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REVIEW

The conserved *miR-8/miR-200* microRNA family and their role in invertebrate and vertebrate neurogenesis

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Abstract Since their discovery in the early 1990s, microRNAs have emerged as key components of the post-transcriptional regulation of gene expression. MicroRNAs occur in the plant and animal kingdoms, with the numbers of microRNAs encoded in the genome increasing together with the evolutionary expansion of the phyla. By base-pairing with complementary sequences usually located within the 3′ untranslated region, microRNAs target mRNAs for degradation, destabilization and/or translational inhibition. Because one microRNA can have many, if not hundreds, of target mRNAs and because one mRNA can, in turn, be targeted by many microRNAs, these small single-stranded RNAs can exert extensive pleiotropic functions during the development, adulthood and ageing of an organism. Specific functions of an increasing number of microRNAs have been described for the invertebrate and vertebrate nervous systems. Among these, the *miR-8*/*miR-200* microRNA family has recently emerged as an important regulator of neurogenesis and gliogenesis and of adult neural homeostasis in the central nervous system of fruit flies, zebrafish and rodents. This highly conserved microRNA family consists of a single ortholog in the fruit fly (*miR-8*) and five members in vertebrates (*miR-200a*, *miR-200b*, *miR-200c*, *miR-141* and *miR-429*). Here, we review our current knowledge about the functions of the *miR-8*/*miR-200* microRNA family during invertebrate and vertebrate neural development and adult homeostasis and, in particular, about their role in the regulation of neural stem/progenitor cell proliferation, cell cycle exit, transition to a neural precursor/neuroblast state, neuronal differentiation and cell survival and during glial cell growth and differentiation into mature oligodendrocytes.

Keywords *miR-8* · *miR-200* · Neurogenesis · Gliogenesis · Central nervous system

Introduction

MicroRNAs (miRNAs or miRs) constitute a subclass of the large family of non-coding RNAs (ncRNAs) comprising those transcripts that are not translated into a polypeptide or protein sequence (for a review, see Qureshi and Mehler 2012). As their name indicates, miRNAs are small single-stranded RNAs that are encoded in the genome either as a gene with its own promoter (in the canonical miRNA pathway) or as part of the intron of another gene, the so-called mirtrons (in the non-canonical miRNA pathway; for reviews, see Krol et al. 2010; Yang and Lai 2011; Qureshi and Mehler 2012). The primary transcript (pri-miRNA) of this gene or the mirtron is processed in two consecutive endoribonucleolytic steps by the nuclear RNAse III enzyme DROSHA, which generates the approximately 70-nucleotide (nt)-long hairpin precursor miRNA (pre-miRNA) that is subsequently transported out of the nucleus and by the cytoplasmic RNAse III enzyme DICER1, which cleaves the pre-miRNA into the approximately 18– to 24-nt-long miRNA/miRNA* duplex (for reviews, see Krol et al. 2010; Qureshi and Mehler 2012; Yates et al.

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2013). One strand of this miRNA/miRNA* duplex (the miRNA guide strand), which is generally with the less stably base-paired 5′ end of the duplex, is subsequently incorporated into a larger protein complex, namely the miRNA-induced silencing complex (miRISC), whereas the other strand (the miRNA passenger strand) is usually degraded. Base-pairing between this "mature" single-stranded miRNA and especially of a heptamer or octamer "seed sequence" at the 5′ end of the miRNA and the complementary sequence of the target mRNA, usually located within the 3′ untranslated region (3′ UTR), induces the degradation, destabilization, or translational inhibition of the target mRNA by mechanisms that are still not fully understood (for reviews, see Huntzinger and Izaurralde 2011; Pasquinelli 2012; Yates et al. 2013).

MicroRNAs have emerged as key regulators of gene expression during development, adulthood and ageing, especially in the central nervous system (CNS; for a review, see Qureshi and Mehler 2012). Accordingly, the dysregulation of miRNA biogenesis and/or function has been associated with a variety of human diseases but most prominently with diseases in which tissue or cell homeostasis is disrupted, such as various types of cancer and neurological (including neurodegenerative), cardiovascular and neurodevelopmental/psychiatric disorders (for reviews, see Esteller 2011; Im and Kenny 2012; Abe and Bonini 2013). MicroRNAs can act as "ON-OFF switches" of gene expression, i.e., the activity of the miRNA leads to the complete degradation or translational inhibition of the target mRNA and results in a complementary expression pattern for the miRNA and its target, or as "fine-tuners" of gene expression, i.e., the activity of the miRNA results in smaller changes in target mRNA stability and/or translation and usually leads to the co-expression of the miRNA and its target (for a review, see Flynt and Lai 2008). In neural tissues, miRNAs are thus ideally suited to control the transition between various cellular or developmental states, such as the transition from a proliferating/self-renewing neural stem/progenitor cell to its postmitotic offspring, the selection of a particular cell fate within a specific lineage, and the terminal differentiation of this postmitotic progeny, but they are also suited to control cell and tissue homeostasis, such as the maintenance (survival) of these cells (for reviews, see Moss 2007; Ivey and Srivastava 2010; Pauli et al. 2011). Among the miRNAs that have gained particular prominence in CNS development and homeostasis are *let-7*, *miR-9* and *miR-124* (for reviews, see Coolen and Bally-Cuif 2009; McNeill and Van Vactor 2012; see also other contributions to this Special Issue). We will focus here on another miRNA family, namely the *miR-8*/*miR-200* family, which has recently emerged as an additional important regulator of neurogenesis and gliogenesis in invertebrates and vertebrates.

The conserved *miR-8*/*miR-200* microRNA family has pleiotropic functions in invertebrates and vertebrates

Chromosomal location, gene structure, mature miRNA sequence and evolutionary relationships of the *miR-8*/*miR-200* family

MicroRNAs belonging to the *miR-200* family have been highly conserved throughout deuterostome evolution and are found across all vertebrate classes, including fish, amphibians, reptiles, birds and mammals (Wheeler et al. 2009). Invertebrate species such as the fruit fly *Drosophila melanogaster* possess only one ortholog of this family, *miR-8* (Fig. 1a; Wheeler et al. 2009). The vertebrate *miR-200* family comprises five members, namely *miR-200a*, *miR-200b*, *miR-200c*, *miR-141* and *miR-429*, which are organized in two gene clusters (for reviews, see Brabletz and Brabletz 2010; Feng et al. 2014). The tricistronic *miR-200b*/*a*/*429* cluster is transcribed from a common promoter and is located on mouse chromosome 4 and human chromosome 1p36 (Fig. 1a, b). The bicistronic *miR-200c*/*141* cluster also has a common promoter and is located on mouse chromosome 6 and human chromosome 12p13 (Fig. 1a, b). Both gene clusters encode mature *miR-200* miRNAs whose seed sequence differs by only 1 nt. Mature *miR-200* miRNAs are therefore classified into two seed sequence subgroups (Fig. 1c): subgroup I comprises *miR-200a* and *miR-141*, which have the seed sequence AACACUG; subgroup II comprises *miR-200b*, *miR-200c* and *miR-429*, which have the seed sequence AAUA CUG. *Drosophila miR-8* has the same seed sequence as subgroup II (Fig. 1c). The slight difference between the two seed sequence subgroups indicates that they each regulate a different population of target mRNAs (Uhlmann et al. 2010). Indeed, a total of 375 target genes (mRNAs) are predicted by three of the publicly available miRNA target prediction tools (Targetscan: Friedman et al. 2009; microRNA: Betel et al. 2008; miRmap: Vejnar and Zdobnov 2012) for seed sequence subgroup I (exemplified by *mmu-miR-200a*) and a total of 448 target genes are predicted by these tools for seed sequence subgroup II (exemplified by *mmu-miR-200b*; Fig. 2a). Of these, only 66 predicted target genes are common to both subgroups (Fig. 2a), suggesting that each seed sequence subgroup mostly regulates a different set of target genes (mRNAs).

Interestingly and despite the high sequence conservation of the mature *miR-8*/*miR-200* miRNAs, the evolutionary relationship between their precursor (pre-miRNA/hairpin) sequences is less straight-forward (Fig. 2b). Although pre-miRNA sequences belonging to *miR-200a*, *miR-200b* and *miR-429* are found in all deuterostomes, *pre-miR-200c* and *pre-miR-141* sequences are only detected in cephalochordates, teleosts and mammals or in tunicates, teleosts and mammals, respectively, suggesting that these miRNAs and/or the

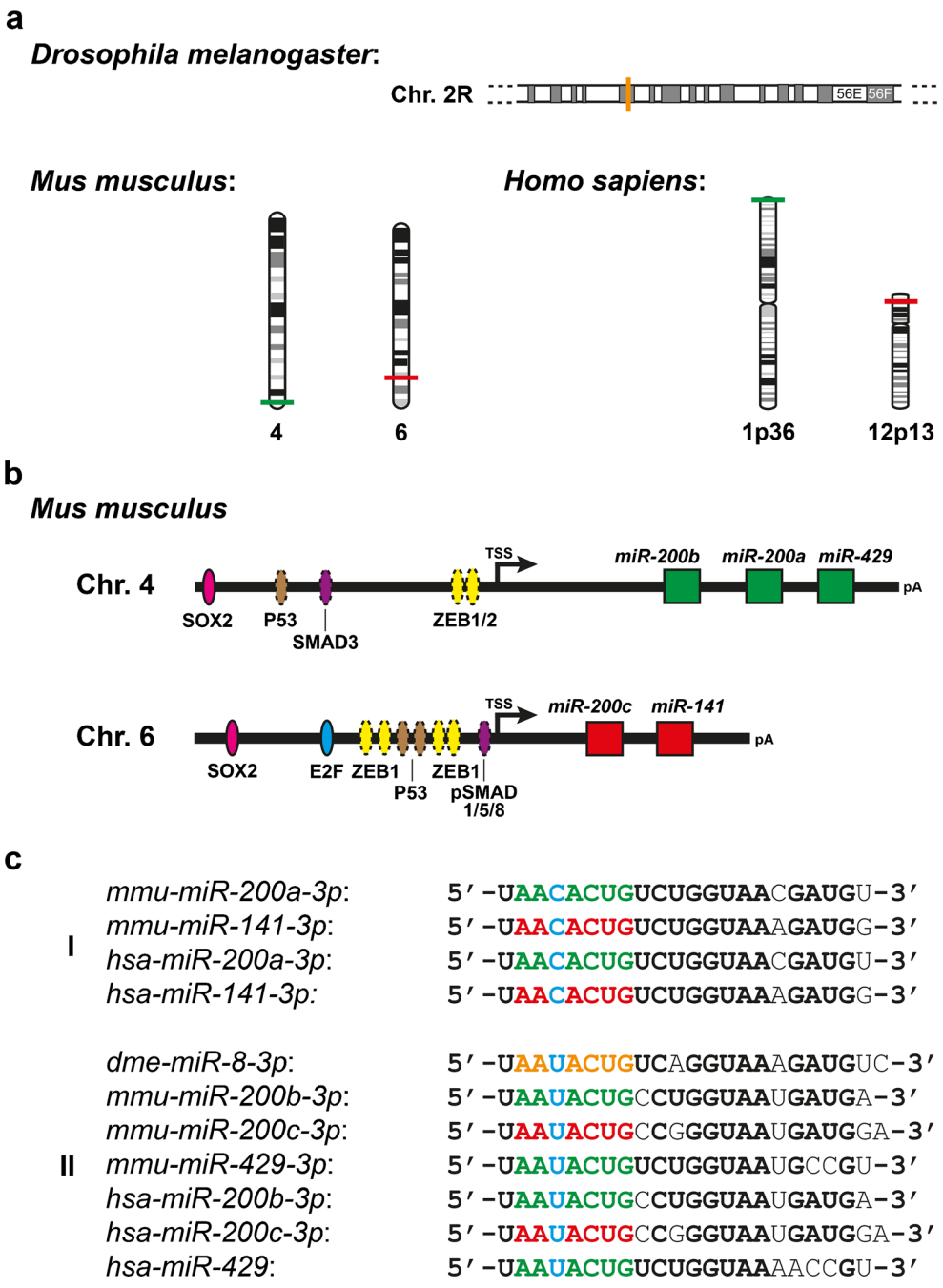

Fig. 1 Chromosomal location, gene structure and mature sequence alignment of the conserved *miR-8*/*miR-200* miRNA family. **a** Position of the *miR-8* gene (*top*, *orange bar*) on chromosome 2R (*Chr. 2R*) in *Drosophila melanogaster* and of the *miR-200b*/*a*/*429* (*green bars*) and *miR-200c*/*141* (*red bars*) gene clusters on chromosomes 4 and 6, respectively, in the mouse (*Mus musculus*), or on chromosome 1p36 and 12p13, respectively, in human (*Homo sapiens*). **b** Structure of the *miR-200b*/*a*/*429* (*top*) and *miR-200c*/*141* (*bottom*) gene cluster in the mouse. *Green boxes* indicate the position of the sequences encoding the *miR-200b*, *miR-200a* and *miR-429* pre-miRNAs and *red boxes* indicate the position of the sequences encoding the *miR-200c* and *miR-141* pre-miRNAs. Each gene cluster is transcribed from a common promoter (*TSS* transcription start site, *pA* polyadenylation signal). Experimentally validated (by chromatin immunoprecipitation, electromobility shift assay, luciferase reporter assays and/or site-directed mutagenesis) binding sites for E2F (*blue oval*; Peng et al. 2012), P53 (*brown ovals*; Kim et al. 2011), SMAD3 or phosphorylated SMAD (pSMAD) 1/5/8 (*purple ovals*; Ahn et al. 2012; Cao et al. 2013), SOX2 (*pink ovals*; Peng et al. 2012; Wang et al. 2013) and ZEB1/2 (*yellow ovals*; Bracken et al. 2008; Burk et al. 2008; Mizuguchi et al. 2012) are shown in the promoter regions of the *miR-200b*/*a*/*429* and *miR-200c*/*141* gene cluster (*stippled lines* activation of the *miR-200* promoter region by the corresponding transcription factor has not been demonstrated in neural cells). Not drawn to scale. **c** Conservation of the mature mouse (*mmu*) and human (*hsa*) miRNA sequences for seed sequence subgroup I (*top*), comprising *miR-200a* and *miR-141* and of the mature *Drosophila* (*dme*), mouse (*mmu*) and human (*hsa*) miRNA sequences for seed sequence subgroup II (*bottom*), comprising *miR-8*, *miR-200b*, *miR-200c* and *miR-429* (*bold* letters conserved nucleotides). The seed sequence is shown in *orange* (*miR-8*), *green* (*miR-200b*/*a*/*429* cluster) and *red* (*miR-200c*/*141* cluster) *letters* and the 1 nt that differs between the two seed sequence subgroups is presented in *blue*. Chromosomal positions were retrieved from Ensembl (release 74; Flicek et al. 2013); mature miRNA sequences were retrieved from miRBase (Kozomara and Griffiths-Jones 2014)

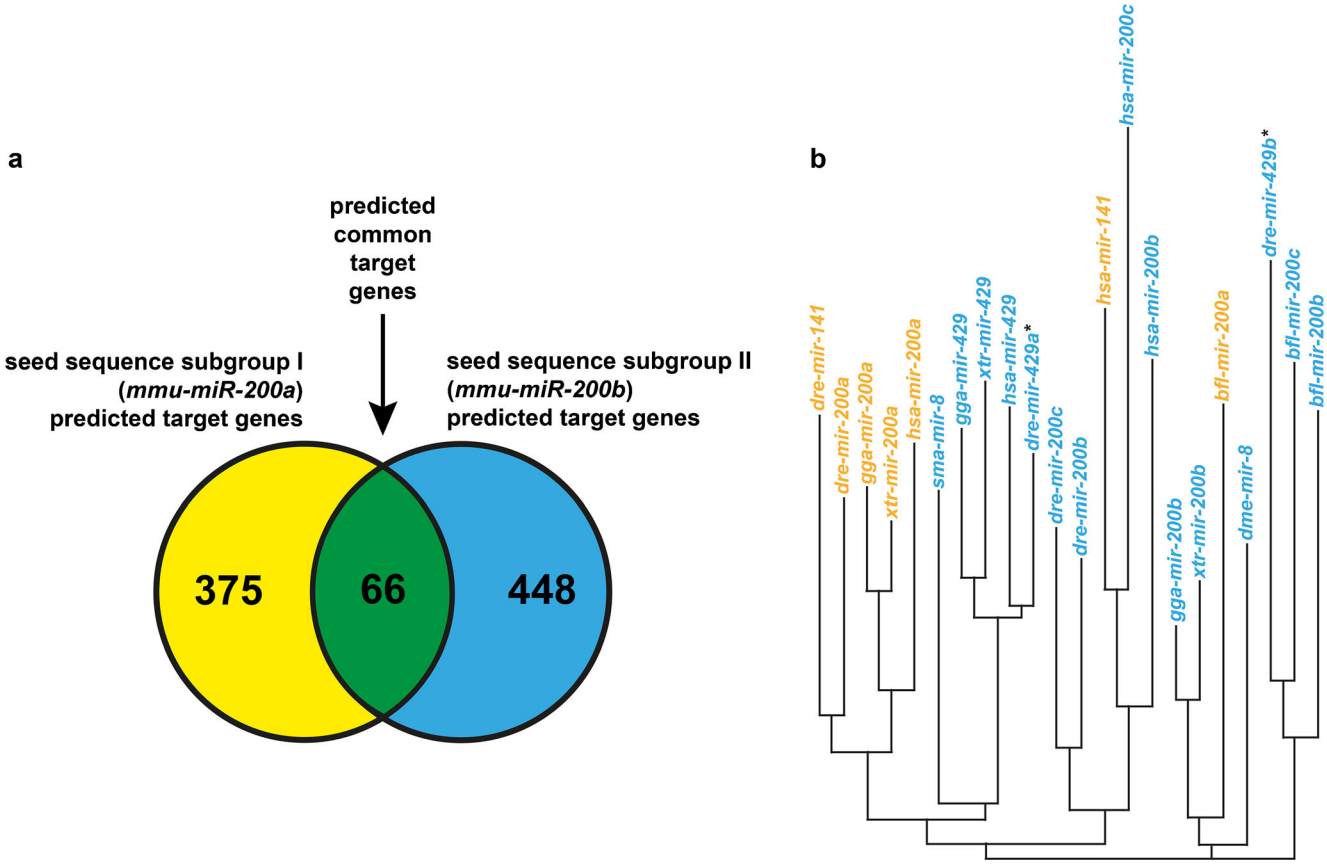

Fig. 2 Proportion of common and specific target genes (mRNAs) for the two *miR-8*/*miR-200* seed sequence subgroups and phylogenetic relationships between the *miR-8*/*miR-200* precursors. **a** Venn diagram illustrating the number of predicted target genes (mRNAs) for either seed sequence subgroup of the *miR-8*/*miR-200* family and the number of target genes common to both seed sequence subgroups. Target predictions for the seed sequence subgroup I are based on the predicted targets of *mmu-miR-200a* and, for the seed sequence subgroup II, on the predicted targets of *mmu-miR-200b*. Datasets from three publicly available miRNA target prediction tools (Targetscan, www.targetscan.org; microRNA, www.microrna.org; miRmap, mirmap.ezlab.org) were combined and filtered for only those targets that are predicted by all three prediction tools. **b** Phylogenetic tree showing the evolutionary relationships between the *miR-8*/*miR-200* pre-miRNAs (*hairpins*) of selected model species. Pre-miRNAs in *yellow* belong to seed sequence subgroup I and, in *blue*, to subgroup II; *asterisks* indicate that two *miR-429* (*a*/*b*) miRNA genes are present in the zebrafish genome, probably because of the whole genome duplication in the teleost lineage. Pre-miRNA sequences of different species were retrieved from miRBase (Kozomara and Griffiths-Jones 2014). Multiple species ClustalW nucleotide alignment of the various pre-miRNAs and generation of the phylogenetic tree by using the neighbor-joining algorithm were performed with the ClustalX 2.1 program (Larkin et al. 2007). Data were imported in the Phylip format to the Archaeopteryx program (Han and Zmasek 2009) for visualization of the phylogenetic tree [*bfl* lancelet (*Branchiostoma floridae*), *dme* fruit fly (*Drosophila melanogaster*), *dre* zebrafish (*Danio rerio*), *gga* chick (*Gallus gallus*), *hsa* human (*Homo sapiens*), *sma* trematode (*Schistosoma mansoni*), *xtr* frog (*Xenopus tropicalis*)]

corresponding gene cluster have been secondarily lost during deuterostome evolution (Fig. 2 and data not shown). Furthermore, *miR-8*/*miR-200* miRNAs belonging to either seed sequence subgroup do not show a clear phylogenetic relationship (Fig. 2b), suggesting that the 1-nt difference between the two subgroups arose independently in different lineages.

Evolutionarily conserved and pleiotropic functions of the *miR-8*/*miR-200* microRNA family

Overall, the sequence of the mature *miR-8*/*miR-200* miRNAs has been highly conserved across phyla (Fig. 1c), suggesting that at least some functions of these miRNAs have also been conserved during evolution. Indeed, *Drosophila miR-8* and human *miR-200* members were originally found to fine-tune the protein levels of atrophin and its human ortholog RERE (arginine glutamic acid dipeptide [RE] repeats), respectively and thereby to prevent neurodegeneration in the fly's CNS and possibly also in the human brain (Karres et al. 2007). Subsequently, *miR-8*/*miR-200* miRNAs were described as repressors of members of the Wingless (Wg) signaling pathway in the *Drosophila* eye and in murine mesenchymal stem cells, respectively, their function being the regulation of eye size and the differentiation of mesenchymal stem cells into adipocytes (Kennell et al. 2008). *Drosophila mir-8* and the human *miR-200* family also suppress the expression of an inhibitor of insulin/phosphoinositide-3 kinase (PI3K)

signaling in fat body and human liver cells, respectively, an action required for the control of body size and fat body/liver cell growth and proliferation (Hyun et al. 2009). Finally, *miR-8* and the human *miR-200* family restrict the expression of SERRATE and its human ortholog JAGGED1, two ligands of the Notch receptor, thereby suppressing metastatic tumour growth in the fruit fly and of metastatic human prostate and breast cancer cells (Brabletz et al. 2011; Vallejo et al. 2011). The mammalian *miR-200* family has meanwhile gained particular prominence because of its regulation of epithelial-to-mesenchymal transition (EMT), "stemness" and somatic cell reprogramming into induced pluripotent stem cells (iPSCs; Lin et al. 2009; Shimono et al. 2009; Wellner et al. 2009; Gill et al. 2011; Chen et al. 2012; Wang et al. 2013); for reviews, see Peter 2009; Brabletz and Brabletz 2010) and because of its tumour-suppressive role in a wide array of human cancers (for reviews, see Peter 2009; Brabletz and Brabletz 2010; Hill et al. 2013; Feng et al. 2014). The vertebrate *miR-200* family has also been implicated in pharyngeal taste bud development in the zebrafish (Kapsimali et al. 2011) and in submandibular gland morphogenesis (Rebustini et al. 2012) and tooth development and renewal (Cao et al. 2013) in the mouse. These data, apart from revealing the high conservation of *miR-8*/*miR-200* targets and mechanistic actions throughout evolution, have also uncovered the vast pleiotropy of this miRNA family. In addition to their previously mentioned functions, four recent publications have shown that *miR-8* and the *miR-200* family are important regulators of various aspects of invertebrate and vertebrate gliogenesis and/or neurogenesis, which will be reviewed below.

Glial *miR-8*/*miR-200* regulates neurogenic niche signals and gliogenesis

Glial *miR-8* regulates glial cell size and proliferation of *Drosophila* optic lobe neural stem cells

Apart from its highly dynamic expression in the fruit fly larva (Karres et al. 2007), a recent report has shown that *miR-8* is specifically expressed in a newly discovered surface-associated glial cell type, which has been termed "optic-lobe-associated cortex glia", underlying the subperineurial glia (the blood–brain-barrier) of the *Drosophila* brain and optic lobes (Morante et al. 2013). The overexpression of *Drosophila miR-8* or human *miR-200c* in glial cells increases cell-autonomously the size of the surface glial cell bodies but suppresses their proliferation in the fruit fly (Morante et al. 2013). This has subsequently been traced back to the regulation, by *miR-8*, of endoreplication without cell division in surface glial cells via an unknown mechanism (Morante et al. 2013). Notably, the complete lack or knock-down and the overexpression of *Drosophila miR-8* in optic-lobe-associated cortex glia cells also has non-cell-autonomous effects on the surrounding neuroepithelium: in the absence or after knock-down of *miR-8*, the proliferation of neural stem cells (NSCs) is increased and occurs at ectopic sites and these cells transit precociously to neuroblasts (neural precursors) in the larval neuroepithelium, whereas the overexpression of *miR-8* in surface glial cells has the opposite effect (Morante et al. 2013). In search of a mechanistic link between the expression of *miR-8* in optic-lobe-associated cortex glia cells and the control of NSC proliferation and neuroblast transition in the underlying neuroepithelium, the authors found that Spitz, a secreted transforming growth factor alpha (TGFa)-like ligand for the epidermal growth factor receptor (EGFR), is specifically expressed in these cells. Overexpression of Spitz in optic-lobe-associated cortex glia cells or a constitutively active EGFR in neuroepithelial cells leads to a similar phenotype (increased NSC proliferation, premature and ectopic neuroblasts) as in *miR-8*-deficient flies, whereas the knock-down of Spitz in optic-lobe-associated cortex glia cells or the overexpression of a dominant negative EGFR in neuroepithelial cells strongly decreases the size of the neuroepithelium, thus resembling the phenotype after *miR-8* overexpression (Morante et al. 2013). Spitz is indeed a direct target of *miR-8* and the expression of a Spitz transgene lacking the 3′ UTR (and thus incapable of being regulated by *miR-8*) completely rescues the defects after *miR-8* overexpression in glial cells (Morante et al. 2013). Together, the data from this study show that *miR-8* cell-autonomously controls the endoreplicative growth of surface-associated glial cells and represses the expression of a secreted neurogenic signal (the TGFa-like ligand Spitz) in a specialized subset of surface glial cells, thereby regulating non-cell-autonomously the proliferation of responsive (EGFR-expressing) NSCs and their proper transition to the neural precursor (neuroblast) state in the *Drosophila* brain and optic lobes (Fig. 3a). Because TGFa- and EGFR-mediated signaling have also been implicated in the control of NSC proliferation in the rodent brain (for a review, see Shi et al. 2008), we need to determine whether such a non-cell-autonomous function of *miR-200* in (radial) glial cells is conserved in the vertebrate neurogenic niche(s) (Fig. 3a). Interestingly, further reduction of Spitz protein levels in *miR-8*-overexpressing glial cells enhances the neuroepithelial growth defects and induces a non-apoptotic form of cell death in the mutant brains, suggesting that a perturbed control of Spitz protein dosage by *miR-8* is also involved in

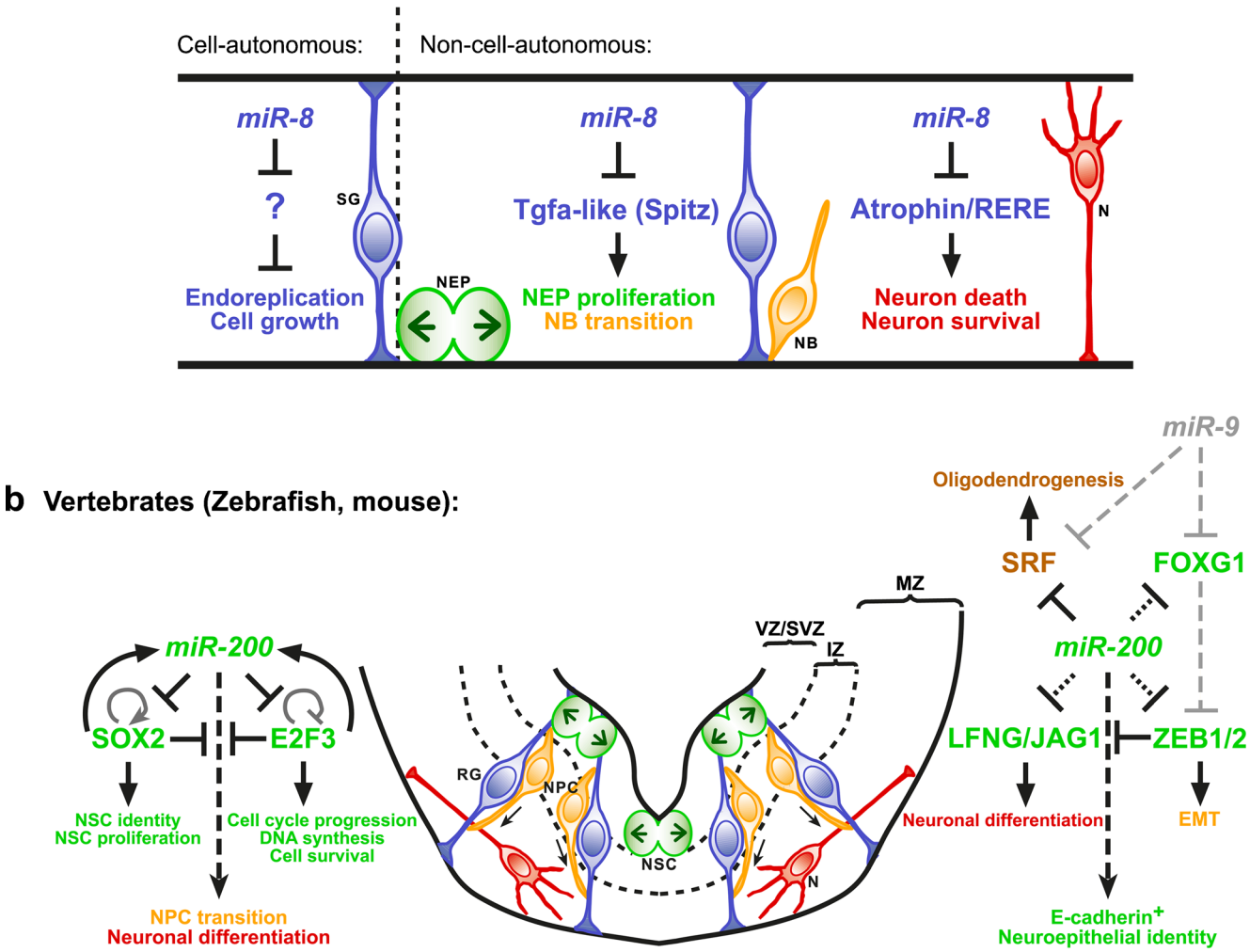

Fig. 3 Functions of *miR-8*/*miR-200* miRNAs in invertebrate and vertebrate neurogenesis and gliogenesis. **a** In the central nervous system (CNS) of the fruit fly (*Drosophila melanogaster*), *miR-8* regulates cell-autonomously the endoreplicative growth of specialized surface glial (*SG*, *blue*) cells by suppressing the expression of an as yet unknown negative regulator of endoreplication in these cells (Morante et al. 2013). Expression of *miR-8* in SG cells also regulates non-cell-autonomously the proliferation of neuroepithelial (*NEP*, *green*) cells and their transition to more committed neuroblasts (*NB*, *yellow*), by down-regulating the levels of a transforming growth factor α (TGFa)-like ligand (*Spitz*), which is secreted from the SG cells and which promotes these two processes in EGFR-expressing NEP cells (Morante et al. 2013). Whether a similar non-cell-autonomous regulatory pathway exists in the vertebrate CNS, possibly controlled by *miR-200* miRNAs expressed in radial glia (*RG*, *blue* in **b**) cells (an essential component of the vertebrate neurogenic niche, Gotz and Huttner 2005; Sild and Ruthazer 2011), remains unclear. Finally, *miR-8* expression in the *Drosophila* CNS fine-tunes the levels of atrophin (*RERE* in mammals), thereby balancing the (apoptotic) death and survival of neural cells, including neurons (*N*, *red*; Karres et al. 2007). **b** In the vertebrate (zebrafish and mice) CNS, *miR-200* family members regulate the proliferation, cell cycle exit and survival of neural stem/progenitor cells (*NSC*, *green*) by suppressing the levels of SOX2 and E2F3 in these cells, thereby facilitating their transition to a migrating neural precursor cell (*NPC*, *yellow*) and subsequent differentiation into a neuron (*N*, *red*; Peng et al. 2012). SOX2 and E2F3 in turn activate the transcription of the *miR-200* gene clusters (Peng et al. 2012; Wang et al. 2013) and self-activate (SOX2, Tomioka et al. 2002) or repress (E2F3, Adams et al. 2000) their own expression in NSCs. *miR-200* miRNAs might also repress the expression of NOTCH signaling members (LFNG, Choi et al. 2008; JAG1, Vallejo et al. 2011) in NSCs, thus inhibiting their precocious differentiation and of ZEB1/2 transcription factors mediating EMT in other contexts (Lamouille et al. 2013), thereby maintaining the neuroepithelial (E-cadherin^{+}) identity of NSCs. Additionally, *miR-200* members suppress the expression of serum response factor (*SRF*, *brown*) in OPCs (not shown), thereby inhibiting their premature differentiation into mature oligodendrocytes (Buller et al. 2012) and down-regulate directly or indirectly the expression of FOXG1 in NSCs/NPCs (Choi et al. 2008); FOXG1 is a transcription factor required for proper telencephalic neural development (Danesin and Houart 2012). *Zeb2*, *Srf* and *Foxg1* mRNAs are also targeted by *miR-9*. A cross-section through the ventral midbrain is shown (*IZ* intermediate zone, *MZ* mantle zone, *SVZ* subventricular zone, *VZ* ventricular zone)

neurodegenerative processes (Morante et al. 2013). The latter finding might reconcile, at least in part, the initial data that the lack of *miR-8* induces neurodegeneration in the *Drosophila* brain (Karres et al. 2007).

Glial *miR-200* regulates the differentiation of oligodendrocyte precursor cells

Another, direct role of *miR-200* family members in controlling the progression of oligodendrogenesis in rats in vivo and cell cultures in vitro has been described by Buller et al. (2012). Expression of serum response factor (SRF), a ubiquitous transcription factor with widespread functions in neuronal differentiation, maturation, migration and activity (for a review, see Knoll and Nordheim 2009), is strongly up-regulated in differentiating white matter oligodendrocytes after ischemic stroke and this correlates with a down-regulation of *miR-200b* expression in the same cells (Buller et al. 2012). Similarly, the expression of SRF increases, whereas the transcription of *miR-200c* decreases as oligodendrocyte precursor cells (OPCs) are differentiating into oligodendrocytes in vitro (Buller et al. 2012). The *Srf* 3′ UTR is directly targeted by *miR-200b* and *miR-9* and co-transfection of immortalized OPCs with mimics of *miR-200b* and *miR-9* suppresses their differentiation into oligodendrocytes (Buller et al. 2012). These data indicate that *miR-200* miRNAs modulate the transition from a proliferating OPC to a differentiating oligodendrocyte, at least in part, by negatively regulating the expression of SRF in these cells (Fig. 3b; Knoll and Nordheim 2009).

Neural *miR-200* regulates olfactory neurogenesis in zebrafish and mice

The expression of *miR-200* miRNAs is highly enriched in the developing and adult olfactory neuroepithelium of rodents (Fig. 4b; Choi et al. 2008). Depletion of mature miRNAs (including the *miR-200* family) in mature olfactory and vomeronasal sensory neurons, as achieved by the conditional ablation of the endoribonuclease *Dicer1* gene in these cells (*OMP-Cre*; $Dicer^{loxP/loxP}$ mice), neither affects the molecular identity, turnover and survival of the sensory neurons, nor the olfactory projections and behavior of the mutant mice (Choi et al. 2008). The conditional ablation of the *Dicer1* gene and consequent depletion of mature miRNAs in neural progenitors, including olfactory progenitors (*Foxg1-Cre*; $Dicer^{loxP/loxP}$ mice), however, leads to the prenatal lethality of the mutant embryos (Choi et al. 2008). This phenotype is coupled to a strong reduction of the olfactory neuroepithelium, including the olfactory progenitor cells, to their disrupted differentiation into mature olfactory sensory neurons at midgestational stages and to the complete loss of this tissue at late gestation (Choi et al. 2008). Notably, the initial patterning and specification of the olfactory neuroepithelium, the proliferation of olfactory progenitor cells and the generation of non-neural respiratory epithelium are not affected but the apoptotic death of olfactory progenitor cells is strongly increased in the *Foxg1-Cre*; $Dicer^{loxP/loxP}$ mutant embryos (Choi et al. 2008). Mature olfactory sensory neurons are also depleted in maternal-zygotic *dicer* zebrafish mutants whose early defects have been rescued by the co-injection of *miR-430* (MZ$dicer^{+}$ ^{miR430} mutants) but in contrast to the mouse, an enlargement of the *foxg1*-expressing olfactory neuroepithelium is observed in these zebrafish mutants (Choi et al. 2008). The genomic organisation and mature sequence of the *miR-200* family is highly conserved between zebrafish and mouse and *miR-200* expression is also enriched in the olfactory tissues of this teleost species (Choi et al. 2008). Accordingly, the antisense morpholino oligonucleotide-mediated knock-down of the entire *miR-200* family in zebrafish embryos results in a similar olfactory phenotype as in MZ$dicer^{+miR430}$ zebrafish and *Foxg1-Cre*; $Dicer^{loxP/loxP}$ mouse mutants (Choi et al. 2008). The only notable difference between the mouse and zebrafish mutants is the early thinning and later complete disappearance of the $Foxg1^{+}$ olfactory epithelium in the *Foxg1-Cre*; $Dicer^{loxP/loxP}$ mouse embryos, probably because of an increased apoptosis of these cells, whereas *foxg1* expression appears to be increased in the olfactory neuroepithelium of the MZ$dicer^{+miR430}$ and *miR-200* morphant zebrafish, probably because of an initial expansion of the $foxg1^{+}$ olfactory progenitors that are unable to differentiate into mature olfactory sensory neurons and despite the apparently also increased apoptotic death of these cells at later stages (Choi et al. 2008). The reason for these discrepancies is unclear at present. Nevertheless, these data indicate that the *miR-200* family plays a major and phylogenetically conserved role in olfactory tissue development. In search of potential targets of the *miR-200* miRNAs in this context, the authors found that zebrafish *lfng* (lunatic fringe/LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase; a modulator of the Notch signaling pathway; Louvi and Artavanis-Tsakonas 2006) and *zfhx1* (zinc finger E-box binding homeobox, also known as ZEB1; a modulator of TGFb signaling and EMT; Conidi et al. 2011) might be direct *miR-200* targets that mediate at least some of the proneural effects of this miRNA family in the developing olfactory neuroepithelium of the zebrafish embryo (Fig. 3b; Choi et al. 2008).

This report is the first indication that the *miR-200* family plays an important role in controlling the transition from a proliferating neural progenitor to a postmitotic and differentiating neuron, i.e., the process that genuinely is known as “neurogenesis”. In addition, the *miR-200* family appears to regulate the proper survival of proliferating neural progenitors during embryonic (neurogenic) stages. The presence of the *miR-200* family, by contrast, is dispensable for the initial patterning and specification events in the embryonic olfactory neuroepithelium and for the proper survival and function of the mature olfactory sensory neurons.

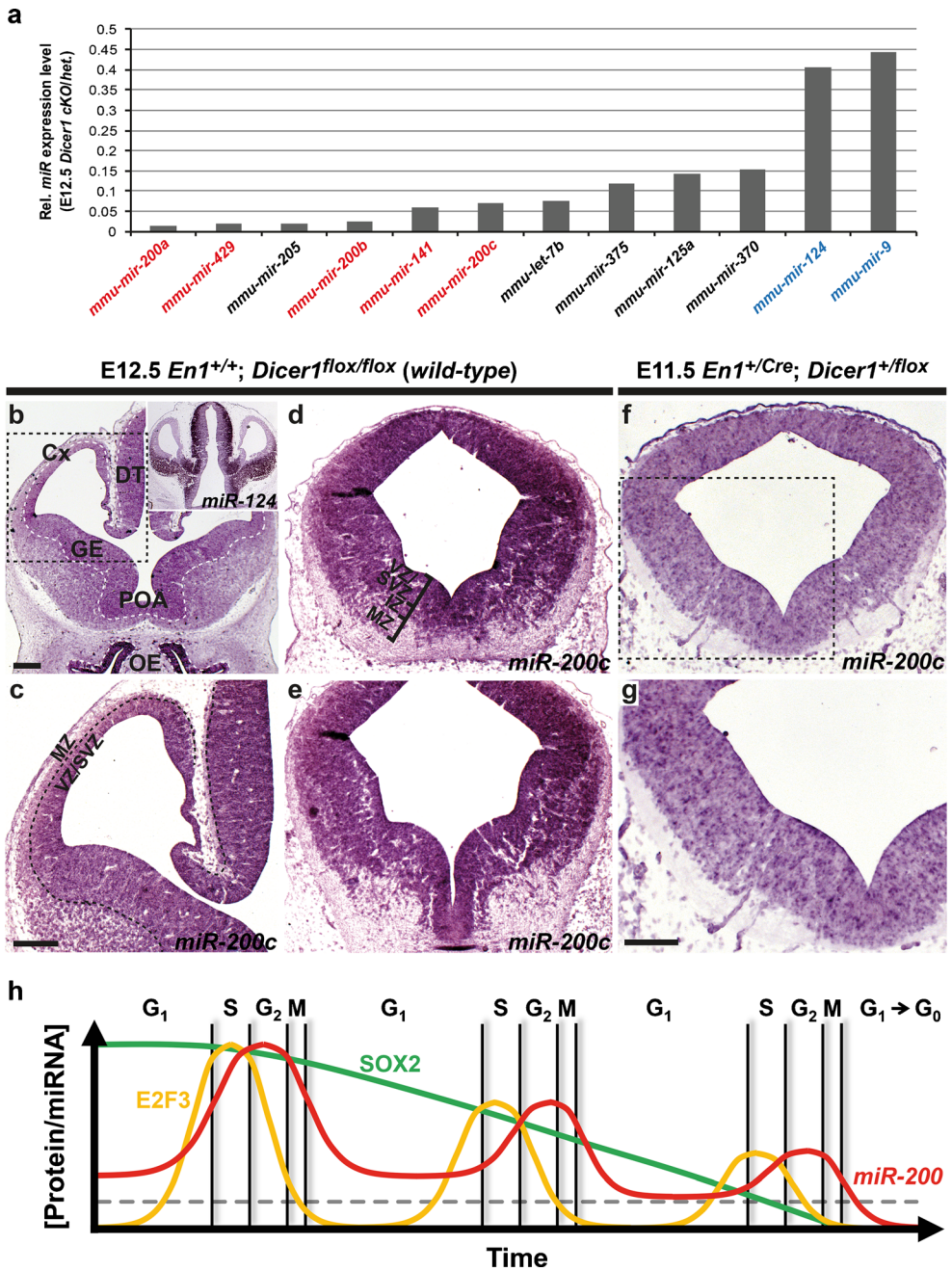

Fig. 4 Dampening of expression fluctuations and gradual reduction of SOX2, E2F3 and *miR-200* levels by an autoregulatory and unilateral negative feedback loop. **a** Expression levels (from next-generation sequencing) of the top ten most down-regulated miRNAs and of *miR-124* and *miR-9* (*blue*) in the mid-/hindbrain region (MHR) of embryonic day 12.5 (E12.5) $En1^{+/Cre}$; $Dicer1^{flox/flox}$ (*Dicer1 cKO*) mouse embryos relative to $En1^{+/Cre}$; $Dicer1^{+/flox}$ (*het.* heterozygote) embryos. The five *miR-200* family members (*red*) were among the most strongly depleted miRNAs after *Dicer1* ablation in the MHR (ratios: *miR-200a*, 0.014; *miR-429*, 0.019; *miR-205*, 0.02; *miR-200b*, 0.026; *miR-141*, 0.059; *miR-200c*, 0.072; *let-7b*, 0.077; *miR-375*, 0.12; *miR-125a*, 0.142; *miR-370*, 0.152; *miR-124*, 0.407; *miR-9*, 0.444). **b**–**g** Representative overviews (**b**,**d**–**f**) and close-up views (**c**, **g**) of the forebrain (**b**, **c**), midbrain (**d**, **f**, **g**) and hindbrain (**e**) in coronal sections from E12.5 $En1^{+/+}$; $Dicer1^{flox/flox}$ (wild-type; **b**–**e**) and E11.5 $En1^{+/Cre}$; $Dicer1^{+/flox}$ (heterozygote; **f**, **g**) mouse embryos, hybridized with a locked nucleic-acid-modified and digoxigenin-labeled *mmu-mir-200c* or *mmu-mir-124* (*inset* in **b**) oligonucleotide in situ probe as described in Peng et al. (2012). Note that *miR-200c* expression is strongest in the olfactory epithelium (*OE*) and weaker in the brain neuroepithelium of the wild-type embryo and is restricted to the ventricular/subventricular zone (*VZ/SVZ*) and intermediate zone (*IZ*) containing neural progenitors and precursors but is mostly absent in the mantle zone (*MZ*) containing postmitotic neurons expressing *miR-124* (**b**–**e**). Note also the non-uniform spotted expression pattern of *miR-200c* in neuroepithelial cells of a heterozygous $Dicer1^{+/-}$ mouse embryo (**f**, **g**). **c**, **g** Higher magnifications of the *boxed areas* in **b**, **f**, respectively (*Cx* cortex, *DT* dorsal thalamus, *GE* ganglionic eminences, *POA* preoptic area). *Bars* 250 μm (**b**), 100 μm (**c**, **g**). **h** Representation of the expected time-course of SOX2 (*green*) and E2F3 (*yellow*) protein levels and of *miR-200* (*red*) miRNA levels in cycling neural stem/progenitor cells (*broken gray line* threshold level at which the transcription of *Sox2* and *E2f3* and concomitantly of the two *miR-200* gene clusters, is switched off completely). G_1, S, G_2, M, G_0 are the phases of the cell cycle. Original data were published in Peng et al. (2012)

Neural *miR-200* regulates ventral mid-/hindbrain neurogenesis in mice

Loss of DICER1-processed mature miRNAs leads to cell cycle exit and neuronal differentiation defects in the developing murine mid-/hindbrain region

The boundary between the midbrain and rostral hindbrain harbors one of the principal signaling centers during vertebrate embryonic development, the isthmic organizer (IsO; for reviews, see Wurst and Bally-Cuif 2001; Vieira et al. 2010). The IsO controls the correct patterning of the mid-/hindbrain region (MHR) and the emergence of key neuronal populations in this region, including dopaminergic, glutamatergic, serotonergic, cholinergic and noradrenergic nuclei, which have been implicated in the control and modulation of motor, cognitive and emotional/affective behaviors (for a review, see Zervas et al. 2005). Depletion of mature miRNAs by the conditional ablation of the murine *Dicer1* gene in the MHR (*Wnt1–Cre*; $Dicer1^{flox/flox}$ mice; Huang et al. 2010; $En1^{+/Cre}$; $Dicer1^{flox/flox}$ mice; Peng et al. 2012) leads to perinatal lethality of the mutant pups and results in a progressive loss of this region during embryonic development; this appears to be mostly attributable to the apoptotic cell death of the corresponding neural tissues (Huang et al. 2010; Peng et al. 2012). However, the establishment of the IsO, the patterning of the MHR and the initial specification of some of the key neuronal populations are not affected by the absence of *Dicer1* and mature miRNAs in this region (Huang et al. 2010; Peng et al. 2012), thus resembling the conditional *Dicer1* ablation phenotype in the olfactory neuroepithelium (Choi et al. 2008). Although the ventral MHR tissues are affected by apoptotic cell death to a much lower extent than the dorsal MHR tissues (Peng et al. 2012), neural progenitors located in the ventral MHR fail to generate the appropriate amount of neuronal progeny, resulting in strongly reduced numbers of midbrain dopaminergic and glutamatergic (red nucleus) neurons and of rostral hindbrain serotonergic neurons at midgestational stages in the *Dicer1* mutant embryos (Huang et al. 2010; Peng et al. 2012). In search of a mechanistic explanation for this phenomenon, we found that the cell cycle exit of neural progenitors located in the ventral MHR and their subsequent differentiation into the corresponding neuronal populations are strongly impaired in $En1^{+/Cre}$; $Dicer1^{flox/flox}$ embryos (Peng et al. 2012). This has opened up a new avenue for addressing the role of mature miRNAs in the control of neural progenitor proliferation, cell cycle exit and neuronal differentiation in the ventral MHR of the mouse embryo.

The *miR-200* family regulates cell cycle exit and neuronal differentiation of proliferating and multipotent neural stem/progenitor cells by targeting SOX2 and E2F3 expression in these cells

Expression profiling by next-generation sequencing has revealed all five members of the *miR-200* family among the most strongly depleted miRNAs in the MHR of the $En1^{+/Cre}$; $Dicer1^{flox/flox}$ embryos (Fig. 4a; Peng et al. 2012). The expression of mature *miR-200* miRNAs also declines as neuronal differentiation progresses in the MHR in vivo and in differentiating mouse and human embryonic stem cells (ESCs) in vitro (Gill et al. 2011; Peng et al. 2012; Du et al. 2013; Huang et al. 2013). Because *miR-200c* is the most highly expressed *miR-200* family member in neural tissues and differentiating pluripotent ESCs (Peng et al. 2012; Huang et al. 2013), subsequent functional analyses have focused on this *miR-200* miRNA. In the MHR and other brain regions, *miR-200c* is expressed in neural progenitors and precursors located in the ventricular (VZ) and subventricular (SVZ) zones of the neuroepithelium but is mostly absent in the mantle zone (MZ) containing postmitotic and differentiating neurons (Fig. 4b-e; Peng et al. 2012). The two transcription factors SOX2 (SRY [sex determining region Y]-box 2) and E2F3 (E2F transcription factor 3) have been identified as two transcriptional activators of the *miR-200c/141* gene cluster (Fig. 1b) and direct targets of *miR-200c* (Peng et al. 2012). The numbers of cycling (S-phase) SOX2- and E2F3-expressing cells are almost doubled and these cells do not generate the appropriate amount of Tubb3 (beta-III-tubulin)-expressing neuronal offspring in the ventral MHR neuroepithelium of the $En1^{+/Cre}$; $Dicer1^{flox/flox}$ embryos (Peng et al. 2012). The "sponge" vector-mediated depletion (Ebert and Sharp 2010) of all *miR-200* miRNAs in primary ventral MHR cell cultures indeed increases the numbers of SOX2- and E2F3-expressing (proliferating) neural progenitor cells and decreases the numbers of differentiating $Tubb3^+$ neurons, thus phenocopying the defects of the *Dicer1* mutant embryos, whereas the opposite effect (decreased $SOX2^+$ and $E2F3^+$ cell numbers and increased numbers of $Tubb3^+$ neurons) is observed after the overexpression of the *miR-200c/141* cluster in these cells (Peng et al. 2012).

Taken together, these data corroborate the previous findings that the *miR-200* family plays a prominent role in the generic regulation of invertebrate and vertebrate neurogenesis and neural progenitor survival but is not involved in the control of early neural patterning and cell fate specification events. Furthermore, they indicate that at least part of this regulatory activity is mediated by a unilateral negative feedback loop between the *miR-200* miRNAs and the two transcription factors SOX2 and E2F3 (Fig. 3b). SOX2 is a

member of the SOXB1 subgroup of SRY-related transcriptional regulators characterized by a highly conserved high-mobility group (HMG) DNA-binding domain (for reviews, see Pevny and Nicolis 2010; Kamachi and Kondoh 2013). In addition to being part of the core transcriptional network regulating ESC pluripotency and somatic cell reprogramming to pluripotent cells (for reviews, see Kashyap et al. 2009; Kamachi and Kondoh 2013), SOX2 is expressed in neural stem and progenitor cells of the vertebrate CNS throughout development and adulthood (Ellis et al. 2004). The overexpression of SOX2 maintains the proliferating neural stem/progenitor cell identity and inhibits their differentiation into postmitotic neurons (Graham et al. 2003), all of which strongly resembles some of the defects detected in the *En1*$^{+/Cre}$; *Dicer1*$^{flox/flox}$ embryos and after *miR-200* knockdown in primary ventral MHR cells (Peng et al. 2012). Conversely, the repression of the SOX2 transcriptional activator function or reduction of *Sox2* gene dosage causes cell cycle exit, the delamination of neural stem/progenitor cells from the VZ and the initiation of their premature differentiation into neurons (Graham et al. 2003; Cavallaro et al. 2008), thus resembling some of the defects seen after *miR-200c*/*141* overexpression in primary ventral MHR cells (Peng et al. 2012). E2F3 belongs to the E2F family of transcription factors with a highly homologous DNA-binding domain and has been mostly viewed as an E2F member (together with E2F1 and E2F2) with transcriptional activator function (for reviews, see DeGregori 2002; Chen et al. 2009b). E2F3 plays an important role in the control of cell survival/apoptosis and cell cycle progression/DNA synthesis in all types of cells, including neural progenitors, in vivo and in vitro (Humbert et al. 2000; Danielian et al. 2008; Tsai et al. 2008; Chen et al. 2009a; Chong et al. 2009), although context-dependent and redundant functions of the E2F family have made it difficult to establish this conclusively (for reviews, see DeGregori 2002; Chen et al. 2009b). The overexpression of E2F1 induces the re-entry of quiescent cells into S-phase and DNA synthesis (Johnson et al. 1993), whereas deletion of the *E2f3* gene alone leads to variable deficits in cell survival and proliferation that are augmented in the absence of the other two activating E2F transcription factors (E2F1/2; Humbert et al. 2000; Danielian et al. 2008; Tsai et al. 2008; Chen et al. 2009a; Chong et al. 2009). The deregulation of E2F3 protein levels in the ventral MHR of the *En1*$^{+/Cre}$; *Dicer1*$^{flox/flox}$ embryos indeed leads to the aberrant accumulation of E2F3 in cycling (S-phase) neural progenitor cells (Peng et al. 2012) and is thus highly likely to contribute to the proliferation/cell cycle exit, neuronal differentiation and survival defects observed in the *Dicer1* mutant embryos and in primary ventral MHR cells after *miR-200* depletion (up-regulated E2F3 expression) or after *miR-200c*/*141* overexpression (down-regulated E2F3 expression).

A *miR-200*-controlled autoregulatory and unilateral negative feedback loop mediates the transition from a proliferating and multipotent neural stem/progenitor cell to a postmitotic and differentiating neuron

As mentioned above, the regulatory function of SOX2 and E2F is highly dose-dependent, with the strongest effects being seen after the overexpression of SOX2 or E2F1 (Johnson et al. 1993; Graham et al. 2003) or after the complete loss of SOX2 (Taranova et al. 2006; Cavallaro et al. 2008; for a review, see Pevny and Nicolis 2010) or of all three activating E2F transcription factors (Humbert et al. 2000; Chen et al. 2009a; Chong et al. 2009). Moreover, the SOX2 and E2F3 transcription factors, in cooperation with their binding partners, can self-activate or -repress, respectively, their own promoters (Adams et al. 2000; Tomioka et al. 2002; for reviews, see DeGregori 2002; Kondoh and Kamachi 2010). The maintenance of adequate SOX2 and E2F protein levels within a (neural progenitor) cell is thus essential for the proper homeostasis of this cell and for its transition to a non-proliferative (postmitotic) status. The two mouse *miR-200* gene clusters are bound and activated by SOX2 (Fig. 1b; Peng et al. 2012; Wang et al. 2013) and the mouse *miR-200c*/*141* gene cluster is also activated by E2F3 (Peng et al. 2012). High basal levels of SOX2 in neural stem/progenitor cells (Graham et al. 2003; Cavallaro et al. 2008) and rising levels of E2F3 protein as these cells transit from the G_1 to the S-phase of the cell cycle (Leone et al. 1998; Adams et al. 2000; Tsai et al. 2008) are therefore predicted to maintain and repress, respectively, their own expression but, at the same time, to activate the transcription of the two *miR-200* gene clusters in these cells. Increasing levels of *miR-200* miRNAs are in turn predicted to down-regulate the protein levels of SOX2 and E2F3 in neural stem/progenitor cells, leading to an attenuation of SOX2, E2F3 and *miR-200* expression in these cells over time, despite a cyclic reactivation of *E2f3* mRNA/protein expression (Leone et al. 1998) and possibly also of the *miR-200c*/*141* gene cluster (Fig. 4h; Peng et al. 2012). Mature *miR-200* miRNAs indeed appear to be relatively unstable in neural cells, as demonstrated by the finding that this miRNA family is among the top ten most strongly depleted mature miRNAs in neural tissues of the DICER1 mutant MHR at E12.5, approximately 3 days after ablation of the *Dicer1* gene in this region (Peng et al. 2012) and in contrast to the apparently more stable mature *miR-9* (Bonev et al. 2012) and *miR-124* miRNAs in these tissues (Fig. 4a). Furthermore, we detected a non-uniform spotted expression of *miR-200c* in the MHR neural tissues of heterozygote *En1*$^{+/Cre}$; *Dicer1*$^{+/flox}$ embryos (Fig. 4f, g), in which only half of the *Dicer1* gene dosage is present in these tissues, thus suggesting a cyclic expression and rapid turnover of this mature miRNA, at least in the MHR neuroepithelium. The self-regulatory and unilateral negative feedback loop between SOX2, E2F3 and *miR-200*

might thus act, on the one hand, by dampening the potential expression oscillations of two components of this feedback loop (E2F3 and *miR-200*, Fig. 4h) in a similar manner to that recently described for the *lin-4* miRNA and its target, the transcription factor *lin-14*, during larval development in the nematode worm *Caenorhabditis elegans* (Kim et al. 2013) and for *miR-9* and its target *Hes1* during neural differentiation of an immortalized murine neural progenitor cell line (Bonev et al. 2012). On the other hand, this self-regulatory and unilateral negative feedback loop is expected to gradually reduce the SOX2/E2F3 protein and *miR-200* miRNA levels in neural stem/progenitor cells (Fig. 4h) and to suppress completely their expression once the SOX2/E2F3 protein levels drop below a certain threshold (Mukherji et al. 2011; for a review, see Ebert and Sharp 2012). However, and in contrast to the more stable mature *miR-9* (Fig. 4a; Bonev et al. 2012), mature *miR-200* miRNAs are not expected to accumulate over time in neural stem/progenitor cells because of their lower stability in these cells (Fig. 4a; Peng et al. 2012), indicating that additional signals are required for the precise timing of (ventral MHR) neural stem/progenitor cell cycle exit and neuronal differentiation. Such a regulatory network might ensure the orderly transition from a proliferating and multipotent neural stem/progenitor cell to a postmitotic and differentiating neuron (Fig. 3b; Flynt and Lai 2008; Ebert and Sharp 2012). An additional layer of complexity might be added to this autoregulatory and unilateral negative feedback loop between SOX3/E2F3 and *miR-200* miRNAs because the *pre-miR-200c* stem-loop contains a conserved sequence motif that is bound by the RNA-binding protein and pluripotency factor LIN28 (for a review, see Shyh-Chang and Daley 2013) in murine ESCs, thereby inhibiting its DICER1-mediated processing into the mature *miR-200c* miRNA and targeting it for degradation (Heo et al. 2009). *Lin28* expression in the developing mouse neuroepithelium is highest before the onset of neurogenesis and declines thereafter (Yang and Moss 2003; Yokoyama et al. 2008; Balzer et al. 2010). This suggests that the processing of the *pre-miR-200c* into the mature and functional *miR-200c* miRNA might be suppressed in early SOX2-expressing neuroepithelial cells, thus ensuring their continuous self-renewal and might only become activated once these cells have down-regulated the expression of LIN28 at later developmental stages. These data also suggest that the various *miR-200* family members might be differentially regulated at the post-transcriptional level in this context. Furthermore, the activation of *miR-200* miRNAs and their regulation of SOX2 expression might in turn be controlled by the fibroblast growth factor (FGF)/FGF receptor (FGFR) and Delta/Notch signaling cascades, as suggested from recent work on pharyngeal taste bud development in the zebrafish embryo (Kapsimali et al. 2011). The FGF/FGFR and Delta/Notch signaling pathways play crucial roles during neural development, particularly in the maintenance of the self-renewing neural progenitor state and in the initiation of neural precursor differentiation (for reviews, see Louvi and Artavanis-Tsakonas 2006; Mason 2007).

In search of a common theme: do *miR-8*/*miR-200* miRNAs act as generic regulators of neurogenesis/gliogenesis and the neuroepithelial cell identity?

Despite the limited data concerning the function of *miR-8*/*miR-200* miRNAs during invertebrate and vertebrate neural development and adult homeostasis, some common aspects seem to emerge about the role of this miRNA family in the neurogenic and gliogenic context:

1. Transcription of *Drosophila miR-8* and vertebrate *miR-200* miRNAs appears to be confined to neural cells within the neurogenic niche(s), i.e., either to specialized glial cells (*miR-8* in optic-lobe-associated cortex glia; *miR-200b*/*c* in OPCs) or to neural stem/progenitor cells (*miR-200*; Fig. 3a, b; Choi et al. 2008; Buller et al. 2012; Peng et al. 2012; Morante et al. 2013). With the exception of the olfactory neuroepithelium (Choi et al. 2008), *miR-200* expression seems to be down-regulated as soon as these cells transit to a postmitotic and differentiating (maturing) oligodendrocyte or neuron. Whether *miR-200* members are also expressed in specialized glial cells such as radial glia or astrocytes (for reviews, see Gotz and Huttner 2005; Sild and Ruthazer 2011) in the vertebrate neurogenic niche remains to be determined.
2. Within the neurogenic niche(s), the *miR-8*/*miR-200* family appears to play an important role in the non-cell-autonomous (*miR-8*) or cell-autonomous (*miR-200*) regulation of neurogenesis by controlling the survival, proliferation, cell cycle exit and transition to a more committed neural precursor (neuroblast) state of neural stem/progenitor cells and consequently also their differentiation into mature neurons (Fig. 3a, b; Choi et al. 2008; Peng et al. 2012; Morante et al. 2013), suggesting that these are the principal functions of the *miR-8*/*miR-200* family in neural tissues. Additionally, the *miR-8*/*miR-200* family has been implicated in the regulation of gliogenesis by controlling cell-autonomously the endoreplicative growth of surface glial cells in *Drosophila* (Morante et al. 2013) and the differentiation of OPCs into mature oligodendrocytes (Fig. 3a, b; Buller et al. 2012).
3. Within the neurogenic niche(s), the *miR-8*/*miR-200* miRNAs appear to be generally co-expressed with their mRNA targets, suggesting that this miRNA family acts mostly as a "tuning miRNA" in the context of invertebrate and vertebrate neuro- and gliogenesis. In particular, the vertebrate *miR-200* family might fine-tune the levels of

the corresponding proteins in neural cells, including glial and neural stem/progenitor and precursor cells, with only minor phenotypic consequences in its absence. In support of this view, *mmu-miR-200c/141* knock-out mice are postnatally viable (Park et al. 2012; Cao et al. 2013), precluding the existence of strong neurological defects in these mice. Nevertheless, the overexpression or complete depletion of *Drosophila miR-8* has severe deleterious effects on larval development and adult homeostasis of the mutant flies (Karres et al. 2007; Hyun et al. 2009; Vallejo et al. 2011; Morante et al. 2013), suggesting that the complete ablation of both *mmu-miR-200* gene clusters is required to detect a phenotypic outcome in the mutant mice.

4. The *miR-8*/*miR-200* targets in the context of invertebrate and vertebrate neuro- and gliogenesis appear to be multifaceted (Fig. 3a, b, Table 1): neural stem/progenitor cell proliferation and cell cycle progression are controlled by SOX2, E2F3, or a TGFa-like ligand and the down-regulation of these targets by *miR-8*/*miR-200* members facilitates the cell cycle exit of neural stem/progenitor cells and/or their transition to a more committed neural precursor (neuroblast) state (Peng et al. 2012; Morante et al. 2013). Differentiation of these neural (including glial) precursor cells into mature neurons or oligodendrocytes might also be controlled by the *miR-8*/*miR-200*-mediated down-regulation of the previous factors but is negatively regulated by the *miR-200*-mediated suppression of LFNG, ZEB1 and SRF expression in these cells (Choi et al. 2008; Buller et al. 2012), thus avoiding their premature differentiation and/or delamination from the neurogenic niche. The survival of neural stem/progenitor cells and their progeny are in turn regulated by the *miR-8*/*miR-200* family through a fine-tuning of atrophin/RERE and/or E2F3 protein levels in these cells (Karres et al. 2007; Peng et al. 2012).
5. The *miR-200* family has emerged as a key regulator of the epithelial cell identity and of the process generally known as EMT in other non-neural contexts (for reviews, see Brabletz and Brabletz 2010; Lamouille et al. 2013). In these contexts, *miR-200* miRNAs promote the expression of E-cadherin and suppress the transition to a migratory mesenchymal cell fate by repressing the expression of the ZEB1 and ZEB2 (SIP1) homeobox transcription factors (Lamouille et al. 2013). As previously noted by Morante et al. (2013), the transition from a proliferating neuroepithelial (neural stem/progenitor) cell to a differentiating and migrating neural precursor/neuroblast and/or neuron strongly resembles EMT. The expression of E- and N-cadherin is indeed required in neural stem/progenitor cells to sustain their self-renewal and to inhibit their premature cell cycle exit and differentiation into neurons (Karpowicz et al. 2009; Zhang et al. 2010; for a review, see Chen et al. 2013). On the other hand, ZEB1/2 are widely expressed in neural progenitors of the developing mouse and human embryo and in postmitotic neurons at later developmental stages and in adulthood (Darling et al. 2003; Bassez et al. 2004). ZEB2 (SIP1) has meanwhile been implicated in the regulation of neurogenesis and gliogenesis in various brain regions in vivo (Seuntjens et al. 2009; McKinsey et al. 2013; for a review, see Conidi et al. 2011) and in the transition of differentiating mouse ESCs to a "definitive" neural stem/progenitor cell fate in vitro (Dang et al. 2012). Another, as yet unexplored, function of the *miR-200* miRNAs in neural stem/progenitor cells might therefore be the preservation of their particular neuroepithelial properties within the neurogenic niche(s) and suppression of "EMT" by fine-tuning the ZEB1/2 protein levels in these cells (Fig. 3b). In support of this idea, we detected a slight but highly reproducible midbrain morphogenesis defect, consisting in a flattened ventral midbrain and broadened mediolateral extension of the mesencephalic ventricle, in the $En1^{+/Cre}$; $Dicer1^{flox/flox}$ embryos (Peng et al. 2012); this might also be attributed to the loss of *miR-200* expression in the mutant neuroepithelial tissue.

Future directions

The findings summarized in this review currently suggest a major role of the *miR-8*/*miR-200* family in the cell-autonomous and non-cell-autonomous regulation of neurogenesis and gliogenesis in the developing invertebrate and vertebrate CNS, including the regulation of neurogenic niche-derived signals, niche-contained neurogenic and/or gliogenic processes themselves and niche-intrinsic adhesive properties. However, several questions remain to be answered about the detailed regulatory mechanism and other or later functions of this miRNA family, particularly during vertebrate neural development and homeostasis.

First, the pre- or perinatal lethality of *Foxg1-Cre*; $Dicer^{loxP/loxP}$, *Wnt1–Cre*; $Dicer1^{flox/flox}$ and $En1^{+/Cre}$; $Dicer1^{flox/flox}$ mice and strong disruption of neuroepithelial tissues in these embryos preclude the examination of a later involvement of *miR-200* family members in gliogenic or adult neurogenic processes. Therefore, further analyses with probably more sophisticated tools are required to establish whether this miRNA family is involved in other developmental processes such as gliogenesis or homeostatic processes during adulthood or after brain injury, as suggested by the specific enrichment of *miR-200c* and *miR-141* miRNAs in isolated microglia cells from the rat brain (Jovicic et al. 2013). Moreover, the finding that *miR-8*-expressing optic-lobe-associated cortex glia cells persist in the adult *Drosophila* brain (Morante et al. 2013)

Table 1 Experimentally validated targets of the *miR-8*/*miR-200* miRNA family with an established or putative function during neuro-/gliogenesis and/or neural homeostasis (*BMI1* Bmi1 polycomb ring finger oncogene, *CTNNB1* beta-catenin, *Jag1* jagged 1, *MH* mid-/hindbrain, *NOG* noggin, *PTPN13* protein tyrosine phosphatase non-receptor type 13, *TGFb2* transforming growth factor beta 2)

Target[a]	Targeted by *miR-8*/*miR-200* family member	Species	Tissue/cells[b]	Function[c]	Reference(s)
Atrophin/RERE	*miR-8*, *miR-200b*, *miR-429*	Fruit fly, Human	CNS, S2 cells	Proapoptotic, prosurvival	Karres et al. 2007
BMI1 (Molofsky et al. 2005)	*miR-200c*	Human, Mouse	MMTV-Wnt1 breast and pancreatic cancer cells, HEK-293 T cells, ESCs	Cell proliferation	Shimono et al. 2009; Wellner et al. 2009
CTNNB1 (Ciani and Salinas 2005)	*miR-200a*	Human	Nasopharyngeal carcinoma and meningioma cells	Cell growth, cell migration	Saydam et al. 2009; Xia et al. 2010
E2F3	*miR-200c*	Mouse	CNS, primary ventral MH cells, COS-7 cells	Cell cycle progression, differentiation, survival	Peng et al. 2012
JAG1 (Louvi and Artavanis-Tsakonas 2006)	*miR-200c*, *miR-141*	Human	Metastatic prostate and colon cancer cells	Cell proliferation, survival	Vallejo et al. 2011
LFNG	*miR-200a*, *miR-200b*	Zebrafish	CNS	Cell differentiation	Choi et al. 2008
NOG (Liu and Niswander 2005)	*miR-200c*	Mouse	Dental epithelium, LS-8 oral epithelial-like cells	Cell differentiation, EMT	Cao et al. 2013
PTPN13 (Savaskan et al. 2005)	*mir-200c*	Human	Epithelial and mesenchymal cancer cells, HEK-293 T cells	Proapoptotic	Schickel et al. 2010
SOX2	*miR-200c*	Mouse, Human	CNS, primary ventral MH cells, COS-7 cells, ESCs	Cell cycle progression, differentiation	Wellner et al. 2009; Peng et al. 2012
SRF	*miR-200b*	Rat, Mouse	CNS, primary and immortalized OPCs	Cell differentiation	Buller et al. 2012
TGFa-like (Spitz)	*miR-8*	Fruit fly	CNS, surface glial cells, S2 cells	Cell proliferation, neurogenesis	Morante et al. 2013
TGFb2 (Krieglstein et al. 2011)	*miR-141*	Human	Colorectal cancer cells	EMT	Burk et al. 2008
TUBB3 (Katsetos et al. 2003)	*miR-200c*	Human	Endometrial and ovarian cancer cells	? (tumor progression)	Cochrane et al. 2009
ZEB1	All	Zebrafish, Human, Mouse	CNS, colorectal, epithelial and mesenchymal cancer cells, mammary carcinoma cells, MDCK, HeLa, HEK-293 T and NIH-3 T3 cells, ESCs	EMT, cell differentiation	Burk et al. 2008; Choi et al. 2008; Gregory et al. 2008; Korpal et al. 2008; Park et al. 2008; Wang et al. 2013
ZEB2 (SIP1) (Conidi et al. 2011)	All	Human, Mouse	Epithelial and mesenchymal cancer cells, mammary carcinoma cells, MDCK, HeLa, HEK-293 T and and NIH-3 T3 cells, ESCs	EMT	Gregory et al. 2008; Korpal et al. 2008; Park et al. 2008; Wang et al. 2013

[a] Putative targets of the *miR-8*/*miR-200* family in the context of neural development and homeostasis (*bold face*) are those mRNAs/proteins with a known expression and/or function in neural tissues (references are given *in parenthesis*) but for which such an interaction with *miR-8*/*miR-200* family members has not yet been demonstrated in neural tissues.

[b] "Tissue/cells" refers to the tissue or cell type in which this miRNA-mRNA interaction has been demonstrated according to the reference(s) listed *right*.

[c] Regulatory function of the *miR-8*/*miR-200* family member(s) in the reported contexts. Listed are only those targets for which a direct interaction of mRNA-binding sites (BSs), usually located within the 3′ UTR, with the corresponding miRNA has been validated experimentally by using the luciferase reporter ("sensor") assays and site-directed mutagenesis or target protection of the BS(s).

suggests a possible role for the *mir-8*/*miR-200* family during adult neurogenesis; this has not been explored so far.

Second, the overt phenotype of *Dicer1* mutant mice and MZ*dicer*$^{+miR430}$ zebrafish mutants is caused by the depletion of many, if not all, mature miRNAs and not just one miRNA (family) present in that particular organism or tissue at the relevant stages. The *miR-8*/*miR-200* family thus probably cooperates with other miRNAs or miRNA families in the regulation of invertebrate and vertebrate neuro- and gliogenesis. One prospective "cooperation partner" for the *miR-8*/*miR-200* family in this context is *miR-9*, a miRNA that has similar functions in the regulation of neural progenitor proliferation and facilitation of the transition to a more committed neural precursor state (for a review, see Coolen et al. 2013). Indeed, two experimentally validated targets (*Srf*; Buller et al. 2012; *Zeb2* Gregory et al. 2008; Korpal et al. 2008; Park et al. 2008; Wang et al. 2013) and one putative target (the forkhead box G1 transcription factor *Foxg1*; Choi et al. 2008) of the *miR-200* family are also targeted by *miR-9* (Fig. 3b; Shibata et al. 2008; Buller et al. 2012; Kropivsek et al. 2014). The extent of unique versus redundant (overlapping with other miRNAs) functions of the *miR-8*/*miR-200* family during invertebrate and vertebrate neuro- and gliogenesis therefore still remains to be determined.

Third, a substantial amount of work is still required to unravel the identity of all potential targets of the *miR-8*/*miR-200* family in neural tissues. Several other targets of this miRNA family have been experimentally validated in non-neural contexts (listed in Table 1), including secreted members of the TGFb and bone morphogenetic protein (BMP) signaling pathways and ligands for the Notch receptor (Burk et al. 2008; Vallejo et al. 2011; Cao et al. 2013). These proteins play crucial roles during neural development and homeostasis (for reviews, see Louvi and Artavanis-Tsakonas 2006; Krieglstein et al. 2011) and suggest a non-cell-autonomous function of *miR-200* miRNAs in the vertebrate neurogenic niche but have not been explored as *miR-8*/*miR-200* targets in this context.

Fourth, the potential low stability and oscillatory expression of mature *miR-200* miRNAs in conjunction with their targets and, at the same time, transcriptional activators SOX2 and E2F3 still has to be experimentally established in (MHR) neural tissues. Because of the rather low levels of mature *miR-200* miRNAs in these tissues, more sophisticated tools need to be generated for the in situ detection of these miRNAs and their precursors and of their mRNA targets, including destabilized reporter constructs and/or transgenic mice.

Lastly, a central and universal function of the *miR-8*/*miR-200* family might be the generic instruction of an epithelial (including neuroepithelial) cell fate in all types of somatic (differentiated) and pluripotent (self-renewing) cells and the preservation of the epithelial integrity in these tissues. Although current evidence hints at such a global function of this miRNA family in vertebrates and potentially also invertebrates (see point 5 in "In search of a common theme"), the involvement of *miR-8*/*miR-200* miRNAs in the regulation of neuroepithelial cell identity (e.g., by promoting E-cadherin expression) and in the suppression of EMT in these cells (e.g., by targeting the ZEB1/2 transcription factors) awaits to be determined in the context of neural development and homeostasis in vivo. Notably, in vitro experiments suggest a generic non-neural-specific function of the *miR-200* miRNAs in this regard: *miR-200*-mediated repression of ZEB1/2 expression in differentiating human ESCs promotes mesendodermal or epidermal cell fates and suppresses neural induction in these cells (Du et al. 2013; Liao et al. 2013), whereas *miR-200*-mediated repression of ZEB1/2 promotes the mesenchymal-to-epithelial conversion (reprogramming) of somatic cells to pluripotent stem cells (Wang et al. 2013).

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References

Abe M, Bonini NM (2013) MicroRNAs and neurodegeneration: role and impact. Trends Cell Biol 23:30–36

Adams MR, Sears R, Nuckolls F, Leone G, Nevins JR (2000) Complex transcriptional regulatory mechanisms control expression of the E2F3 locus. Mol Cell Biol 20:3633–3639

Ahn SM, Cha JY, Kim J, Kim D, Trang HT, Kim YM, Cho YH, Park D, Hong S (2012) Smad3 regulates E-cadherin via miRNA-200 pathway. Oncogene 31:3051–3059

Balzer E, Heine C, Jiang Q, Lee VM, Moss EG (2010) LIN28 alters cell fate succession and acts independently of the let-7 microRNA during neurogliogenesis in vitro. Development 137:891–900

Bassez G, Camand OJ, Cacheux V, Kobetz A, Dastot-Le Moal F, Marchant D, Catala M, Abitbol M, Goossens M (2004) Pleiotropic and diverse expression of ZFHX1B gene transcripts during mouse and human development supports the various clinical manifestations of the "Mowat-Wilson" syndrome. Neurobiol Dis 15:240–250

Betel D, Wilson M, Gabow A, Marks DS, Sander C (2008) The microRNA.org resource: targets and expression. Nucleic Acids Res 36:D149–D153

Bonev B, Stanley P, Papalopulu N (2012) MicroRNA-9 modulates Hes1 ultradian oscillations by forming a double-negative feedback loop. Cell Rep 2:10–18

Brabletz S, Brabletz T (2010) The ZEB/miR-200 feedback loop—a motor of cellular plasticity in development and cancer? EMBO Rep 11:670–677

Brabletz S, Bajdak K, Meidhof S, Burk U, Niedermann G, Firat E, Wellner U, Dimmler A, Faller G, Schubert J et al (2011) The ZEB1/miR-200 feedback loop controls Notch signalling in cancer cells. EMBO J 30:770–782

Bracken CP, Gregory PA, Kolesnikoff N, Bert AG, Wang J, Shannon MF, Goodall GJ (2008) A double-negative feedback loop between ZEB1-SIP1 and the microRNA-200 family regulates epithelial-mesenchymal transition. Cancer Res 68:7846–7854

Buller B, Chopp M, Ueno Y, Zhang L, Zhang RL, Morris D, Zhang Y, Zhang ZG (2012) Regulation of serum response factor by miRNA-

200 and miRNA-9 modulates oligodendrocyte progenitor cell differentiation. Glia 60:1906–1914
Burk U, Schubert J, Wellner U, Schmalhofer O, Vincan E, Spaderna S, Brabletz T (2008) A reciprocal repression between ZEB1 and members of the miR-200 family promotes EMT and invasion in cancer cells. EMBO Rep 9:582–589
Cao H, Jheon A, Li X, Sun Z, Wang J, Florez S, Zhang Z, McManus MT, Klein OD, Amendt BA (2013) The Pitx2:miR-200c/141:noggin pathway regulates Bmp signaling and ameloblast differentiation. Development 140:3348–3359
Cavallaro M, Mariani J, Lancini C, Latorre E, Caccia R, Gullo F, Valotta M, DeBiasi S, Spinardi L, Ronchi A et al (2008) Impaired generation of mature neurons by neural stem cells from hypomorphic Sox2 mutants. Development 135:541–557
Chen D, Pacal M, Wenzel P, Knoepfler PS, Leone G, Bremner R (2009a) Division and apoptosis of E2f-deficient retinal progenitors. Nature 462:925–929
Chen HZ, Tsai SY, Leone G (2009b) Emerging roles of E2Fs in cancer: an exit from cell cycle control. Nat Rev Cancer 9:785–797
Chen J, Wang G, Lu C, Guo X, Hong W, Kang J, Wang J (2012) Synergetic cooperation of microRNAs with transcription factors in iPS cell generation. PLoS One 7:e40849
Chen S, Lewallen M, Xie T (2013) Adhesion in the stem cell niche: biological roles and regulation. Development 140:255–265
Choi PS, Zakhary L, Choi WY, Caron S, Alvarez-Saavedra E, Miska EA, McManus M, Harfe B, Giraldez AJ, Horvitz HR et al (2008) Members of the miRNA-200 family regulate olfactory neurogenesis. Neuron 57:41–55
Chong JL, Wenzel PL, Saenz-Robles MT, Nair V, Ferrey A, Hagan JP, Gomez YM, Sharma N, Chen HZ, Ouseph M et al (2009) E2f1-3 switch from activators in progenitor cells to repressors in differentiating cells. Nature 462:930–934
Ciani L, Salinas PC (2005) WNTs in the vertebrate nervous system: from patterning to neuronal connectivity. Nat Rev Neurosci 6:351–362
Cochrane DR, Spoelstra NS, Howe EN, Nordeen SK, Richer JK (2009) MicroRNA-200c mitigates invasiveness and restores sensitivity to microtubule-targeting chemotherapeutic agents. Mol Cancer Ther 8: 1055–1066
Conidi A, Cazzola S, Beets K, Coddens K, Collart C, Cornelis F, Cox L, Joke D, Dobreva MP, Dries R et al (2011) Few Smad proteins and many Smad-interacting proteins yield multiple functions and action modes in TGFbeta/BMP signaling in vivo. Cytokine Growth Factor Rev 22:287–300
Coolen M, Bally-Cuif L (2009) MicroRNAs in brain development and physiology. Curr Opin Neurobiol 19:461–470
Coolen M, Katz S, Bally-Cuif L (2013) miR-9: a versatile regulator of neurogenesis. Front Cell Neurosci 7:220
Danesin C, Houart C (2012) A Fox stops the Wnt: implications for forebrain development and diseases. Curr Opin Genet Dev 22: 323–330
Dang LT, Wong L, Tropepe V (2012) Zfhx1b induces a definitive neural stem cell fate in mouse embryonic stem cells. Stem Cells Dev 21: 2838–2851
Danielian PS, Friesenhahn LB, Faust AM, West JC, Caron AM, Bronson RT, Lees JA (2008) E2f3a and E2f3b make overlapping but different contributions to total E2f3 activity. Oncogene 27:6561–6570
Darling DS, Stearman RP, Qi Y, Qiu MS, Feller JP (2003) Expression of Zfhep/deltaEF1 protein in palate, neural progenitors, and differentiated neurons. Gene Expr Patterns 3:709–717
DeGregori J (2002) The genetics of the E2F family of transcription factors: shared functions and unique roles. Biochim Biophys Acta 1602:131–150
Du ZW, Ma LX, Phillips C, Zhang SC (2013) miR-200 and miR-96 families repress neural induction from human embryonic stem cells. Development 140:2611–2618
Ebert MS, Sharp PA (2010) MicroRNA sponges: progress and possibilities. RNA 16:2043–2050
Ebert MS, Sharp PA (2012) Roles for microRNAs in conferring robustness to biological processes. Cell 149:515–524
Ellis P, Fagan BM, Magness ST, Hutton S, Taranova O, Hayashi S, McMahon A, Rao M, Pevny L (2004) SOX2, a persistent marker for multipotential neural stem cells derived from embryonic stem cells, the embryo or the adult. Dev Neurosci 26:148–165
Esteller M (2011) Non-coding RNAs in human disease. Nat Rev Genet 12:861–874
Feng X, Wang Z, Fillmore R, Xi Y (2014) MiR-200, a new star miRNA in human cancer. Cancer Lett 344:166–173
Flicek P, Ahmed I, Amode MR, Barrell D, Beal K, Brent S, Carvalho-Silva D, Clapham P, Coates G, Fairley S et al (2013) Ensembl 2013. Nucleic Acids Res 41:D48–D55
Flynt AS, Lai EC (2008) Biological principles of microRNA-mediated regulation: shared themes amid diversity. Nat Rev Genet 9:831–842
Friedman RC, Farh KK, Burge CB, Bartel DP (2009) Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 19:92–105
Gill JG, Langer EM, Lindsley RC, Cai M, Murphy TL, Kyba M, Murphy KM (2011) Snail and the microRNA-200 family act in opposition to regulate epithelial-to-mesenchymal transition and germ layer fate restriction in differentiating ESCs. Stem Cells 29:764–776
Gotz M, Huttner WB (2005) The cell biology of neurogenesis. Nat Rev Mol Cell Biol 6:777–788
Graham V, Khudyakov J, Ellis P, Pevny L (2003) SOX2 functions to maintain neural progenitor identity. Neuron 39:749–765
Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, Vadas MA, Khew-Goodall Y, Goodall GJ (2008) The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol 10:593–601
Han MV, Zmasek CM (2009) phyloXML: XML for evolutionary biology and comparative genomics. BMC Bioinformatics 10:356
Heo I, Joo C, Kim YK, Ha M, Yoon MJ, Cho J, Yeom KH, Han J, Kim VN (2009) TUT4 in concert with Lin28 suppresses microRNA biogenesis through pre-microRNA uridylation. Cell 138:696–708
Hill L, Browne G, Tulchinsky E (2013) ZEB/miR-200 feedback loop: at the crossroads of signal transduction in cancer. Int J Cancer 132: 745–754
Huang T, Liu Y, Huang M, Zhao X, Cheng L (2010) Wnt1-cre-mediated conditional loss of Dicer results in malformation of the midbrain and cerebellum and failure of neural crest and dopaminergic differentiation in mice. J Mol Cell Biol 2:152–163
Huang HN, Chen SY, Hwang SM, Yu CC, Su MW, Mai W, Wang HW, Cheng WC, Schuyler SC, Ma N et al (2013) miR-200c and GATA binding protein 4 regulate human embryonic stem cell renewal and differentiation. Stem Cell Res 12:338–353
Humbert PO, Verona R, Trimarchi JM, Rogers C, Dandapani S, Lees JA (2000) E2f3 is critical for normal cellular proliferation. Genes Dev 14:690–703
Huntzinger E, Izaurralde E (2011) Gene silencing by microRNAs: contributions of translational repression and mRNA decay. Nat Rev Genet 12:99–110
Hyun S, Lee JH, Jin H, Nam J, Namkoong B, Lee G, Chung J, Kim VN (2009) Conserved MicroRNA miR-8/miR-200 and its target USH/FOG2 control growth by regulating PI3K. Cell 139:1096–1108
Im HI, Kenny PJ (2012) MicroRNAs in neuronal function and dysfunction. Trends Neurosci 35:325–334
Ivey KN, Srivastava D (2010) MicroRNAs as regulators of differentiation and cell fate decisions. Cell Stem Cell 7:36–41
Johnson DG, Schwarz JK, Cress WD, Nevins JR (1993) Expression of transcription factor E2F1 induces quiescent cells to enter S phase. Nature 365:349–352
Jovicic A, Roshan R, Moisoi N, Pradervand S, Moser R, Pillai B, Luthi-Carter R (2013) Comprehensive expression analyses of neural cell-

type-specific miRNAs identify new determinants of the specification and maintenance of neuronal phenotypes. J Neurosci 33:5127–5137

Kamachi Y, Kondoh H (2013) Sox proteins: regulators of cell fate specification and differentiation. Development 140:4129–4144

Kapsimali M, Kaushik AL, Gibon G, Dirian L, Ernest S, Rosa FM (2011) Fgf signaling controls pharyngeal taste bud formation through miR-200 and Delta-Notch activity. Development 138:3473–3484

Karpowicz P, Willaime-Morawek S, Balenci L, DeVeale B, Inoue T, Kooy D van der (2009) E-Cadherin regulates neural stem cell self-renewal. J Neurosci 29:3885–3896

Karres JS, Hilgers V, Carrera I, Treisman J, Cohen SM (2007) The conserved microRNA miR-8 tunes atrophin levels to prevent neurodegeneration in *Drosophila*. Cell 131:136–145

Kashyap V, Rezende NC, Scotland KB, Shaffer SM, Persson JL, Gudas LJ, Mongan NP (2009) Regulation of stem cell pluripotency and differentiation involves a mutual regulatory circuit of the NANOG, OCT4, and SOX2 pluripotency transcription factors with polycomb repressive complexes and stem cell microRNAs. Stem Cells Dev 18: 1093–1108

Katsetos CD, Herman MM, Mork SJ (2003) Class III beta-tubulin in human development and cancer. Cell Motil Cytoskeleton 55:77–96

Kennell JA, Gerin I, MacDougald OA, Cadigan KM (2008) The microRNA miR-8 is a conserved negative regulator of Wnt signaling. Proc Natl Acad Sci U S A 105:15417–15422

Kim T, Veronese A, Pichiorri F, Lee TJ, Jeon YJ, Volinia S, Pineau P, Marchio A, Palatini J, Suh SS et al (2011) p53 regulates epithelial-mesenchymal transition through microRNAs targeting ZEB1 and ZEB2. J Exp Med 208:875–883

Kim D, Grun D, Oudenaarden A van (2013) Dampening of expression oscillations by synchronous regulation of a microRNA and its target. Nat Genet 45:1337–1344

Knoll B, Nordheim A (2009) Functional versatility of transcription factors in the nervous system: the SRF paradigm. Trends Neurosci 32: 432–442

Kondoh H, Kamachi Y (2010) SOX-partner code for cell specification: regulatory target selection and underlying molecular mechanisms. Int J Biochem Cell Biol 42:391–399

Korpal M, Lee ES, Hu G, Kang Y (2008) The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. J Biol Chem 283:14910–14914

Kozomara A, Griffiths-Jones S (2014) miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res 42:D68–D73

Krieglstein K, Zheng F, Unsicker K, Alzheimer C (2011) More than being protective: functional roles for TGF-beta/activin signaling pathways at central synapses. Trends Neurosci 34:421–429

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

Kropivsek K, Pickford J, Carter DA (2014) Postnatal dynamics of Zeb2 expression in rat brain: analysis of novel 3′ UTR sequence reveals a miR-9 interacting site. J Mol Neurosci 52:138–147

Lamouille S, Subramanyam D, Blelloch R, Derynck R (2013) Regulation of epithelial-mesenchymal and mesenchymal-epithelial transitions by microRNAs. Curr Opin Cell Biol 25:200–207

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R et al (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948

Leone G, DeGregori J, Yan Z, Jakoi L, Ishida S, Williams RS, Nevins JR (1998) E2F3 activity is regulated during the cell cycle and is required for the induction of S phase. Genes Dev 12:2120–2130

Liao X, Xue H, Wang YC, Nazor KL, Guo S, Trivedi N, Peterson SE, Liu Y, Loring JF, Laurent LC (2013) Matched miRNA and mRNA signatures from an hESC-based in vitro model of pancreatic differentiation reveal novel regulatory interactions. J Cell Sci 126:3848–3861

Lin CH, Jackson AL, Guo J, Linsley PS, Eisenman RN (2009) Myc-regulated microRNAs attenuate embryonic stem cell differentiation. EMBO J 28:3157–3170

Liu A, Niswander LA (2005) Bone morphogenetic protein signalling and vertebrate nervous system development. Nat Rev Neurosci 6:945–954

Louvi A, Artavanis-Tsakonas S (2006) Notch signalling in vertebrate neural development. Nat Rev Neurosci 7:93–102

Mason I (2007) Initiation to end point: the multiple roles of fibroblast growth factors in neural development. Nat Rev Neurosci 8:583–596

McKinsey GL, Lindtner S, Trzcinski B, Visel A, Pennacchio LA, Huylebroeck D, Higashi Y, Rubenstein JL (2013) Dlx1&2-dependent expression of Zfhx1b (Sip1, Zeb2) regulates the fate switch between cortical and striatal interneurons. Neuron 77:83–98

McNeill E, Van Vactor D (2012) MicroRNAs shape the neuronal landscape. Neuron 75:363–379

Mizuguchi Y, Specht S, Lunz JG 3rd, Isse K, Corbitt N, Takizawa T, Demetris AJ (2012) Cooperation of p300 and PCAF in the control of microRNA 200c/141 transcription and epithelial characteristics. PLoS One 7:e32449

Molofsky AV, He S, Bydon M, Morrison SJ, Pardal R (2005) Bmi-1 promotes neural stem cell self-renewal and neural development but not mouse growth and survival by repressing the p16Ink4a and p19Arf senescence pathways. Genes Dev 19:1432–1437

Morante J, Vallejo DM, Desplan C, Dominguez M (2013) Conserved miR-8/miR-200 defines a glial niche that controls neuroepithelial expansion and neuroblast transition. Dev Cell 27:174–187

Moss EG (2007) Heterochronic genes and the nature of developmental time. Curr Biol 17:R425–R434

Mukherji S, Ebert MS, Zheng GX, Tsang JS, Sharp PA, Oudenaarden A van (2011) MicroRNAs can generate thresholds in target gene expression. Nat Genet 43:854–859

Park CY, Jeker LT, Carver-Moore K, Oh A, Liu HJ, Cameron R, Richards H, Li Z, Adler D, Yoshinaga Y et al (2012) A resource for the conditional ablation of microRNAs in the mouse. Cell Rep 1:385–391

Park SM, Gaur AB, Lengyel E, Peter ME (2008) The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. Genes Dev 22:894–907

Pasquinelli AE (2012) MicroRNAs and their targets: recognition, regulation and an emerging reciprocal relationship. Nat Rev Genet 13: 271–282

Pauli A, Rinn JL, Schier AF (2011) Non-coding RNAs as regulators of embryogenesis. Nat Rev Genet 12:136–149

Peng C, Li N, Ng YK, Zhang J, Meier F, Theis FJ, Merkenschlager M, Chen W, Wurst W, Prakash N (2012) A unilateral negative feedback loop between miR-200 microRNAs and Sox2/E2F3 controls neural progenitor cell-cycle exit and differentiation. J Neurosci 32:13292–13308

Peter ME (2009) Let-7 and miR-200 microRNAs: guardians against pluripotency and cancer progression. Cell Cycle 8:843–852

Pevny LH, Nicolis SK (2010) Sox2 roles in neural stem cells. Int J Biochem Cell Biol 42:421–424

Qureshi IA, Mehler MF (2012) Emerging roles of non-coding RNAs in brain evolution, development, plasticity and disease. Nat Rev Neurosci 13:528–541

Rebustini IT, Hayashi T, Reynolds AD, Dillard ML, Carpenter EM, Hoffman MP (2012) miR-200c regulates FGFR-dependent epithelial proliferation via Vldlr during submandibular gland branching morphogenesis. Development 139:191–202

Savaskan E, Ravid R, Meier F, Muller-Spahn F, Jockers R (2005) Immunohistochemical localization of Fas-associated phosphatase-1 (FAP-1) in Alzheimer disease hippocampus. Appl Immunohistochem Mol Morphol 13:190–193

Saydam O, Shen Y, Wurdinger T, Senol O, Boke E, James MF, Tannous BA, Stemmer-Rachamimov AO, Yi M, Stephens RM et al (2009) Down regulated microRNA-200a in meningiomas promotes tumor growth by reducing E-cadherin and activating the Wnt/beta-catenin signaling pathway. Mol Cell Biol 29:5923–5940

Schickel R, Park SM, Murmann AE, Peter ME (2010) miR-200c regulates induction of apoptosis through CD95 by targeting FAP-1. Mol Cell 38:908–915

Seuntjens E, Nityanandam A, Miquelajauregui A, Debruyn J, Stryjewska A, Goebbels S, Nave KA, Huylebroeck D, Tarabykin V (2009) Sip1 regulates sequential fate decisions by feedback signaling from postmitotic neurons to progenitors. Nat Neurosci 12:1373–1380

Shi Y, Sun G, Zhao C, Stewart R (2008) Neural stem cell self-renewal. Crit Rev Oncol Hematol 65:43–53

Shibata M, Kurokawa D, Nakao H, Ohmura T, Aizawa S (2008) MicroRNA-9 modulates Cajal-Retzius cell differentiation by suppressing Foxg1 expression in mouse medial pallium. J Neurosci 28: 10415–10421

Shimono Y, Zabala M, Cho RW, Lobo N, Dalerba P, Qian D, Diehn M, Liu H, Panula SP, Chiao E et al (2009) Down regulation of miRNA-200c links breast cancer stem cells with normal stem cells. Cell 138: 592–603

Shyh-Chang N, Daley GQ (2013) Lin28: primal regulator of growth and metabolism in stem cells. Cell Stem Cell 12:395–406

Sild M, Ruthazer ES (2011) Radial glia: progenitor, pathway, and partner. Neuroscientist 17:288–302

Taranova OV, Magness ST, Fagan BM, Wu Y, Surzenko N, Hutton SR, Pevny LH (2006) SOX2 is a dose-dependent regulator of retinal neural progenitor competence. Genes Dev 20:1187–1202

Tomioka M, Nishimoto M, Miyagi S, Katayanagi T, Fukui N, Niwa H, Muramatsu M, Okuda A (2002) Identification of Sox-2 regulatory region which is under the control of Oct-3/4-Sox-2 complex. Nucleic Acids Res 30:3202–3213

Tsai SY, Opavsky R, Sharma N, Wu L, Naidu S, Nolan E, Feria-Arias E, Timmers C, Opavska J, Bruin A de et al (2008) Mouse development with a single E2F activator. Nature 454:1137–1141

Uhlmann S, Zhang JD, Schwager A, Mannsperger H, Riazalhosseini Y, Burmester S, Ward A, Korf U, Wiemann S, Sahin O (2010) miR-200bc/429 cluster targets PLCgamma1 and differentially regulates proliferation and EGF-driven invasion than miR-200a/141 in breast cancer. Oncogene 29:4297–4306

Vallejo DM, Caparros E, Dominguez M (2011) Targeting Notch signalling by the conserved miR-8/200 microRNA family in development and cancer cells. EMBO J 30:756–769

Vejnar CE, Zdobnov EM (2012) MiRmap: comprehensive prediction of microRNA target repression strength. Nucleic Acids Res 40:11673–11683

Vieira C, Pombero A, Garcia-Lopez R, Gimeno L, Echevarria D, Martinez S (2010) Molecular mechanisms controlling brain development: an overview of neuroepithelial secondary organizers. Int J Dev Biol 54:7–20

Wang G, Guo X, Hong W, Liu Q, Wei T, Lu C, Gao L, Ye D, Zhou Y, Chen J et al (2013) Critical regulation of miR-200/ZEB2 pathway in Oct4/Sox2-induced mesenchymal-to-epithelial transition and induced pluripotent stem cell generation. Proc Natl Acad Sci U S A 110:2858–2863

Wellner U, Schubert J, Burk UC, Schmalhofer O, Zhu F, Sonntag A, Waldvogel B, Vannier C, Darling D, Hausen A zur et al (2009) The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. Nat Cell Biol 11:1487–1495

Wheeler BM, Heimberg AM, Moy VN, Sperling EA, Holstein TW, Heber S, Peterson KJ (2009) The deep evolution of metazoan microRNAs. Evol Dev 11:50–68

Wurst W, Bally-Cuif L (2001) Neural plate patterning: upstream and downstream of the isthmic organizer. Nat Rev Neurosci 2:99–108

Xia H, Ng SS, Jiang S, Cheung WK, Sze J, Bian XW, Kung HF, Lin MC (2010) miR-200a-mediated down regulation of ZEB2 and CTNNB1 differentially inhibits nasopharyngeal carcinoma cell growth, migration and invasion. Biochem Biophys Res Commun 391:535–541

Yang DH, Moss EG (2003) Temporally regulated expression of Lin-28 in diverse tissues of the developing mouse. Gene Expr Patterns 3:719–726

Yang JS, Lai EC (2011) Alternative miRNA biogenesis pathways and the interpretation of core miRNA pathway mutants. Mol Cell 43:892–903

Yates LA, Norbury CJ, Gilbert RJ (2013) The long and short of microRNA. Cell 153:516–519

Yokoyama S, Hashimoto M, Shimizu H, Ueno-Kudoh H, Uchibe K, Kimura I, Asahara H (2008) Dynamic gene expression of Lin-28 during embryonic development in mouse and chicken. Gene Expr Patterns 8:155–160

Zervas M, Blaess S, Joyner AL (2005) Classical embryological studies and modern genetic analysis of midbrain and cerebellum development. Curr Top Dev Biol 69:101–138

Zhang J, Woodhead GJ, Swaminathan SK, Noles SR, McQuinn ER, Pisarek AJ, Stocker AM, Mutch CA, Funatsu N, Chenn A (2010) Cortical neural precursors inhibit their own differentiation via N-cadherin maintenance of beta-catenin signaling. Dev Cell 18:472–479