat. Cell Biol.,* **2008**, *10,* 987-993.

[100] 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,* 365-371.

[101] Makeyev, E.V.; Zhang, J.; Carrasco, M.A.; Maniatis, T. The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. *Mol. Cell,* **2007**, *27,* 435-448.

[102] Yoo, A.S.; Staahl, B.T.; Chen, L.; Crabtree, G.R. MicroRNA-mediated switching of chromatin-remodelling complexes in neural development. *Nature,* **2009**, *460,* 642-646.

[103] Stefani, G. and Slack, F.J. Small non-coding RNAs in animal development. *Nat. Rev. Mol. Cell Biol.,* **2008**, *9,* 219-230.

[104] Chen, J.F.; Mandel, E.M.; Thomson, J.M.; Wu, Q.; Callis, T.E.; Hammond, S.M.; Conlon, F.L.; Wang, D.Z. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat. Genet.,* **2006**, *38,* 228-233.

[105] Ivey, K.N.; Muth, A.; Arnold, J.; King, F.W.; Yeh, R.F.; Fish, J.E.; Hsiao, E.C.; Schwartz, R.J.; Conklin, B.R.; Bernstein, H.S.; Srivastava, D. MicroRNA regulation of cell lineages in mouse and human embryonic stem cells. *Cell Stem Cell,* **2008**, *2,* 219-229.

[106] Liu, N.; Bezprozvannaya, S.; Williams, A.H.; Qi, X.; Richardson, J.A.; Bassel-Duby, R.; Olson, E.N. microRNA-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart. *Genes Dev.,* **2008**, *22,* 3242-3254.

[107] Zhao, Y.; Ransom, J.F.; Li, A.; Vedantham, V.; von, D.M.; Muth, A.N.; Tsuchihashi, T.; McManus, M.T.; Schwartz, R.J.; Srivastava, D. Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. *Cell,* **2007**, *129,* 303-317.

[108] Chen, J.F.; Tao, Y.; Li, J.; Deng, Z.; Yan, Z.; Xiao, X.; Wang, D.Z. microRNA-1 and microRNA-206 regulate skeletal muscle satellite cell proliferation and differentiation by repressing Pax7. *J. Cell Biol.,* **2010**, *190,* 867-879.

[109] Dey, B.K.; Gagan, J.; Dutta, A. miR-206 and -486 induce myoblast differentiation by downregulating Pax7. *Mol. Cell Biol.,* **2011**, *31,* 203-214.

[110] 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,* 3407-3422.

[111] 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,* 641-648.

[112] Beveridge, N.J.; Tooney, P.A.; Carroll, A.P.; Tran, N.; Cairns, M.J. Down-regulation of miR-17 family expression in response to retinoic acid induced neuronal differentiation. *Cell Signal.,* **2009**, *21,* 1837-1845.

[113] Lin, S.T. and Fu, Y.H. miR-23 regulation of lamin B1 is crucial for oligodendrocyte development and myelination. *Dis. Model. Mech.,* **2009**, *2,* 178-188.

[114] Cheng, L.C.; Pastrana, E.; Tavazoie, M.; Doetsch, F. miR-124 regulates adult neurogenesis in the subventricular zone stem cell niche. *Nat Neurosci.,* **2009**, *12,* 399-408.

[115] Farrell, B.C.; Power, E.M.; Mc Dermott, K.W. Developmentally regulated expression of Sox9 and microRNAs 124, 128 and 23 in neuroepithelial stem cells in the developing spinal cord. *Int. J Dev Neurosci.,* **2011**, *29,* 31-36.

[116] Gao, F.B. Context-dependent functions of specific microRNAs in neuronal development. *Neural Dev,* **2010**, *5,* 25.

[117] Visvanathan, J.; Lee, S.; Lee, B.; Lee, J.W.; Lee, S.K. The microRNA miR-124 antagonizes the anti-neural REST/SCP1 pathway during embryonic CNS development. *Genes Dev,* **2007**, *21,* 744-749.

[118] Le, M.T.; Xie, H.; Zhou, B.; Chia, P.H.; Rizk, P.; Um, M.; Udolph, G.; Yang, H.; Lim, B.; Lodish, H.F. MicroRNA-125b promotes neuronal differentiation in human cells by repressing multiple targets. *Mol Cell Biol,* **2009**, *29,* 5290-5305.

[119] Evangelisti, C.; Florian, M.C.; Massimi, I.; Dominici, C.; Giannini, G.; Galardi, S.; Bue, M.C.; Massalini, S.; McDowell, H.P.; Messi, E.; Gulino, A.; Farace, M.G.; Ciafre, S.A. MiR-128 up-regulation inhibits Reelin and DCX expression and reduces neuroblastoma cell motility and invasiveness. *FASEB J,* **2009**, *23,* 4276-4287.

[120] Godlewski, J.; Nowicki, M.O.; Bronisz, A.; Williams, S.; Otsuki, A.; Nuovo, G.; Raychaudhury, A.; Newton, H.B.; Chiocca, E.A.; Lawler, S. Targeting of the Bmi-1 oncogene/stem cell renewal factor by microRNA-128 inhibits glioma proliferation and self-renewal. *Cancer Res,* **2008**, *68,* 9125-9130.

[121] Choi, P.S.; Zakhary, L.; Choi, W.Y.; Caron, S.; varez-Saavedra, E.; Miska, E.A.; McManus, M.; Harfe, B.; Giraldez, A.J.; Horvitz, H.R.; Schier, A.F.; Dulac, C. Members of the miRNA-200 family regulate olfactory neurogenesis. *Neuron,* **2008**, *57,* 41-55.

[122] Zhao, X.; He, X.; Han, X.; Yu, Y.; Ye, F.; Chen, Y.; Hoang, T.; Xu, X.; Mi, Q.S.; Xin, M.; Wang, F.; Appel, B.; Lu, Q.R. MicroRNA-mediated control of oligodendrocyte differentiation. *Neuron,* **2010**, *65,* 612-626.

[123] Ferretti, E.; De, S.E.; Miele, E.; Laneve, P.; Po, A.; Pelloni, M.; Paganelli, A.; Di, M.L.; Caffarelli, E.; Screpanti, I.; Bozzoni, I.; Gulino, A. Concerted microRNA control of Hedgehog signalling in cerebellar neuronal progenitor and tumour cells. *EMBO J,* **2008**, *27,* 2616-2627.

[124] Zhao, Y.; Samal, E.; Srivastava, D. Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. *Nature,* **2005**, *436,* 214-220.

[125] Anderson, C.; Catoe, H.; Werner, R. MIR-206 regulates connexin43 expression during skeletal muscle development. *Nucleic Acids Res,* **2006**, *34,* 5863-5871.

[126] van, R.E.; Sutherland, L.B.; Liu, N.; Williams, A.H.; McAnally, J.; Gerard, R.D.; Richardson, J.A.; Olson, E.N. A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. *Proc Natl Acad Sci U S A,* **2006**, *103,* 18255-18260.

[127] Wong, C.F. and Tellam, R.L. MicroRNA-26a targets the histone methyltransferase Enhancer of Zeste homolog 2 during myogenesis. *J Biol Chem,* **2008**, *283,* 9836-9843.

[128] Crist, C.G.; Montarras, D.; Pallafacchina, G.; Rocancourt, D.; Cumano, A.; Conway, S.J.; Buckingham, M. Muscle stem cell behavior is modified by microRNA-27 regulation of Pax3 expression. *Proc Natl Acad Sci U S A,* **2009**, *106,* 13383-13387.

[129] Zhang, C. MicroRNA-145 in vascular smooth muscle cell biology: a new therapeutic target for vascular disease. *Cell Cycle,* **2009**, *8,* 3469-3473.

[130] Cordes, K.R.; Sheehy, N.T.; White, M.P.; Berry, E.C.; Morton, S.U.; Muth, A.N.; Lee, T.H.; Miano, J.M.; Ivey, K.N.; Srivastava, D. miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. *Nature,* **2009**, *460,* 705-710.

[131] Yamamoto, M. and Kuroiwa, A. Hoxa-11 and Hoxa-13 are involved in repression of MyoD during limb muscle development. *Dev Growth Differ.,* **2003**, *45,* 485-498.

[132] Kim, K.; Lee, Y.S.; Harris, D.; Nakahara, K.; Carthew, R.W. The RNAi pathway initiated by Dicer-2 in Drosophila. *Cold Spring Harb. Symp. Quant. Biol,* **2006**, *71,* 39-44.

[133] Liu, H.; Cao, Y.D.; Ye, W.X.; Sun, Y.Y. Effect of microRNA-206 on cytoskeleton remodelling by downregulating Cdc42 in MDA-MB-231 cells. *Tumori,* **2010**, *96,* 751-755.

[134] Winbanks, C.E.; Wang, B.; Beyer, C.; Koh, P.; White, L.; Kantharidis, P.; Gregorevic, P. TGF-{beta} regulates miR-206 and miR-29 to control myogenic differentiation through regulation of histone deacetylase 4 (HDAC4). *J Biol Chem,* **2011**.

[135] Callis, T.E.; Pandya, K.; Seok, H.Y.; Tang, R.H.; Tatsuguchi, M.; Huang, Z.P.; Chen, J.F.; Deng, Z.; Gunn, B.; Shumate, J.; Willis, M.S.; Selzman, C.H.; Wang, D.Z.

MicroRNA-208a is a regulator of cardiac hypertrophy and conduction in mice. *J Clin Invest,* **2009**, *119,* 2772-2786.

[136] Liu, J.; Luo, X.J.; Xiong, A.W.; Zhang, Z.D.; Yue, S.; Zhu, M.S.; Cheng, S.Y. MicroRNA-214 promotes myogenic differentiation by facilitating exit from mitosis via down-regulation of proto-oncogene N-ras. *J Biol Chem,* **2010**, *285,* 26599-26607.

[137] Cardinali, B.; Castellani, L.; Fasanaro, P.; Basso, A.; Alema, S.; Martelli, F.; Falcone, G. Microrna-221 and microrna-222 modulate differentiation and maturation of skeletal muscle cells. *PLoS. One.,* **2009**, *4,* e7607.

[138] Small, E.M.; O'Rourke, J.R.; Moresi, V.; Sutherland, L.B.; McAnally, J.; Gerard, R.D.; Richardson, J.A.; Olson, E.N. Regulation of PI3-kinase/Akt signaling by muscle-enriched microRNA-486. *Proc Natl Acad Sci U S A,* **2010**, *107,* 4218-4223.

[139] Takahashi, K. and Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell,* **2006**, *126,* 663-676.

[140] Yu, J.; Vodyanik, M.A.; Smuga-Otto, K.; ntosiewicz-Bourget, J.; Frane, J.L.; Tian, S.; Nie, J.; Jonsdottir, G.A.; Ruotti, V.; Stewart, R.; Slukvin, I.I.; Thomson, J.A. Induced pluripotent stem cell lines derived from human somatic cells. *Science,* **2007**, *318,* 1917-1920.

[141] Murchison, E.P.; Partridge, J.F.; Tam, O.H.; Cheloufi, S.; Hannon, G.J. Characterization of Dicer-deficient murine embryonic stem cells. *Proc. Natl. Acad. Sci. U. S. A,* **2005**, *102,* 12135-12140.

[142] Wang, Y.; Medvid, R.; Melton, C.; Jaenisch, R.; Blelloch, R. DGCR8 is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal. *Nat. Genet.,* **2007**, *39,* 380-385.

[143] Blagosklonny, M.V. and Pardee, A.B. The restriction point of the cell cycle. *Cell Cycle,* **2002**, *1,* 103-110.

[144] Faast, R.; White, J.; Cartwright, P.; Crocker, L.; Sarcevic, B.; Dalton, S. Cdk6-cyclin D3 activity in murine ES cells is resistant to inhibition by p16(INK4a). *Oncogene,* **2004**, *23,* 491-502.

[145] Sinkkonen, L.; Hugenschmidt, T.; Berninger, P.; Gaidatzis, D.; Mohn, F.; rtus-Revel, C.G.; Zavolan, M.; Svoboda, P.; Filipowicz, W. MicroRNAs control de novo DNA methylation through regulation of transcriptional repressors in mouse embryonic stem cells. *Nat. Struct. Mol. Biol.,* **2008**, *15,* 259-267.

[146] Melton, C.; Judson, R.L.; Blelloch, R. Opposing microRNA families regulate self-renewal in mouse embryonic stem cells. *Nature,* **2010**, *463,* 621-626.

[147] Hanina, S.A.; Mifsud, W.; Down, T.A.; Hayashi, K.; O'Carroll, D.; Lao, K.; Miska, E.A.; Surani, M.A. Genome-wide identification of targets and function of individual MicroRNAs in mouse embryonic stem cells. *PLoS. Genet.,* **2010**, *6,* e1001163.

[148] Barroso-Deljesus, A.; Lucena-Aguilar, G.; Sanchez, L.; Ligero, G.; Gutierrez-Aranda, I.; Menendez, P. The Nodal inhibitor Lefty is negatively modulated by the microRNA miR-302 in human embryonic stem cells. *FASEB J.,* **2011**.

[149] Rosa, A. and Brivanlou, A.H. A regulatory circuitry comprised of miR-302 and the transcription factors OCT4 and NR2F2 regulates human embryonic stem cell differentiation. *EMBO J.,* **2011**, *30,* 237-248.

[150] Tay, Y.; Zhang, J.; Thomson, A.M.; Lim, B.; Rigoutsos, I. MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. *Nature,* **2008**, *455,* 1124-1128.

[151] Wellner, U.; Schubert, J.; Burk, U.C.; Schmalhofer, O.; Zhu, F.; Sonntag, A.; Waldvogel, B.; Vannier, C.; Darling, D.; zur, H.A.; Brunton, V.G.; Morton, J.; Sansom, O.; Schuler, J.; Stemmler, M.P.; Herzberger, C.; Hopt, U.; Keck, T.; Brabletz, S.; Brabletz, T. The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. *Nat. Cell Biol.,* **2009**, *11,* 1487-1495.

[152] Xu, N.; Papagiannakopoulos, T.; Pan, G.; Thomson, J.A.; Kosik, K.S. MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells. *Cell,* **2009**, *137,* 647-658.

[153] Barroso-Deljesus, A.; Romero-Lopez, C.; Lucena-Aguilar, G.; Melen, G.J.; Sanchez, L.; Ligero, G.; Berzal-Herranz, A.; Menendez, P. Embryonic stem cell-specific miR302-367 cluster: human gene structure and functional characterization of its core promoter. *Mol. Cell Biol.,* **2008**, *28,* 6609-6619.

[154] Card, D.A.; Hebbar, P.B.; Li, L.; Trotter, K.W.; Komatsu, Y.; Mishina, Y.; Archer, T.K. Oct4/Sox2-regulated miR-302 targets cyclin D1 in human embryonic stem cells. *Mol. Cell Biol.,* **2008**, *28,* 6426-6438.

[155] Judson, R.L.; Babiarz, J.E.; Venere, M.; Blelloch, R. Embryonic stem cell-specific microRNAs promote induced pluripotency. *Nat. Biotechnol.,* **2009**, *27,* 459-461.

[156] Li, R.; Liang, J.; Ni, S.; Zhou, T.; Qing, X.; Li, H.; He, W.; Chen, J.; Li, F.; Zhuang, Q.; Qin, B.; Xu, J.; Li, W.; Yang, J.; Gan, Y.; Qin, D.; Feng, S.; Song, H.; Yang, D.; Zhang, B.; Zeng, L.; Lai, L.; Esteban, M.A.; Pei, D. A mesenchymal-to-epithelial transition initiates and is required for the nuclear reprogramming of mouse fibroblasts. *Cell Stem Cell,* **2010**, *7,* 51-63.

[157] Li, Z.; Yang, C.S.; Nakashima, K.; Rana, T.M. Small RNA-mediated regulation of iPS cell generation. *EMBO J.,* **2011**.

[158] Lin, S.L.; Chang, D.C.; Lin, C.H.; Ying, S.Y.; Leu, D.; Wu, D.T. Regulation of somatic cell reprogramming through inducible mir-302 expression. *Nucleic Acids Res.,* **2011**, *39,* 1054-1065.

[159] nokye-Danso, F.; Trivedi, C.M.; Juhr, D.; Gupta, M.; Cui, Z.; Tian, Y.; Zhang, Y.; Yang, W.; Gruber, P.J.; Epstein, J.A.; Morrisey, E.E. Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. *Cell Stem Cell,* **2011**, *8,* 376-388.

[160] Lin, S.L.; Chang, D.C.; Chang-Lin, S.; Lin, C.H.; Wu, D.T.; Chen, D.T.; Ying, S.Y. Mir-302 reprograms human skin cancer cells into a pluripotent ES-cell-like state. *RNA.,* **2008**, *14,* 2115-2124.

[161] Subramanyam, D.; Lamouille, S.; Judson, R.L.; Liu, J.Y.; Bucay, N.; Derynck, R.; Blelloch, R. Multiple targets of miR-302 and miR-372 promote reprogramming of human fibroblasts to induced pluripotent stem cells. *Nat. Biotechnol.,* **2011**, *29,* 443-448.

[162] Miyoshi, N.; Ishii, H.; Nagano, H.; Haraguchi, N.; Dewi, D.L.; Kano, Y.; Nishikawa, S.; Tanemura, M.; Mimori, K.; Tanaka, F.; Saito, T.; Nishimura, J.; Takemasa, I.; Mizushima, T.; Ikeda, M.; Yamamoto, H.; Sekimoto, M.; Doki, Y.; Mori, M. Reprogramming of mouse and human cells to pluripotency using mature microRNAs. *Cell Stem Cell,* **2011**, *8,* 633-638.

[163] Subramanyam, D. and Blelloch, R. From microRNAs to targets: pathway discovery in cell fate transitions. *Curr. Opin. Genet. Dev.,* **2011**, *21,* 498-503.

[164] Delaloy, C.; Liu, L.; Lee, J.A.; Su, H.; Shen, F.; Yang, G.Y.; Young, W.L.; Ivey, K.N.; Gao, F.B. MicroRNA-9 coordinates proliferation and migration of human embryonic stem cell-derived neural progenitors. *Cell Stem Cell,* **2010**, *6,* 323-335.

[165] Krichevsky, A.M.; Sonntag, K.C.; Isacson, O.; Kosik, K.S. Specific microRNAs modulate embryonic stem cell-derived neurogenesis. *Stem Cells,* **2006**, *24,* 857-864.

[166] Deo, M.; Yu, J.Y.; Chung, K.H.; Tippens, M.; Turner, D.L. Detection of mammalian microRNA expression by in situ hybridization with RNA oligonucleotides. *Dev. Dyn.,* **2006**, *235,* 2538-2548.

[167] Lim, L.P.; Lau, N.C.; Garrett-Engele, P.; Grimson, A.; Schelter, J.M.; Castle, J.; Bartel, D.P.; Linsley, P.S.; Johnson, J.M. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature,* **2005**, *433,* 769-773.

[168] Ooi, A.G.; Sahoo, D.; Adorno, M.; Wang, Y.; Weissman, I.L.; Park, C.Y. MicroRNA-125b expands hematopoietic stem cells and enriches for the lymphoid-balanced and lymphoid-biased subsets. *Proc. Natl. Acad. Sci. U. S. A,* **2010**.

[169] Farazi, T.A.; Spitzer, J.I.; Morozov, P.; Tuschl, T. miRNAs in human cancer. *J Pathol.,* **2011**, *223,* 102-115.

[170] Marton, S.; Garcia, M.R.; Robello, C.; Persson, H.; Trajtenberg, F.; Pritsch, O.; Rovira, C.; Naya, H.; Dighiero, G.; Cayota, A. Small RNAs analysis in CLL reveals a deregulation of miRNA expression and novel miRNA candidates of putative relevance in CLL pathogenesis. *Leukemia,* **2008**, *22,* 330-338.

[171] Ozen, M.; Creighton, C.J.; Ozdemir, M.; Ittmann, M. Widespread deregulation of microRNA expression in human prostate cancer. *Oncogene,* **2008**, *27,* 1788-1793.

[172] Lu, J.; Getz, G.; Miska, E.A.; varez-Saavedra, E.; Lamb, J.; Peck, D.; Sweet-Cordero, A.; Ebert, B.L.; Mak, R.H.; Ferrando, A.A.; Downing, J.R.; Jacks, T.; Horvitz, H.R.; Golub, T.R. MicroRNA expression profiles classify human cancers. *Nature,* **2005**, *435,* 834-838.

[173] Friedman, R.C.; Farh, K.K.; Burge, C.B.; Bartel, D.P. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res,* **2009**, *19,* 92-105.

[174] Fabbri, M.; Ivan, M.; Cimmino, A.; Negrini, M.; Calin, G.A. Regulatory mechanisms of microRNAs involvement in cancer. *Expert Opin Biol Ther,* **2007**, *7,* 1009-1019.

[175] Johnson, S.M.; Grosshans, H.; Shingara, J.; Byrom, M.; Jarvis, R.; Cheng, A.; Labourier, E.; Reinert, K.L.; Brown, D.; Slack, F.J. RAS is regulated by the let-7 microRNA family. *Cell,* **2005**, *120,* 635-647.

[176] Cimmino, A.; Calin, G.A.; Fabbri, M.; Iorio, M.V.; Ferracin, M.; Shimizu, M.; Wojcik, S.E.; Aqeilan, R.I.; Zupo, S.; Dono, M.; Rassenti, L.; Alder, H.; Volinia, S.; Liu, C.G.; Kipps, T.J.; Negrini, M.; Croce, C.M. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci U S A,* **2005**, *102,* 13944-13949.

[177] Ma, L.; Teruya-Feldstein, J.; Weinberg, R.A. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature,* **2007**, *449,* 682-688.

[178] Costinean, S.; Zanesi, N.; Pekarsky, Y.; Tili, E.; Volinia, S.; Heerema, N.; Croce, C.M. Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(mu)-miR155 transgenic mice. *Proc Natl Acad Sci U S A,* **2006**, *103,* 7024-7029.

[179] O'Connell, R.M.; Rao, D.S.; Chaudhuri, A.A.; Boldin, M.P.; Taganov, K.D.; Nicoll, J.; Paquette, R.L.; Baltimore, D. Sustained expression of microRNA-155 in hematopoietic stem cells causes a myeloproliferative disorder. *J Exp Med.,* **2008**, *205,* 585-594.

[180] Taulli, R.; Bersani, F.; Foglizzo, V.; Linari, A.; Vigna, E.; Ladanyi, M.; Tuschl, T.; Ponzetto, C. The muscle-specific microRNA miR-206 blocks human rhabdomyosarcoma growth in xenotransplanted mice by promoting myogenic differentiation. *J Clin Invest,* **2009**, *119,* 2366-2378.

[181] Garzon, R.; Marcucci, G.; Croce, C.M. Targeting microRNAs in cancer: rationale, strategies and challenges. *Nat Rev Drug Discov,* **2010**, *9,* 775-789.

[182] Calin, G.A.; Ferracin, M.; Cimmino, A.; Di, L.G.; Shimizu, M.; Wojcik, S.E.; Iorio, M.V.; Visone, R.; Sever, N.I.; Fabbri, M.; Iuliano, R.; Palumbo, T.; Pichiorri, F.; Roldo, C.; Garzon, R.; Sevignani, C.; Rassenti, L.; Alder, H.; Volinia, S.; Liu, C.G.; Kipps, T.J.; Negrini, M.; Croce, C.M. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N. Engl. J Med.,* **2005**, *353,* 1793-1801.

[183] Sevignani, C.; Calin, G.A.; Nnadi, S.C.; Shimizu, M.; Davuluri, R.V.; Hyslop, T.; Demant, P.; Croce, C.M.; Siracusa, L.D. MicroRNA genes are frequently located near mouse cancer susceptibility loci. *Proc Natl Acad Sci U S A,* **2007**, *104,* 8017-8022.

[184] Mavrakis, K.J.; Wolfe, A.L.; Oricchio, E.; Palomero, T.; de, K.K.; McJunkin, K.; Zuber, J.; James, T.; Khan, A.A.; Leslie, C.S.; Parker, J.S.; Paddison, P.J.; Tam, W.; Ferrando, A.; Wendel, H.G. Genome-wide RNA-mediated interference screen identifies miR-19 targets in Notch-induced T-cell acute lymphoblastic leukaemia. *Nat Cell Biol,* **2010**, *12,* 372-379.

[185] Huse, J.T.; Brennan, C.; Hambardzumyan, D.; Wee, B.; Pena, J.; Rouhanifard, S.H.; Sohn-Lee, C.; le, S.C.; Agami, R.; Tuschl, T.; Holland, E.C. The PTEN-regulating microRNA miR-26a is amplified in high-grade glioma and facilitates gliomagenesis in vivo. *Genes Dev,* **2009**, *23,* 1327-1337.

[186] Ryan, B.M.; Robles, A.I.; Harris, C.C. Genetic variation in microRNA networks: the implications for cancer research. *Nat Rev Cancer,* **2010**, *10,* 389-402.

[187] Wu, M.; Jolicoeur, N.; Li, Z.; Zhang, L.; Fortin, Y.; L'Abbe, D.; Yu, Z.; Shen, S.H. Genetic variations of microRNAs in human cancer and their effects on the expression of miRNAs. *Carcinogenesis,* **2008**, *29,* 1710-1716.

[188] Raveche, E.S.; Salerno, E.; Scaglione, B.J.; Manohar, V.; Abbasi, F.; Lin, Y.C.; Fredrickson, T.; Landgraf, P.; Ramachandra, S.; Huppi, K.; Toro, J.R.; Zenger, V.E.; Metcalf, R.A.; Marti, G.E. Abnormal microRNA-16 locus with synteny to human 13q14 linked to CLL in NZB mice. *Blood,* **2007**, *109,* 5079-5086.

[189] Jazdzewski, K.; Murray, E.L.; Franssila, K.; Jarzab, B.; Schoenberg, D.R.; de la, C.A. Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. *Proc Natl Acad Sci U S A,* **2008**, *105,* 7269-7274.

[190] Chin, L.J.; Ratner, E.; Leng, S.; Zhai, R.; Nallur, S.; Babar, I.; Muller, R.U.; Straka, E.; Su, L.; Burki, E.A.; Crowell, R.E.; Patel, R.; Kulkarni, T.; Homer, R.; Zelterman, D.; Kidd, K.K.; Zhu, Y.; Christiani, D.C.; Belinsky, S.A.; Slack, F.J.; Weidhaas, J.B. A SNP in a let-7 microRNA complementary site in the KRAS 3' untranslated region increases non-small cell lung cancer risk. *Cancer Res,* **2008**, *68,* 8535-8540.

[191] Jiang, S.; Zhang, H.W.; Lu, M.H.; He, X.H.; Li, Y.; Gu, H.; Liu, M.F.; Wang, E.D. MicroRNA-155 functions as an OncomiR in breast cancer by targeting the suppressor of cytokine signaling 1 gene. *Cancer Res,* **2010**, *70,* 3119-3127.

[192] Takamizawa, J.; Konishi, H.; Yanagisawa, K.; Tomida, S.; Osada, H.; Endoh, H.; Harano, T.; Yatabe, Y.; Nagino, M.; Nimura, Y.; Mitsudomi, T.; Takahashi, T. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. *Cancer Res,* **2004**, *64,* 3753-3756.

[193] Abelson, J.F.; Kwan, K.Y.; O'Roak, B.J.; Baek, D.Y.; Stillman, A.A.; Morgan, T.M.; Mathews, C.A.; Pauls, D.L.; Rasin, M.R.; Gunel, M.; Davis, N.R.; Ercan-Sencicek, A.G.; Guez, D.H.; Spertus, J.A.; Leckman, J.F.; Dure, L.S.; Kurlan, R.; Singer, H.S.; Gilbert, D.L.; Farhi, A.; Louvi, A.; Lifton, R.P.; Sestan, N.; State MW Sequence variants in SLITRK1 are associated with Tourette's syndrome. *Science,* **2005**, *310,* 317-320.

[194] Chang, T.C.; Yu, D.; Lee, Y.S.; Wentzel, E.A.; Arking, D.E.; West, K.M.; Dang, C.V.; Thomas-Tikhonenko, A.; Mendell, J.T. Widespread microRNA repression by Myc contributes to tumorigenesis. *Nat Genet.,* **2008**, *40,* 43-50.

[195] Suzuki, H.I.; Yamagata, K.; Sugimoto, K.; Iwamoto, T.; Kato, S.; Miyazono, K. Modulation of microRNA processing by p53. *Nature,* **2009**, *460,* 529-533.

[196] Iorio, M.V.; Ferracin, M.; Liu, C.G.; Veronese, A.; Spizzo, R.; Sabbioni, S.; Magri, E.; Pedriali, M.; Fabbri, M.; Campiglio, M.; Menard, S.; Palazzo, J.P.; Rosenberg, A.; Musiani, P.; Volinia, S.; Nenci, I.; Calin, G.A.; Querzoli, P.; Negrini, M.; Croce, C.M. MicroRNA gene expression deregulation in human breast cancer. *Cancer Res,* **2005**, *65,* 7065-7070.

[197] Shen, J.; Ambrosone, C.B.; DiCioccio, R.A.; Odunsi, K.; Lele, S.B.; Zhao, H. A functional polymorphism in the miR-146a gene and age of familial breast/ovarian cancer diagnosis. *Carcinogenesis,* **2008**, *29,* 1963-1966.

[198] Taganov, K.D.; Boldin, M.P.; Chang, K.J.; Baltimore, D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci U S A,* **2006**, *103,* 12481-12486.

[199] Xu, B.; Feng, N.H.; Li, P.C.; Tao, J.; Wu, D.; Zhang, Z.D.; Tong, N.; Wang, J.F.; Song, N.H.; Zhang, W.; Hua, L.X.; Wu, H.F. A functional polymorphism in Pre-miR-146a gene is associated with prostate cancer risk and mature miR-146a expression in vivo. *Prostate,* **2010**, *70,* 467-472.

[200] Saito, Y.; Liang, G.; Egger, G.; Friedman, J.M.; Chuang, J.C.; Coetzee, G.A.; Jones, P.A. Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. *Cancer Cell,* **2006**, *9,* 435-443.

[201] Chuang, J.C. and Jones, P.A. Epigenetics and microRNAs. *Pediatr. Res,* **2007**, *61,* 24R-29R.

[202] Lehmann, U.; Hasemeier, B.; Romermann, D.; Muller, M.; Langer, F.; Kreipe, H. [Epigenetic inactivation of microRNA genes in mammary carcinoma]. *Verh. Dtsch. Ges. Pathol.,* **2007**, *91,* 214-220.

[203] Meng, F.; Glaser, S.S.; Francis, H.; Demorrow, S.; Han, Y.; Passarini, J.D.; Stokes, A.; Cleary, J.P.; Liu, X.; Venter, J.; Kumar, P.; Priester, S.; Hubble, L.; Stoloch, D.; Sharma, J.; Liu, C.G.; Alpini, G. Functional Analysis of microRNAs in Human Hepatocellular Cancer Stem Cells. *J Cell Mol Med.,* **2011**.

[204] Trang, P.; Medina, P.P.; Wiggins, J.F.; Ruffino, L.; Kelnar, K.; Omotola, M.; Homer, R.; Brown, D.; Bader, A.G.; Weidhaas, J.B.; Slack, F.J. Regression of murine lung tumors by the let-7 microRNA. *Oncogene,* **2010**, *29,* 1580-1587.

[205] Lee, Y.S. and Dutta, A. The tumor suppressor microRNA let-7 represses the HMGA2 oncogene. *Genes Dev,* **2007**, *21,* 1025-1030.

[206] Sampson, V.B.; Rong, N.H.; Han, J.; Yang, Q.; Aris, V.; Soteropoulos, P.; Petrelli, N.J.; Dunn, S.P.; Krueger, L.J. MicroRNA let-7a down-regulates MYC and reverts MYC-induced growth in Burkitt lymphoma cells. *Cancer Res,* **2007**, *67,* 9762-9770.

[207] Ma, L.; Young, J.; Prabhala, H.; Pan, E.; Mestdagh, P.; Muth, D.; Teruya-Feldstein, J.; Reinhardt, F.; Onder, T.T.; Valastyan, S.; Westermann, F.; Speleman, F.; Vandesompele, J.; Weinberg, R.A. miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. *Nat Cell Biol,* **2010**, *12,* 247-256.

[208] Hossain, A.; Kuo, M.T.; Saunders, G.F. Mir-17-5p regulates breast cancer cell proliferation by inhibiting translation of AIB1 mRNA. *Mol Cell Biol,* **2006**, *26,* 8191-8201.

[209] Petrocca, F.; Visone, R.; Onelli, M.R.; Shah, M.H.; Nicoloso, M.S.; de, M., I; Iliopoulos, D.; Pilozzi, E.; Liu, C.G.; Negrini, M.; Cavazzini, L.; Volinia, S.; Alder, H.; Ruco, L.P.; Baldassarre, G.; Croce, C.M.; Vecchione, A. E2F1-regulated microRNAs impair TGFbeta-dependent cell-cycle arrest and apoptosis in gastric cancer. *Cancer Cell,* **2008**, *13,* 272-286.

[210] Xiao, C.; Srinivasan, L.; Calado, D.P.; Patterson, H.C.; Zhang, B.; Wang, J.; Henderson, J.M.; Kutok, J.L.; Rajewsky, K. Lymphoproliferative disease and autoimmunity

in mice with increased miR-17-92 expression in lymphocytes. *Nat Immunol.,* **2008**, *9,* 405-414.

[211] O'Donnell, K.A.; Wentzel, E.A.; Zeller, K.I.; Dang, C.V.; Mendell, J.T. c-Myc-regulated microRNAs modulate E2F1 expression. *Nature,* **2005**, *435,* 839-843.

[212] Frankel, L.B.; Christoffersen, N.R.; Jacobsen, A.; Lindow, M.; Krogh, A.; Lund, A.H. Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. *J Biol Chem,* **2008**, *283,* 1026-1033.

[213] Velu, C.S.; Baktula, A.M.; Grimes, H.L. Gfi1 regulates miR-21 and miR-196b to control myelopoiesis. *Blood,* **2009**, *113,* 4720-4728.

[214] Loffler, D.; Brocke-Heidrich, K.; Pfeifer, G.; Stocsits, C.; Hackermuller, J.; Kretzschmar, A.K.; Burger, R.; Gramatzki, M.; Blumert, C.; Bauer, K.; Cvijic, H.; Ullmann, A.K.; Stadler, P.F.; Horn, F. Interleukin-6 dependent survival of multiple myeloma cells involves the Stat3-mediated induction of microRNA-21 through a highly conserved enhancer. *Blood,* **2007**, *110,* 1330-1333.

[215] Zhu, S.; Wu, H.; Wu, F.; Nie, D.; Sheng, S.; Mo, Y.Y. MicroRNA-21 targets tumor suppressor genes in invasion and metastasis. *Cell Res,* **2008**, *18,* 350-359.

[216] Meng, F.; Henson, R.; Wehbe-Janek, H.; Ghoshal, K.; Jacob, S.T.; Patel, T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology,* **2007**, *133,* 647-658.

[217] Zhu, S.; Si, M.L.; Wu, H.; Mo, Y.Y. MicroRNA-21 targets the tumor suppressor gene tropomyosin 1 (TPM1). *J Biol Chem,* **2007**, *282,* 14328-14336.

[218] Poliseno, L.; Salmena, L.; Riccardi, L.; Fornari, A.; Song, M.S.; Hobbs, R.M.; Sportoletti, P.; Varmeh, S.; Egia, A.; Fedele, G.; Rameh, L.; Loda, M.; Pandolfi, P.P. Identification of the miR-106b~25 microRNA cluster as a proto-oncogenic PTEN-targeting intron that cooperates with its host gene MCM7 in transformation. *Sci Signal.,* **2010**, *3,* ra29.

[219] Sander, S.; Bullinger, L.; Klapproth, K.; Fiedler, K.; Kestler, H.A.; Barth, T.F.; Moller, P.; Stilgenbauer, S.; Pollack, J.R.; Wirth, T. MYC stimulates EZH2 expression by repression of its negative regulator miR-26a. *Blood,* **2008**, *112,* 4202-4212.

[220] Mott, J.L.; Kobayashi, S.; Bronk, S.F.; Gores, G.J. mir-29 regulates Mcl-1 protein expression and apoptosis. *Oncogene,* **2007**, *26,* 6133-6140.

[221] Fabbri, M.; Garzon, R.; Cimmino, A.; Liu, Z.; Zanesi, N.; Callegari, E.; Liu, S.; Alder, H.; Costinean, S.; Fernandez-Cymering, C.; Volinia, S.; Guler, G.; Morrison, C.D.; Chan, K.K.; Marcucci, G.; Calin, G.A.; Huebner, K.; Croce, C.M. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proc Natl Acad Sci U S A,* **2007**, *104,* 15805-15810.

[222] Cole, K.A.; Attiyeh, E.F.; Mosse, Y.P.; Laquaglia, M.J.; Diskin, S.J.; Brodeur, G.M.; Maris, J.M. A functional screen identifies miR-34a as a candidate neuroblastoma tumor suppressor gene. *Mol Cancer Res,* **2008**, *6,* 735-742.

[223] Liu, C.; Kelnar, K.; Liu, B.; Chen, X.; Calhoun-Davis, T.; Li, H.; Patrawala, L.; Yan, H.; Jeter, C.; Honorio, S.; Wiggins, J.F.; Bader, A.G.; Fagin, R.; Brown, D.; Tang, D.G. The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. *Nat Med.,* **2011**, *17,* 211-215.

[224] Pigazzi, M.; Manara, E.; Baron, E.; Basso, G. miR-34b targets cyclic AMP-responsive element binding protein in acute myeloid leukemia. *Cancer Res,* **2009**, *69,* 2471-2478.

[225] Craig, V.J.; Cogliatti, S.B.; Imig, J.; Renner, C.; Neuenschwander, S.; Rehrauer, H.; Schlapbach, R.; Dirnhofer, S.; Tzankov, A.; Muller, A. Myc-mediated repression of microRNA-34a promotes high grade transformation of B-cell lymphoma by dysregulation of FoxP1. *Blood,* **2011**.

[226] Li, N.; Fu, H.; Tie, Y.; Hu, Z.; Kong, W.; Wu, Y.; Zheng, X. miR-34a inhibits migration and invasion by down-regulation of c-Met expression in human hepatocellular carcinoma cells. *Cancer Lett.,* **2009**, *275,* 44-53.

[227] Yamakuchi, M.; Ferlito, M.; Lowenstein, C.J. miR-34a repression of SIRT1 regulates apoptosis. *Proc Natl Acad Sci U S A,* **2008**, *105,* 13421-13426.

[228] Fabbri, M.; Bottoni, A.; Shimizu, M.; Spizzo, R.; Nicoloso, M.S.; Rossi, S.; Barbarotto, E.; Cimmino, A.; Adair, B.; Wojcik, S.E.; Valeri, N.; Calore, F.; Sampath, D.; Fanini, F.; Vannini, I.; Musuraca, G.; Dell'Aquila, M.; Alder, H.; Davuluri, R.V.; Rassenti, L.Z.; Negrini, M.; Nakamura, T.; Amadori, D.; Kay, N.E.; Rai, K.R.; Keating, M.J.; Kipps, T.J.; Calin, G.A.; Croce, C.M. Association of a microRNA/TP53 feedback circuitry with pathogenesis and outcome of B-cell chronic lymphocytic leukemia. *JAMA,* **2011**, *305,* 59-67.

[229] Park, S.M.; Gaur, A.B.; Lengyel, E.; Peter, M.E. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. *Genes Dev,* **2008**, *22,* 894-907.

[230] Iliopoulos, D.; Lindahl-Allen, M.; Polytarchou, C.; Hirsch, H.A.; Tsichlis, P.N.; Struhl, K. Loss of miR-200 inhibition of Suz12 leads to polycomb-mediated repression required for the formation and maintenance of cancer stem cells. *Mol Cell,* **2010**, *39,* 761-772.

[231] Si, M.L.; Zhu, S.; Wu, H.; Lu, Z.; Wu, F.; Mo, Y.Y. miR-21-mediated tumor growth. *Oncogene,* **2007**, *26,* 2799-2803.

[232] Kent, O.A.; Chivukula, R.R.; Mullendore, M.; Wentzel, E.A.; Feldmann, G.; Lee, K.H.; Liu, S.; Leach, S.D.; Maitra, A.; Mendell, J.T. Repression of the miR-143/145 cluster by oncogenic Ras initiates a tumor-promoting feed-forward pathway. *Genes Dev,* **2010**, *24,* 2754-2759.

[233] Thompson, R.C.; Herscovitch, M.; Zhao, I.; Ford, T.J.; Gilmore, T.D. NF-kappaB down-regulates expression of the B-lymphoma marker CD10 through a miR-155/PU.1 pathway. *J Biol Chem,* **2011**, *286,* 1675-1682.

[234] Costinean, S.; Sandhu, S.K.; Pedersen, I.M.; Tili, E.; Trotta, R.; Perrotti, D.; Ciarlariello, D.; Neviani, P.; Harb, J.; Kauffman, L.R.; Shidham, A.; Croce, C.M. Src homology 2 domain-containing inositol-5-phosphatase and CCAAT enhancer-binding

protein beta are targeted by miR-155 in B cells of Emicro-MiR-155 transgenic mice. *Blood,* **2009**, *114,* 1374-1382.

[235] Dar, A.A.; Majid, S.; de, S.D.; Nosrati, M.; Bezrookove, V.; Kashani-Sabet, M. miR-205 suppresses melanoma cell proliferation and induces senescence via regulation of E2F1. *J Biol Chem,* **2011**.

[236] Ma, S.; Tang, K.H.; Chan, Y.P.; Lee, T.K.; Kwan, P.S.; Castilho, A.; Ng, I.; Man, K.; Wong, N.; To, K.F.; Zheng, B.J.; Lai, P.B.; Lo, C.M.; Chan, K.W.; Guan, X.Y. miR-130b Promotes CD133(+) liver tumor-initiating cell growth and self-renewal via tumor protein 53-induced nuclear protein 1. *Cell Stem Cell,* **2010**, *7,* 694-707.

[237] le, S.C.; Nagel, R.; Egan, D.A.; Schrier, M.; Mesman, E.; Mangiola, A.; Anile, C.; Maira, G.; Mercatelli, N.; Ciafre, S.A.; Farace, M.G.; Agami, R. Regulation of the p27(Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. *EMBO J,* **2007**, *26,* 3699-3708.

[238] Wu, Z.S.; Wu, Q.; Wang, C.Q.; Wang, X.N.; Huang, J.; Zhao, J.J.; Mao, S.S.; Zhang, G.H.; Xu, X.C.; Zhang, N. miR-340 inhibition of breast cancer cell migration and invasion through targeting of oncoprotein c-Met. *Cancer,* **2011**.

[239] Tavazoie, S.F.; Alarcon, C.; Oskarsson, T.; Padua, D.; Wang, Q.; Bos, P.D.; Gerald, W.L.; Massague, J. Endogenous human microRNAs that suppress breast cancer metastasis. *Nature,* **2008**, *451,* 147-152.

[240] Voorhoeve, P.M.; le, S.C.; Schrier, M.; Gillis, A.J.; Stoop, H.; Nagel, R.; Liu, Y.P.; van, D.J.; Drost, J.; Griekspoor, A.; Zlotorynski, E.; Yabuta, N.; De, V.G.; Nojima, H.; Looijenga, L.H.; Agami, R. A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. *Adv. Exp Med. Biol,* **2007**, *604,* 17-46.

[241] Kumar, M.S.; Lu, J.; Mercer, K.L.; Golub, T.R.; Jacks, T. Impaired microRNA processing enhances cellular transformation and tumorigenesis. *Nat Genet.,* **2007**, *39,* 673-677.

[242] Fukuda, T.; Yamagata, K.; Fujiyama, S.; Matsumoto, T.; Koshida, I.; Yoshimura, K.; Mihara, M.; Naitou, M.; Endoh, H.; Nakamura, T.; Akimoto, C.; Yamamoto, Y.; Katagiri, T.; Foulds, C.; Takezawa, S.; Kitagawa, H.; Takeyama, K.; O'Malley, B.W.; Kato, S. DEAD-box RNA helicase subunits of the Drosha complex are required for processing of rRNA and a subset of microRNAs. *Nat Cell Biol,* **2007**, *9,* 604-611.

[243] Davis, B.N.; Hilyard, A.C.; Lagna, G.; Hata, A. SMAD proteins control DROSHA-mediated microRNA maturation. *Nature,* **2008**, *454,* 56-61.

[244] Paroo, Z.; Ye, X.; Chen, S.; Liu, Q. Phosphorylation of the human microRNA-generating complex mediates MAPK/Erk signaling. *Cell,* **2009**, *139,* 112-122.

[245] Hatfield, S. and Ruohola-Baker, H. microRNA and stem cell function. *Cell Tissue Res,* **2008**, *331,* 57-66.

[246] Gupta, P.B.; Onder, T.T.; Jiang, G.; Tao, K.; Kuperwasser, C.; Weinberg, R.A.; Lander, E.S. Identification of selective inhibitors of cancer stem cells by high-throughput screening. *Cell,* **2009**, *138,* 645-659.

[247] Neveu, P.; Kye, M.J.; Qi, S.; Buchholz, D.E.; Clegg, D.O.; Sahin, M.; Park, I.H.; Kim, K.S.; Daley, G.Q.; Kornblum, H.I.; Shraiman, B.I.; Kosik, K.S. MicroRNA profiling reveals two distinct p53-related human pluripotent stem cell states. *Cell Stem Cell,* **2010**, *7,* 671-681.

[248] Krek, A.; Grun, D.; Poy, M.N.; Wolf, R.; Rosenberg, L.; Epstein, E.J.; MacMenamin, P.; da, P., I; Gunsalus, K.C.; Stoffel, M.; Rajewsky, N. Combinatorial microRNA target predictions. *Nat Genet.,* **2005**, *37,* 495-500.

Legend

Fig.1 miRNA biogenesis . The introns from full-length miRNA transcripts are excised to form pri-miRNA, which then undergoes Drosha processing. The DGCR8 acts as a molecular marker for Drosha cleavage by binding to the pri-miRNA at the single-stranded regions . After Drosha cleavage, the pre-miRNA is exported from the nucleus into the cytoplasm by Exportin-5 or RAN GTP and it is the processed by Dicer into the mature miRNA. After RISC incorporation, miRNAs either inhibit translation or promote degradation of their target mRNA transcripts, depending on the degree of complementarity.