Species-Specific miRNAs in Human Brain Development and Disease

Kanella Prodromidou* and Rebecca Matsas

Laboratory of Cellular and Molecular Neurobiology—Stem Cells, Department of Neurobiology, Hellenic Pasteur Institute, Athens, Greece

Identification of the unique features of human brain development and function can be critical towards the elucidation of intricate processes such as higher cognitive functions and human-specific pathologies like neuropsychiatric and behavioral disorders. The developing primate and human central nervous system (CNS) are distinguished by expanded progenitor zones and a protracted time course of neurogenesis, leading to the expansion in brain size, prominent gyral anatomy, distinctive synaptic properties, and complex neural circuits. Comparative genomic studies have revealed that adaptations of brain capacities may be partly explained by human-specific genetic changes that impact the function of proteins associated with neocortical expansion, synaptic function, and language development. However, the formation of complex gene networks may be most relevant for brain evolution. Indeed, recent studies identified distinct human-specific gene expression patterns across developmental time occurring in brain regions linked to cognition. Interestingly, such modules show species-specific divergence and are enriched in genes associated with neuronal development and synapse formation whilst also being implicated in neuropsychiatric diseases.

microRNAs represent a powerful component of gene-regulatory networks by promoting spatiotemporal post-transcriptional control of gene expression in the human and primate brain. It has also been suggested that the divergence in miRNA expression plays an important role in shaping gene expression divergence among species. Primate-specific and human-specific miRNAs are principally involved in progenitor proliferation and neurogenic processes but also associate with human cognition, and neurological disorders. Human embryonic or induced pluripotent stem cells and brain organoids, permitting experimental access to neural cells and differentiation stages that are otherwise difficult or impossible to reach in humans, are an essential means for studying species-specific brain miRNAs. Single-cell sequencing approaches can further decode refined miRNA-mRNA interactions during developmental transitions. Elucidating species-specific miRNA regulation will shed new light into the mechanisms that control spatiotemporal events during human brain development and disease, an important step towards fostering novel, holistic and effective therapeutic approaches for neural disorders. In this review, we discuss species-specific regulation of miRNA function, its contribution to the evolving features of the human brain and in neurological disease, with respect also to future therapeutic approaches.

Keywords: RNA sequencing (RNAseq), primate, neurogenesis, evolution, gene networks, brain, miRNAs, human
INTRODUCTION

Human brain development presents unique features and underlies the intricately coordinated spatiotemporal expression of thousands of genes. This elaborate mechanism entails the timely acquisition of diverse cellular identities further orchestrating regional specialization and inter-connectivity in the brain. microRNAs (miRNAs) are powerful post-transcriptional regulators, increasingly recognized as important components of fundamental neurodevelopmental processes and related disorders (Adlakha and Saini, 2014). miRNAs act to repress the translation or degrade their mRNA targets to modulate and fine-tune gene expression levels. One of the early roles ascribed to miRNAs was their contribution to developmental transitions by suppressing transcripts associated with the previous stage. An alternative, but complementary hypothesis suggests that miRNAs act reduce the variance in the expression level of their target genes, conferring increased robustness to signaling decisions during development (Hornstein and Shomron, 2006; Ebert and Sharp, 2012). Importantly, it is appreciated that miRNA-driven regulation contributed critically to gene expression changes on the human evolutionary lineage affecting genes involved in progenitor proliferation and neuronal generation and function (Nowakowski et al., 2013; Arcila et al., 2014). miRNA-mediated regulation in the developing brain presents primate distinct aspects, including over 100 primate-specific and 14 human-specific miRNAs that have been identified (Berezikov, 2011; Hu et al., 2011).

In this review we present current data on species-specific miRNA regulation and discuss miRNAs as hubs of critical brain transcriptional processes during human neural development. Finally, we stress the requirement to delineate the functional significance of miRNA-driven transcriptome changes at the single-cell level, as an important step towards resolving the complex regulatory network operating during human neurogenesis. Towards this direction, brain organoids and application of single-cell sequencing methodologies constitute invaluable tools to address causality between the emergence of novel miRNAs and rewiring of transcriptional programs during the evolution of brain complexity.

DISTINCT FEATURES OF HUMAN BRAIN DEVELOPMENT

Although brain development follows the same principles across mammals, the primate and human central nervous system (CNS) is distinguished by highly derived features. These include expanded progenitor zones (Smart et al., 2002) accompanied by enhanced and tightly controlled proliferative and/or neurogenic potential of progenitor cells (Otani et al., 2016; Sousa et al., 2017a,b), further associated with molecular changes and an increased diversity of neural cell types (Bystron et al., 2006; Lui et al., 2011; Gulden and Şestan, 2014; Taverna et al., 2014; Bae et al., 2015; Dehay et al., 2015). Human brain development is characterized by a relatively protracted time course of neurogenesis, followed by an extraordinary numeric expansion of the neuronal cell population (Rockel et al., 1980; Hutslер et al., 2005; Marin-Padilla, 2014; Otani et al., 2016) and the emergence of sophisticated neural circuits of connectivity reflected in prominent changes in gyral anatomy (Rogers et al., 2010; Hofman, 2012; Figure 1).

Brain evolution produced changes in morphology, abundance, and function of cell types. For example, differences between rodent and human neuronal features include distinctive membrane (Wang et al., 2015; Eyal et al., 2016) and synaptic properties (Molnár et al., 2008, 2016; Testa-Silva et al., 2010; Verhoog et al., 2013; Szegedi et al., 2016). Deviations between humans and non-human primates (NHPs) have also been reported regarding the morphology and number of glial cells in the brain (Oberheim et al., 2012; Bianchi et al., 2013; Herculano-Houzel et al., 2016). In addition, excitatory projection neurons in humans show more elaborate dendritic arborization, and contain a greater number and density of spines compared to non-human primates (NHPs; Duan et al., 2003; Elston et al., 2011). Along this line, a subgroup of modified pyramidal neurons, known as spindle or von Economo neurons, mainly found in the fronto-insular and anterior cingulate cortex, are larger and more numerous in humans than in other apes (Allman et al., 2010). Although their function remains elusive, they have been implicated in brain disorders with social-emotional deficits, such as autism spectrum disorders (ASDs), schizophrenia (SCZ), frontotemporal dementia and Alzheimer’s disease (AD; Yang et al., 2019). Evidence also exists for species-specific differences in serotonergic transmission, dopamine innervation and the regional localization and abundance of certain subclasses of inhibitory neurons and their axonal projections in the neocortex of humans and NHPs (Sherwood et al., 2004; Raghanti et al., 2008, 2016). Similarly, a specialized GABAergic neuron subtype has recently been identified with a molecular and anatomical signature specific to humans (Boldog et al., 2018).

GENE NETWORKS ASSOCIATED WITH PRIMATE- AND HUMAN-SPECIFIC BRAIN ADAPTATIONS

Comparative genomic studies revealed that adapted brain specializations and capacities may be partly explained by human-specific gene conversions that impact the function of proteins associated with neocortical expansion, synaptic function, and language development. Characteristic examples include genes that have undergone human-specific duplication like the cortical development gene Slit-Robo Rho GTPase activating protein 2 (SRGAP2) which induces branching of neurons and neurite outgrowth (Dennis et al., 2012). Another case is ARHGAP11 which is expressed in basal progenitors and promotes neocortical expansion by increasing neuron numbers and brain folding (Florio et al., 2015). FOXP2 is also a gene that has undergone surprisingly rapid evolution in the primate lineage leading to humans and encodes a homeodomain protein essential for normal human speech (Lai et al., 2001). Similarly, ASPM (Gai et al., 2016), Microcephalin (Evans et al., 2004) and AHI1 genes (Ferland et al., 2014) that have undergone "positive
selection,” encode for human proteins that associate with normal cerebral cortical size and axon guidance.

Importantly, gene co-expression analyses have revealed that transcriptional regulation and complexity in the neocortex have dramatically increased on the human lineage (Konopka et al., 2012; Silbereis et al., 2016). The allometric expansion of the brain is primarily accompanied by changes in patterns of gene networks and neuronal activity leading to the structural reorganization of the connectome and possibly harboring new behavioral and cognitive phenotypes (Buckner and Krienen, 2013). In this regard, recent transcriptome studies have provided crucial information on divergent patterns of molecular expression that are most relevant in evolutionary terms and can be used to uncover human specializations of brain structure and function.

Specifically, fetal gene co-expression modules have been identified that showed substantial regional differences in the developing human neocortex (Johnson et al., 2009; Miller et al., 2014; Pletikos et al., 2014). Enriched genes were shown to be critical for neuronal processes, such as differentiation, maturation, axonal projection, and synapse formation. These molecular networks also displayed divergence between humans and rhesus macaques (Figure 1; Pletikos et al., 2014), highlighting evolving biological processes involved in the patterning and differentiation of neural circuits in discrete areas (Miller et al., 2014; Hoerder-Suabedissen and Molnár, 2015).

Further work revealed human-distinct temporal progression of neurodevelopmental processes predominantly during the early and mid-fetal period. The identified gene modules were linked to synapse formation, neuronal differentiation, oligodendrocyte maturation, and myelination, and exhibited heterochronic or heterotopic expression in brain regions including the prefrontal cortex, which is central to human cognition and behavior (Figure 1). Interestingly, divergent spatiotemporal expression patterns included also genes associated with ASDs and schizophrenia (Konopka et al., 2012; Zhu et al., 2018). These studies highlight gene expression regulation in space and time as an evolution-modified feature that impacts on neurodevelopmental processes as well as in brain complexity and disease. Diverse expression patterns responsible for interspecies differences can be attributed to changes in DNA methylation, histone modifications (Maze et al., 2014), alternative splicing events, promoter-driven transcription regulation (Davuluri et al., 2008; Nilsen and Graveley, 2010) and non-coding RNAs.

**microRNAs ARE POWERFUL POST-TRANSCRIPTIONAL REGULATORS OF GENE EXPRESSION IN THE BRAIN**

miRNAs (miRNAs) are a class of short non-coding RNAs of ~22 nucleotides in length that constitute an important component of the regulatory circuitry determining expression patterns. Mature miRNAs mediate post-transcriptional regulation of gene expression through direct degradation of their target mRNA and/or suppression of translation. miRNAs bind to their mRNA targets by partial complementarity between the mRNA’s 3’UTR and a 6–8 nucleotides long “seed” sequence at the 5’ end of the microRNA. Therefore, a single microRNA can target multiple miRNAs simultaneously, while a single RNA may be regulated by different microRNAs (Klein et al., 2005; Kosik, 2006; Saliminejad et al., 2019).

miRNA biogenesis begins with the transcription of double-stranded primary miRNA (pri-miRNA) short hairpin structures by RNA polymerase II. The pri-miRNA is then cleaved by the RNase-III enzyme, Drosha, producing ~70-bp pre-miRNAs that are exported from the nucleus into the cytoplasm by Exportin 5 (Exp5), a Ran-GTP dependent Nucleo/cytoplasmic cargo

![Figure 1](https://example.com/figure1.png)

**FIGURE 1** | Species-specific evolution of traits associated with brain development during the divergence to primate and human lineages. Although brain development follows the same basic principles across mammals, evolution has resulted in the appearance of species-specific features. Characteristics of primate divergence include allometric increase in brain size, expansion of progenitor zones along with increased diversity of neural cell types and sophistication of neural circuits reflected in enhanced gyral anatomy. Human brain development is further distinguished by a relatively protracted period of neurogenesis, followed by an extraordinary numeric expansion of the neuronal cell population and by the heterochronic or heterotopic expression of genes associated with synapse formation and myelination in brain regions including the prefrontal cortex, which is central to human cognition and behavior.
transporter. The Dicer enzyme cleaves pre-miRNA sequences into 21–23 nt mature miRNA double-stranded duplexes which are loaded into a pre-RISC (pre-miRNA-induced silencing complex) containing Argonaute (Ago) and other proteins. In the mature miRISC complex the “passenger” strand (complementary strand) is removed leaving just the “guide” strand (mature miRNA strand) which will bind to the mRNA target and instigate inhibition of its expression, reviewed in Winter et al. (2009) and Davis et al. (2015).

The concentration of miRNAs within cells is regulated at different levels. It has been shown that Ago proteins are not only critical for miRNA biogenesis and function, but they also regulate the abundance of mature miRNAs by increasing their stability (Grishok et al., 2001; Diederichs and Haber, 2007; Winter and Diederichs, 2011). Association with mRNA targets also enhances miRNA stability, a phenomenon known as target-mediated miRNA protection, while the introduction of additional target sites can also promote miRNA accumulation (Chatterjee et al., 2011). On the other hand, miRNAs are subject to degradation. It has been shown that miRNA-mRNA interactions not only stabilize but can also destabilize the miRNA and promote its degradation through a process known as target RNA directed miRNA degradation (Ameres et al., 2010; Fuchs Wightman et al., 2018). The degree of sequence complementarity infers the outcome of the miRNA-mRNA interaction, with higher complementarity favoring miRNA degradation and lower complementarity favoring miRNA stabilization. As demonstrated, target miRNAs promote posttranscriptional modifications to the 3′ end of the miRNA involving either the addition of non-templated nucleotides, a process known as “3′-end tailing” or elimination of nucleotides via 3′-to-5′ trimming, both of which control the rate of miRNA decay (Baccarini et al., 2011; Marcinowski et al., 2012).

The ability of miRNAs to fine-tune gene expression levels (Schratt, 2009) constitutes a critical property for controlling spatiotemporal events during brain development. Of the 2,500 mature miRNAs that have been identified in humans (Friedländer et al., 2014), an estimated 70% is expressed in the nervous system (Adlakha and Saini, 2014). miRNAs have emerged as important post-transcriptional regulators of gene expression involved in neurogenesis and neural function in mammalian species (Davis et al., 2015; Nowakowski et al., 2018). A relatively small number of brain miRNAs are well characterized, including miR-92 which targets EOMES (TBR2), a T-box transcription factor that is preferentially expressed in cortical intermediate progenitors and regulates cortical neuron production and expansion, thereby affecting the thickness of the cerebral cortex (Nowakowski et al., 2013). miR-124 and miR-9 have also been shown to affect neural lineage differentiation by downregulating multiple mRNAs (Krichevsky et al., 2006). MiR-9 is a highly brain enriched miRNA that is involved in a negative feedback loop with TLX, a nuclear receptor (Zhao et al., 2009) which controls stem cell proliferation in developing and adult brain (Shi et al., 2004; Liu et al., 2008; Zhang et al., 2008). MiR-9 together with miR-124, one of the most abundant miRNAs in the brain, target REST which opposes neuronal differentiation (Conaco et al., 2006; Visvanathan et al., 2007), while REST itself acts as an inhibitor of miR-124 expression. Moreover, both miRNAs act synergistically to repress BAF53a, a subunit of the neural-progenitor-specific BAF (npBAF) chromatin-remodeling complex, operating during the post-mitotic phase of neuronal development (Yoo et al., 2009). Finally, miR-124 downregulates the RNA-binding protein Ptbp1, a repressor of neuron-specific splicing (Makeyev et al., 2007).

Additionally, miRNAs have been shown to be essential for neural subtype specification. For example, miR-7a promotes oligodendrocyte generation by targeting Pax6 and NeuroD4 (Zhao et al., 2012), while miR-218 is required to establish motor neuron fate (Thiebes et al., 2015). miRNAs have also important roles in synapse formation and plasticity. These include miR-125 which targets the post-synaptic protein PSD-95 in cortical neurons (Mudashetty et al., 2011), the neuron-specific miR-129 which represses Kvl,1, a voltage-gated potassium channel that regulates excitability (Sosanya et al., 2013), and miR-219 which downregulates CamKII, a the major mediator of Long Term Potentiation (LTP) and N-methyl-D-aspartate receptor (NMDA) signaling (Kocerha et al., 2009). Other miRNAs modulate synaptic function upon activation. miR-485 expression is increased following neuronal stimulation to regulate the pre-synaptic protein SV2A and inhibit neurotransmitter release (Cohen et al., 2011). miR-132, on the other hand, accumulates in response to activity in forebrain neurons to regulate dendritic growth, activity-induced spine growth and spine morphology (Magill et al., 2010; Nudelman et al., 2010).

**THE EVOLVING ROLE OF miRNAs IN THE PRIMATE AND HUMAN BRAIN**

**The Evolution of miRNAs**

Evolution of miRNAs is an ongoing process and experimental evidence suggests that along with highly conserved miRNAs, a number of new brain miRNAs have emerged. Many of these are not conserved beyond primates, indicating their recent origin (Berezikov et al., 2006). Following evolutionary adaptations, more than 100 primate-specific miRNAs (that is, miRNAs present only in humans and non-human primates) and 14 human-specific miRNAs (Table 1) have been identified in the developing brain (Berezikov, 2011). Novel miRNAs arise either by the appearance of transcribed hairpin structures or by mutations in the miRNA seed region (Lu et al., 2008). The genomic sources for the acquisition of novel miRNAs are reviewed in detail in “Evolution of microRNA diversity and regulation in animals” (Berezikov, 2011). Briefly, novel miRNAs can emerge by duplication of existing miRNA genes. Alternatively, introns are a frequent source of unstructured transcripts that can gradually evolve into novel intronic miRNAs. **De novo** emergence of miRNAs can also occur, where an evolved transcriptional unit provides a source of the initially unstructured transcript that transitions through the miRNA-like hairpin stage and evolves into a novel miRNA gene. In addition, transposable elements or structured transcripts, such as tRNA and small nucleolar RNA (snRNA), can provide novel transcriptional units for the evolution of miRNA-like hairpins into novel miRNA.
Prodromidou and Matsas

miRNAs in the Evolving Brain

| Precursor id               | Chromosome |
|---------------------------|------------|
| hsa-mir-1302-10           | chr15      |
| hsa-mir-1302-11           | chr19      |
| hsa-mir-1302-2            | chr1       |
| hsa-mir-3156-3            | chr21      |
| hsa-mir-3848              | chr21      |
| hsa-mir-3673              | chr8       |
| hsa-mir-3690              | chrX       |
| hsa-mir-4487              | chr11      |
| hsa-mir-4739              | chr17      |
| hsa-mir-5095              | chr1       |
| hsa-mir-659               | chr22      |
| hsa-mir-941-1             | chr20      |
| hsa-mir-941-3             | chr20      |
| hsa-mir-941-4             | chr20      |

Finally, antisense transcription of existing miRNA loci can lead to the formation of miRNA hairpins with novel mature miRNA sequences.

miRNAs as Key Regulators in Shaping Patterns of Gene Expression in the Developing and Adult Primate and Human Brain

A series of observations support the notion that miRNAs are instrumental contributors to the alterations that may account for the accelerated evolution of the human brain. First, elevated production of mature miRNAs has been observed in the human brain compared to other species, which is attributed to the higher processing efficiency of miRNA precursors in humans (Chakraborty et al., 2018).

It has been proposed that the evolution of miRNA-mediated regulatory networks has contributed to organismal complexity (Berezikov, 2011). This can mechanistically be explained by the ability of miRNAs to advance network functionalities by delivering the extra precision required to constrain the intrinsically noisy gene expression process (Raj and van Oudenaarden, 2008; Herranz and Cohen, 2010). In support, the work of Arcila et al. (2014) on primate-specific miRNAs suggests that integration of novel miRNAs into ancient gene circuitry exerted additional regulation over-proliferation of neural progenitors in cortical germinal areas (Figure 2), a region that demonstrates significant expansion across brain evolution (Arcila et al., 2014).

Furthermore, evolving miRNA-mediated regulation shows critical implications in shaping gene networks and even determining anatomical regions during brain development. Work on primates has revealed that miRNA profiles can resolve discrete areas within the developing cortex while dominant differences were observed between the germinal zone and the differentiated cells in the cortical plate (Arcila et al., 2014). Consistently, studies on the transition from infant to adolescent human brain show differential expression of miRNAs within and between brain regions, with the prefrontal cortex, the region mostly connected with human cognition, exhibiting the greatest number of differentially expressed miRNAs (Ziats and Rennert, 2014). Moreover, miRNA expression displays increased deviations between brain regions over time, indicating the implication of miRNAs in the regional specialization as the brain matures (Ziats and Rennert, 2014). Importantly, common neurodevelopmental disorders associated with genes targeted by these miRNAs (Figure 2; Ziats and Rennert, 2014).

Recent elegant work highlights the impact of miRNAs in regulating regionally divergent transcriptional states in the developing human cortex. To characterize the landscape of miRNA–mRNA interactions during human brain development, Nowakovski et al. developed a new single-cell approach for combined mRNA and miRNA profiling in the same cell across human fetal tissue samples corresponding to peak neurogenesis [Gestational week (GW) 15 and 16.5] and early gliogenesis (GW 19–20.5). Their study revealed that major regulatory molecules like transcription factors, chromatin modifiers, and signaling components are enriched among miRNA targets. Reconstruction of gene-regulatory networks uncovered that miRNA–mRNA interactions often correspond to the acquisition of cell-type identities and undergo dynamic transitions even among closely related cell types during neuronal differentiation and maturation (Figure 2). Further strengthening previous studies, the authors demonstrate that different pathways driven by brain-specific miRNAs are...
related to developmental stage and cortical area specificity (Nowakowski et al., 2018).

Transcriptomic studies founded the notion that miRNA divergence correlates with the divergence of gene expression patterns in the prefrontal cortex and cerebellum among humans, chimpanzees, and macaques. Accordingly, it has been reported that a significant inverse relationship exists between human and chimpanzee miRNA expression divergence and expression divergence of their predicted target genes at both mRNA and protein levels (Hu et al., 2011). Inline, Svante Pääbo and colleagues placed miRNAs among the key regulators that remodeled cortical development (Somel et al., 2011). Using a computational method of analysis, they show that trans-acting regulators and particularly miRNAs drive the pronounced gene expression changes observed in the human prefrontal cortex. As deduced, the developmental profiles of miRNAs, as well as their target genes, show the fastest rates of human-specific evolutionary change.

**HUMAN- AND PRIMATE-SPECIFIC miRNAs IN NEURAL DEVELOPMENT, PHYSIOLOGY AND PATHOLOGY**

Expression, even if at low levels, has been detected in the prefrontal cortex and cerebellum for the majority of the 14 human-specific miRNAs identified so far (Hu et al., 2012). Enrichment analysis of the predicted target genes of human-specific miRNAs attests that these relate to neuronal components and processes, including cytoskeletal elements, metal ion binding, and postsynaptic, dendritic, and somatic functions (Barbash et al., 2014). Separate studies on individual primate- and human-specific miRNAs are limited and confined in human tissue examination complemented by functional analysis using cell lines or mouse models in one case (Table 2). Nevertheless, data so far show that primate-specific miRNAs are principally involved in the regulation of cell cycle dynamics operating during progenitor proliferation and neuronal differentiation while they further associate with neurodevelopmental disorders (Figure 2).

A great ape specific miRNA, miR-2115, is enriched in radial glia and becomes prominently upregulated at GW19–20 in the human germinal zones. It controls cell-cycle dynamics during human cortical development by fine-tuning the expression of ORC4, a known regulator of DNA replication (Nowakowski et al., 2018). miR-1301-3p and miR-1180-3p are primate-specific miRNAs that have been identified in the germinal zones of the visual cortex of the macaque developing brain. miR-1301-3p regulates the mRNA for histone-lysine N-methyltransferases mll1 and mll2 (MixedLineage, Leukemia) that function by resolving silenced bivalent loci in neural precursors for remodeling cortical development (Somel et al., 2011). Using a computational method of analysis, they show that trans-acting regulators and particularly miRNAs drive the pronounced gene expression changes observed in the human prefrontal cortex. As deduced, the developmental profiles of miRNAs, as well as their target genes, show the fastest rates of human-specific evolutionary change.

### Table 2

| miRNA Species | miRNA target(s) | Detection of brain expression | Methodology Proposed associated function and disorder |
|---------------|-----------------|------------------------------|----------------------------------------------------|
| miR-2115      | Primate specific ORC4 | Upregulated at GW19–20 in the human germinal zones | Single-cell RNA sequencing for identification of mRNA | Cell cycle dynamics during human cortical development, neurodevelopmental disorder |
| miR-1301-3p   | Primate specific histone-lysine N-methyltransferases, mll1 and mll2 | Elevated in the brain of depressed individuals | Functional analysis in cell lines and drug testing in human NPCs | Pathophysiology of depression, autism, intellectual disability |
| miR-1180-3p   | Primate specific KANSL1 and DLX1 | Fetal brain of cynomolgus monkeys (Macaca fascicularis, gestation period 165 days) | RNA Sequencing for identification and luciferase assays in human cell lines | GABAergic neurogenesis, autism, intellectual disability |
| miR-1202      | Primate specific GRM4 | Elevated in the brain of depressed individuals | Target validation in human cell lines | Association with AD risk |
| miR-603       | Primate specific E2F1 and LRPAP1 | Target validation in human cell lines | RNA sequencing for expression analysis | Human longevity, neurotransmitter signaling, language and speech |
| miR-941       | Human-specific SMO, SUFU, GLI1, IRS1, PPARGC1A | High expression in the human prefrontal cortex and cerebellum | Target validation in human cell lines | Human longevity, neurotransmitter signaling, language and speech |
In humans, while two of these (miR-299-3p and miR-3p, with high sequence conservation among species, which increased new variants around target genes of human-specific miRNAs are consistent with the theory that the speciation increased new variants around target genes of human-specific miRNAs (Barbash et al., 2014).

These findings in conjunction with the appearance of an increased density of potential binding sites, enhancing the target enrichment for miRNAs (Meunier et al., 2013), but also display untranslated regions (UTRs) which are the main target region for miRNAs. Evolutionary changes and consequently species distinct features also exist for the binding partners of miRNAs. The evolution of miRNA regulation is intimately intertwined with the evolution of their targets, as newly emerging miRNAs integrate into preexisting gene expression circuitries (Arcila et al., 2014). This is particularly pronounced in the brain where neuronal transcripts not only have longer 3′ untranslated regions (UTRs) which are the main target region for miRNAs (Meunier et al., 2013), but also display an increased density of potential binding sites, enhancing their selective advantage for acquiring miRNA-mediated regulation (Cacchiarelli et al., 2008; Barbash et al., 2014). These findings in conjunction with the appearance of increased new variants around target genes of human-specific miRNAs are consistent with the theory that the speciation of hominids was accompanied by an enhancement in the capacity of newly evolved miRNAs to modulate gene expression (Barbash et al., 2014).

Interestingly Hu et al identified five miRNAs, namely miR-184, miR-487a, miR-383, miR-34c-5p, and miR-299-3p, with high sequence conservation among species, which nevertheless show significantly different levels of expression in humans, while two of these (miR-299-3p and miR-184) show preferential expression in cortical neurons (Hu et al., 2012). Functional analysis combining the targets of all five miRNAs revealed enrichment in genes associated with neural processes and specifically with cell proliferation and differentiation, synaptic transmission and neuronal function. Moreover, miR-299-3p targets associate with axon guidance, while miR-184 targets are related to long-term potentiation, which is directly linked to learning and memory formation (Hu et al., 2011). Human-specific miRNA-mediated regulation of certain genes with a critical role in brain function has also been reported. For instance, miR-483–5p, binds in a sequence-exclusive manner to the human epigenetic regulator methyl CpG binding protein 2 (MeCP2) that controls proper neurological function, to modulate its levels in the fetal cortex (Han et al., 2013).

**HUMAN EXPERIMENTAL MODELS FOR INVESTIGATION OF miRNA-SPECIFIC FEATURES OF BRAIN DEVELOPMENT AND ASSOCIATED DISORDERS**

Access to human tissue in combination with high-throughput sequencing techniques provided important insight into the significance of the miRNA-driven transcriptome changes across brain development and evolution. However, it is necessary to validate predicted targets and clarify cell type association, stage-specificity and mode of action for individual miRNAs. As experimental animals cannot fully simulate human brain development and disease, human embryonic stem cells (hESCs) or induced pluripotent stem cells (iPSCs) represent valuable means for advancing human studies (Chambers et al., 2009; Shi et al., 2012). Directed cortical differentiation of human, chimpanzee, and macaque iPSCs (Otani et al., 2016) clearly depicted the divergence in the timing of key developmental events and highlighted the extended proliferation of chimpanzee and human progenitors as compared to macaque, emphasizing the validity of such systems in reproducing species-specific features.

Further studies on hESCs accompanied by miRNA profiling during differentiation of various neuronal subtypes underlined the effect of known miRNAs, such as let-7, miR-124, miR-7, miR-125 and miR-9 in progenitor proliferation, cell fate specification and neuronal commitment and maturation (Delaloy et al., 2010; Boissart et al., 2012; Liu et al., 2012; Cimadamore et al., 2013; Tu et al., 2018). miRNA research on hESC models has also uncovered new targets and roles for these miRNAs. For example, miR-9 which has been described in mouse to promote neuronal lineage differentiation by targeting REST, was shown to also target Stat3, a protein involved in microtubule stability, and to coordinate proliferation and migration during the early stage of maturation of neural progenitors. Similarly, miR-125 with reported neuronal function, was shown to act in earlier stages to promote exit from pluripotency and potentiate neural specification by targeting SMAD4. Moreover, research on patient induced pluripotent stem cells and their neuronal derivatives uncovered the role...
of certain miRNAs in autism and schizophrenia (Halevy et al., 2015; Murai et al., 2016; Mellios et al., 2018).

Notably, given the complexity of the human brain, 3D tissue culture models that embody cellular diversity and spatial organization that mimics brain architecture, are more relevant to understand the critical input of miRNAs in shaping intricate brain gene networks. Brain organoids that grow as 3D aggregates from pluripotent stem cells comprise an exciting new tool for modeling human brain physiology and pathology, for uncovering human-specific traits and for drug discovery and testing. Despite current limitations, particularly batch heterogeneity that impedes consistency, organoids resemble human brain not only at the cellular level, but also in terms of general tissue structure, developmental trajectories and neuronal functionalities (Lancaster et al., 2013; Pašca et al., 2015; Qian et al., 2016; Giandomenico et al., 2016). Cortical organoids in particular that have been more extensively studied, recapitulate the organization of neural progenitor zones to a considerable degree, reflecting developmental events during embryonic stages in vivo (Lancaster et al., 2013; Qian et al., 2016). Such systems can be valuable for functional gain- or loss-of-function studies to elucidate the contribution of ancient or novel miRNAs in primate and/or human-specific processes. Nevertheless, studying neuronal network formation among different brain regions, especially distant ones, remains a challenge. Recent advances in the generation of fused brain organoids from co-culture of individual ones with distinct regional identities may provide a closer-to-the-in-vivo-situation model to investigate the basis of human neural circuitry formation in health and disease (Birey et al., 2017; Xiang et al., 2017, 2019). Alternatively, it is possible to exploit the inherent intrinsic heterogeneity observed within single organoids, as for example in brain organoids containing retinal regions that can respond to light stimuli (Quadrato et al., 2017). However in all cases absence of vascularization restricts oxygen and nutrient supplies limiting organoid survival, consequently affecting the time-scale of such studies. Therefore a combination of different approaches and technologies, including 2D cultures derived from pluripotent stem cells, brain organoids, single-cell transcriptomics, potentially along with miRNA target degradation kinetics analysis to elucidate the miRNA-driven evolutionary variants of the neurodevelopmental program should prove more rewarding.

In relevance, single-cell RNA-sequencing technology has greatly facilitated research efforts in resolving the diversity and developmental trajectories of human brain cell types providing transformative insights into the developmental lineages and functional states of individual cells (Tasic et al., 2016, 2018; Nowakowski et al., 2017; Paul et al., 2017; Hrvatin et al., 2018; Lake et al., 2018; Zhong et al., 2018). Importantly, a number of studies have illustrated the potential to integrate spatial information at single-cell resolution (Ke et al., 2013; Lubeck et al., 2014; Chen et al., 2015; Satija et al., 2015; Salmén et al., 2018). However, when designing such experiments, consideration of the tradeoff between a number of cells sequenced and the read depth per cell is a factor that has to be seriously considered, not the least because of budgetary constraints (Menon, 2018). Additionally, such developments still necessitate the formulation of new computational modalities to integrate the rapidly expanding data sets generated from diverse sources so that biological sense is made (Butler et al., 2018; Stuart and Satija, 2019). Improvements in algorithm design and data analysis are essential to allow unsupervised methods for tracking gene expression and correctly reconstructing trajectories that map cell lineages and developmental transition of cells.

Towards miRNA Based Therapeutics

The more we understand miRNA biology and its contribution to the intricate regulatory networks of gene expression during development, the better will we be able to uncover related disturbances underlying the origin of various neuropsychiatric and behavioral disorders. Indeed, compelling evidence regarding the involvement of miRNAs in human disease has instigated attention into their potential use as therapeutics (van Rooij et al., 2012). miRNA-based strategies include miRNA mimics and inhibitors (antagomiRs) to respectively increase and decrease their expression or, conversely, their target genes. The field currently progresses rapidly as in 2018 the FDA approved the first therapy based on miRNA administration for the treatment of rare progressive polyneuropathy caused by hereditary transthyretin-mediated amyloidosis (Adams et al., 2018; Wood, 2018). Understanding the functions of miRNAs in specific cell types and during different stages throughout life under normal and pathological conditions could improve the specificity and efficacy of miRNA therapeutic strategies. Moreover, delivery to the brain is challenging and the pleiotropic nature of miRNA functions undeniably makes off-target biological effects an important limitation (Junn and Mouradian, 2012). Novel approaches are in the pipeline to design new chemical formulations in order to increase miRNA stability and improve their specificity and permeability, while delivery methods are also being developed to target the brain and decrease unwanted side effects (van Rooij et al., 2012; Sokilde et al., 2015; Rupaimoole and Slack, 2017). In this regard, decoding species-specific regulation of miRNA function will prove an important step for fostering novel, holistic and effective therapeutic approaches for human disorders.

CONCLUSIONS AND FUTURE PERSPECTIVES

Accumulating evidence highlights the emergent role of miRNAs as critical regulators that influence the overall transcriptional landscape in the human brain. In addition, primate- and human-specific regulation by miRNAs during neurodevelopment argues in favor of their involvement in the establishment of species-specific features during brain evolution. A comprehensive understanding of the spatiotemporal miRNA mediated regulation will help resolve the intricate regulatory network operating during human neurogenesis and will grant important cues for the causality of neurodevelopmental disorders. Down this line, 2D- and 3D-human pluripotent-derived experimental setups constitute a useful system for modeling species-specific features of development and for
capturing miRNA-mediated rewiring of the transcriptional program. In conjunction, single-cell sequencing approaches can decode the refined miRNA-mRNA interactions during cell developmental transitions. Taken together, miRNAs constitute an indispensable component of the extended gene regulatory network and evaluating their functional significance in the evolving brain can determine key features of human neuronal development and disease.

AUTHOR CONTRIBUTIONS
KP and RM planned and wrote the manuscript.

REFERENCES
Adams, D., Gonzalez-Duarte, A., O’Riordan, W. D., Yang, C. C., Ueda, M., Kristen, A. V., et al. (2018). Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. *N. Engl. J. Med.* 379, 11–21. doi: 10.1056/NEJMoa1716153
Adikaña, Y. K., and Sami, N. (2014). Brain microRNAs and insights into biological functions and therapeutic potential of brain enriched miRNA-128. *Mol. Cancer* 13:33. doi: 10.1186/1476-4598-13-33
Allman, J. M., Tetreault, N. A., Hakemeen, A. Y., Manaye, K. F., Semendeferi, K., Erwin, J. M., et al. (2010). The von Economo neurons in frontoinsular and anterior cingulate cortex in great apes and humans. *Brain Struct. Funct.* 214, 495–517. doi: 10.1007/s00424-010-0254-0
Ameres, S. L., Horwich, M. D., Hung, J. H., Xu, J., Ghioldy, M., Weng, Z., et al. (2010). Target RNA-directed trimming and tailing of small silencing RNAs. *Science* 328, 1534–1539. doi: 10.1126/science.1187058
Arcila, M. L., Betzeau, M., Cambronne, X. A., Guzman, E., Doerflinger, N., Bounahller, F., et al. (2014). Novel primate miRNAs coevolved with ancient target genes in germinal zone-specific expression patterns. *Neuron* 81, 1255–1262. doi: 10.1016/j.neuron.2014.01.017
Baccarini, A., Chauhan, H., Gardner, T. J., Jayaprakash, A. D., Sachidanandam, R., Adlakha, Y. K., and Saini, N. (2014). Brain microRNAs and insights into biological functions and therapeutic potential of brain enriched miRNA-128. *Mol. Cancer* 13:33. doi: 10.1186/1476-4598-13-33
Bae, B. I., Jayaraman, D., and Walsh, C. A. (2015). Genetic changes shaping the human brain. *Dev. Cell* 32, 433–443. doi: 10.1016/j.devcel.2015.01.035
Barbash, S., Shifman, S., and Soreq, H. (2014). Global coevolution of human microRNAs and their target genes. *Mol. Biol. Evol.* 31, 1237–1247. doi: 10.1093/molbev/msu090
Berezikov, E. (2011). Evolution of microRNA diversity and regulation in animals. *Nature* 478, 366–374. doi: 10.1038/nature10339
Berezikov, E., Thuemmler, F., van Laake, L. W., Kondova, I., Bontrop, R., Cuppen, E., et al. (2006). Diversity of microRNAs in human and chimpanzee brain. *Nature genetics* 38, 1375–1377. doi: 10.1038/ng1914
Bianchi, S., Stimpson, C. D., Bauerfeind, A. L., Schapiro, S. J., Baze, W. B., McArthur, M. J., et al. (2013). Dendritic morphology of pyramidal neurons in human and chimpanzee brain. *Nature genetics* 45, 54–59. doi: 10.1038/nature22330
Boissart, C., Nissan, X., Giraud-Triboult, K., Peschanski, M., and Benchoua, A. (2016). Brain microRNAs and insights into biological functions and therapeutic potential of brain enriched miRNA-128. *Mol. Cancer* 13:33. doi: 10.1186/1476-4598-13-33
Buckner, R. L., and Krienen, F. M. (2013). The evolution of distribution association networks in the human brain. *Trends Cogn. Sci.* 17, 648–665. doi: 10.1016/j.tics.2013.09.017
Butler, A., Hoffmann, P., Smibert, P., Papalexi, E., and Satija, R. (2018). Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nat. Biotechnol.* 36, 411–420. doi: 10:1038/nbt.4096
Bystron, I., Rakic, P., Molnar, Z., and Blakemore, C. (2006). The first neurons of the human cerebral cortex. *Nat. Neurosci.* 9, 880–886. doi: 10.1038/nn1726
Cacchiarelli, D., Santoni, D., and Bozzoni, I. (2008). MicroRNAs as prime players in a combinatorial view of evolution. *RNA Biol.* 5, 120–122. doi: 10:4161/rna.5.3.16569
Chakraborty, S., Islam, M. R., Ali, M. M., and Nabi, A. (2018). Evolutionary divergence of brain-specific precursor miRNAs drives efficient processing and production of mature miRNAs in human. *Neuroscience* 392, 141–159. doi: 10.1016/j.neuroscience.2018.09.010
Chambers, S. M., Fasano, C. A., Papapetrou, E. P., Tomishima, M., Sadelain, M., and Studer, L. (2009). Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat. Biotechnol.* 27, 275–280. doi: 10:1038/nbt.1529
Chatterjee, S., Fasler, M., Büssing, L. and Grosshans, H. (2011). Target-mediated protection of endogenous microRNAs in *C. elegans*. *Dev. Cell* 20, 388–396. doi: 10.1016/j.devcel.2011.02.008
Chen, K. H., Boettiger, A. N., Moffitt, J. R., Wang, S., and Zhuang, X. (2015). RNA imaging. Spatially resolved, highly multiplexed RNA profiling in single cells. *Science* 348:aaa6090. doi: 10.1126/science.aaa6090
Cimadomare, F., Amador-Arjona, A., Chen, C., Huang, C. T., and Terskikh, A. V. (2013). SOX2-LIN28/let-7 pathway regulates proliferation and neurogenesis in neural precursors. *Proc. Natl. Acad. Sci. U S A* 110, E3017–E3026. doi: 10.1073/pnas.1220176110
Cohen, J. E., Lee, P. R., Chen, S., Li, W., and Fields, R. D. (2011). MicroRNA regulation of homeostatic synaptic plasticity. *Proc. Natl. Acad. Sci. U S A* 108, 11650–11655. doi: 10.1073/pnas.1017576108
Conaco, C., Otto, S., Han, J. J., and Mandel, G. (2006). Reciprocal actions of REST and a microRNA promote neuronal identity. *Proc. Natl. Acad. Sci. U S A* 103, 2422–2427. doi: 10.1073/pnas.0511041103
Davis, G. M., Haas, M. A., and Pocock, R. (2015). MicroRNAs: not “fine-tuners” but key regulators of neuronal development and function. *Front. Neural. Sci.* 6, 242–2427. doi: 10.3389/fnins.2015.00245
Davuluri, R. V., Suzuki, Y., Sugano, S., Plass, C., and Huang, T. H. (2008). The functional consequences of alternative promoter use in mammalian genomes. *Trends Genet.* 24, 167–177. doi: 10.1016/j.tig.2008.01.008
Dehay, C., Kennedy, H., and Kosik, K. S. (2015). The outer subventricular zone and primate-specific cortical complexification. *Neuron* 85, 683–694. doi: 10.1016/j.neuron.2014.12.060
Delaloye, C., Liu, L., Lee, J. A., Su, H., Shen, F., Yang, G. Y., et al. (2010). MicroRNA-9 coordinates proliferation and migration of human embryonic stem cell-derived neural progenitors. *Cell Stem Cell* 6, 323–335. doi: 10.1016/j.stem.2010.02.015
Dennis, M. Y., Nuttle, X., Sudmant, P. H., Antonacci, F., Graves, T. A., Nefedov, M., et al. (2012). Evolution of human-specific neural SRGAP2 genes by incomplete segmental duplication. *Cell* 149, 912–922. doi: 10.1016/j.cell.2012.03.033

FUNDING
We acknowledge funding from Stavros Niarchos Foundation to the Hellenic Pasteur Institute as part of the Foundation’s initiative to support the Greek Research Center Ecosystem and the Hellenic Foundation for Research and Innovation 899-PARKINSynapse grant.

ACKNOWLEDGMENTS
We thank the members of the Matsas Lab for stimulating discussions and comments.
Prodromidou and Matsas

miRNAs in the Evolving Brain

Eyal, G., Verhoog, M. B., Testa-Silva, G., Deitcher, Y., Lodder, J. C., Benavides-Ebert, M. S., and Sharp, P. A. (2012). Roles for microRNAs in conferring robustness to biological processes. Cell 149, 515–524. doi: 10.1016/j.cell.2012.04.005

Elston, G. N., Benavides-Piccione, R., Elston, A., Manger, P. R., and De Felipe, J. (2011). Pyramidal cells in prefrontal cortex of primates: marked differences in neuronal structure among species. Front. Neuroanat. 5:2. doi: 10.3389/fnana.2011.00002

Evans, P. D., Anderson, J. R., Vallender, E. J., Choi, S. S., and Lahn, B. T. (2004). Reconstructing the evolutionary history of microcephalin, a gene controlling human brain size. Hum. Mol. Genet. 13, 1139–1145. doi: 10.1093/hmg/ddh126

Eyal, G., Verhoog, M. B., Testa-Silva, G., Deitcher, Y., Lodder, J. C., Benavides-Piccione, R., et al. (2016). Unique membrane properties and enhanced signal processing in human neocortical neurons. Elife 5:e16553. doi: 10.7554/eLife.16553

Ferland, R. J., Eyal, W., Collura, R. V., Tully, L. D., Hill, R. S., Al-Nouri, D., et al. (2004). Abnormal cerebellar development and axonal decussation due to mutations in AHI1 in Joubert syndrome. Nat. Genet. 36, 1008–1013. doi: 10.1038/ng1149

Florio, M., Albert, M., Taverna, E., Namba, T., Brandl, H., Lewitus, E., et al. (2015). Human-specific gene ARHGAP11B promotes basal progenitor amplification and neocortex expansion. Science 347, 1465–1470. doi: 10.1126/science.aaaa175

Friedländer, M. R., Lizano, E., Houben, A. J., Bezdan, D., Banez-Coronel, M., Kudla, G., et al. (2014). Evidence for the biogenesis of more than 1,000 novel human microRNAs. Genome Biol. 15:R57. doi: 10.1186/gb-2014-15-4-r57

Fuchs Wightman, F., Giono, L. E., Fededa, J. P., and de la Mata, M. (2018). Targeting nuclear receptor tailless is required for neurogenesis in the adult subventricular zone. Stem Cell Rev. Rep. 14, 373–379. doi: 10.1007/s12015-018-00435

Gai, M., Bianchi, F. T., Vagnoni, C., Verni, F., Bonaccorsi, S., Pasquero, S., et al. (2016). ASPM and CITK regulate spindle orientation and their therapeutic potential. Pharmacol. Ther. 133, 142–150. doi: 10.1016/j.pharmthera.2011.10.002

Ke, R., Mignardi, M., Pacureanu, A., Svedlund, J., Botling, J., Wahlby, C., et al. (2013). In situ sequencing for RNA analysis in preserved tissue and cells. Nat. Methods 10, 857–860. doi: 10.1038/nmeth.2563

Klein, M. E., Impey, S., and Goodman, R. H. (2005). Role reversal: the regulation of neuronal gene expression by microRNAs. Curr. Opin. Neurobiol. 15, 507–513. doi: 10.1016/j.conb.2005.08.011

Kocerha, J., Faghhi, M. A., Lopez-Toledano, M. A., Huang, J., Ramsey, A. J., Caron, M. G., et al. (2009). MicroRNA-219 modulates NMDA receptor-mediated neurobehavioral dysfunction. Proc. Natl. Acad. Sci. U S A 106, 3507–3512. doi: 10.1073/pnas.0808585106

Konopka, G., Friedrich, T., Davis-Turak, J., Winden, K., Oldham, M. C., Gao, F., et al. (2012). Human-specific transcriptional networks in the brain. Neuron 75, 601–617. doi: 10.1016/j.neuron.2012.05.034

Kosik, K. S. (2006). The neuronal microRNA system. Nat. Rev. Neurosci. 7, 911–920. doi: 10.1038/nrn2037

Krishkevsky, A. M., Sonntag, K. C., Isascón, O., and Kosik, K. S. (2006). Specific microRNAs modulate embryonic stem cell-derived neurogenesis. Stem Cells 24, 857–864. doi: 10.1634/stemcells.2005-0441

Lai, C. S., Fisher, S. E., Hurst, J. A., Vargha-Khadem, F., and Monaco, A. P. (2001). A forkhead-domain gene is mutated in a severe speech and language disorder. Nature 413, 519–523. doi: 10.1038/35097076

Lake, B. B., Chen, S., Sos, B. C., Fan, J., Kaaser, G. E., Yung, Y. C., et al. (2018). Integrative single-cell analysis of transcriptional and epigenetic states in the human adult brain. Nat. Biotechnol. 36, 70–80. doi: 10.1038/nbt.4038

Lancaster, M. A., Renner, M., Martin, C. A., Wenzel, D., Bicknell, L. S., Hurles, M. E., et al. (2013). Cerebral organoids model human brain development and microcephaly. Nature 501, 373–379. doi: 10.1038/nature12517

Lindsay, C. W., and Hopkins, C. R. (2012). Metabotropic glutamate receptor 4 (mGlu4)-positive allosceric modulators for the treatment of Parkinson's disease: historical perspective and review of the patent literature. Expert Opin. Ther. Pat. 22, 461–481. doi: 10.1517/13543776.2012.679437

Liu, H. K., Belt, T., Bock, D., Takacs, A., Wu, H., Lichter, P., et al. (2008). The nuclear receptor tailless is required for neurogenesis in the adult subventricular zone. Genes Dev. 22, 2473–2478. doi: 10.1101/gad.479308

Liu, J., Githinji, J., McLaughlin, B., Wilczek, K., and Nolte, J. (2012). Role of miRNAs in neuronal differentiation from human embryonic stem cell-derived neural stem cells. Stem Cell Rev. Rep. 8, 1129–1137. doi: 10.1007/s12015-012-9411-6

Lopez, J. P., Lim, R., Cruceanu, C., Crapper, L., Casano, C., Labonte, B., et al. (2014). miR-1202 is a primate-specific and brain-enriched microRNA involved in major depression and antidepressant treatment. Nat. Med. 20, 764–768. doi: 10.1038/nm.3582

Lu, J., Shen, Y., Wu, Q., Kumar, S. H., Be, B., Shi, S., et al. (2008). The birth and death of microRNA genes in Drosophila. Nat. Genet. 40, 351–355. doi: 10.1038/ng.73
Lubeck, E., Coskun, A. F., Zhiyentayev, T., Ahmad, M., and Cai, L. (2014). Single-cell in situ RNA profiling by sequential hybridization. *Nat. Methods* 11, 360–361. doi: 10.1038/nmeth.2892

Lui, J. H., Hansen, D. V., and Kriegstein, A. R. (2011). Development and evolution of the human neocortex. *Cell* 146, 18–36. doi: 10.1016/j.cell.2011.06.030

Maeshima, S. T., Cahn, H. R. A., Luikart, B. W., Liow, D. T., Leighton, B. H., Westbrook, G. L., et al. (2010). microRNA-132 regulates dendritic growth and arborization of newborn neurons in the adult hippocampus. *Proc. Natl. Acad. Sci. U S A* 107, 20382–20387. doi: 10.1073/pnas.1015691107

Makeev, E. V., Zhang, J., Carrasco, M. A., and Maniatis, T. (2007). The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. *Mol. Cell* 27, 435–448. doi: 10.1016/j.molcel.2007.07.015

Marcinowski, L., Tanguy, M., Krmputic, A., Radle, B., Lisnic, V. J., Tuddenham, L., et al. (2012). Degradation of cellular mir-27 by a novel, highly abundant viral transcript is important for efficient virus replication in vivo. *PLoS Pathog.* 8:e1002510. doi: 10.1371/journal.ppat.1002510

Marin-Padilla, M. (2014). The mammalian neocortex new pyramidal neuron: a new conception. *Front. Neuroanat.* 7:51. doi: 10.3389/fnana.2013.00051

Maze, I., Chaudhury, D., Dietz, D. M., Von Schimmelmann, M., Kennedy, P. J., Marcinowski, L., Tanguy, M., Krmpotic, A., Radle, B., Lisnic, V. J., Tuddenham, L., Magill, S. T., Cambronne, X. A., Luikart, B. W., Liow, D. T., Leighton, B. H., Westbrook, G. L., et al. (2010). microRNA-132 regulates dendritic growth and arborization of newborn neurons in the adult hippocampus. *Proc. Natl. Acad. Sci. U S A* 107, 20382–20387. doi: 10.1073/pnas.1015691107

Nowakowski, T. J., Rani, N., Golkaram, M., Zhou, H. R., Alvarado, B., Huch, K., et al. (2018). Regulation of cell-type-specific transcriptomes by microRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nat. Biotechnol.* 36, 495–502. doi: 10.1038/nbt.4134

Nathanson, N. M., et al. (2010). Neuronal activity rapidly induces transcriptional landscape of the prenatal human brain. *Proc. Natl. Acad. Sci. U S A* 107, 20382–20387. doi: 10.1073/pnas.1015691107

Obereheim, N. A., Goldman, S. A., and Nedergaard, M. (2012). Heterogeneity of astrocytic form and function. *Methods Mol. Biol.* 814, 23–45. doi: 10.1007/978-1-61779-452-0_3

Otabil, A. M., Sloan, S. A., Clarke, L. E., Tian, Y., Makinson, C. D., Huber, N., et al. (2015). Functional cortical neurons and astrocytes from human pluripotent stem cells in 3D culture. *Nat. Methods* 12, 671–678. doi: 10.1038/nmeth.3415

Pasca, A. M., Sloan, S. A., Clarke, L. E., Tian, Y., Makinson, C. D., Huber, N., et al. (2015). Functional cortical neurons and astrocytes from human pluripotent stem cells in 3D culture. *Nat. Methods* 12, 671–678. doi: 10.1038/nmeth.3415

Paul, A., Crow, M., Raudales, R., He, M., Gillis, J., and Huang, Z. J. (2017). Transcriptional architecture of synaptic communication delineates GABAergic neuron identity. *Cell* 171, 522.e20–539.e20. doi: 10.1016/j.cell.2017.08.032

Pletikos, M., Sousa, A. M., Sedmak, G., Meyer, K. A., Zhu, Y., Cheng, F., et al. (2014). Temporal specification and bilaterality of human neocortical topographic gene expression. *Neuron* 81, 321–332. doi: 10.1016/j.neuron.2013.11.018

Qian, X., Nguyen, H. N., Song, M. M., Hadjono, C., Ogden, S. C., Hammack, C., et al. (2016). Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. *Cell* 165, 1238–1254. doi: 10.1016/j.cell.2016.04.032

Quadrato, G., Nguyen, T., Macosko, E. Z., Sherwood, J. L., Min Yang, S., Berger, D. R., et al. (2017). Cell diversity and network dynamics in photosensitive human brain organoids. *Nature* 545, 48–53. doi: 10.1038/nature22047

Raghanti, M. A., Edler, M. K., Stephenson, A. R., Wilson, L. J., Hopkins, W. D., Ely, J. I., et al. (2016). Human-specific increase of dopaminergic innervation in a striatal region associated with speech and language: a comparative analysis of the primate basal ganglia. *J. Comp. Neurol.* 524, 2117–2129. doi: 10.1002/cne.23937

Raghanti, M. A., Stimpson, C. D., Marcinkiewicz, J. L., Erwin, J. M., Hof, P. R., and Sherwood, C. C. (2008). Differences in cortical serotonin innervation among humans, chimpanzees and macaque monkeys: a comparative study. *Cereb. Cortex* 18, 584–597. doi: 10.1093/cercor/bhm089

Raj, A., and van Oudenaarden, A. (2008). Nature, nurture, or chance: stochastic gene expression and its consequences. *Cell* 135, 216–226. doi: 10.1016/j.cell.2008.09.050

Rokkel, A. J., Horns, R. W., and Powell, T. P. (1980). The basic uniformity in structure of the neocortex. *Brain* 103, 221–244. doi: 10.1093/brain/103.2.221

Rogers, J., Kochunov, P., Zilles, K., Shelvedy, W., Lancaster, J., Thompson, P., et al. (2010). On the genetic architecture of cortical folding and brain volume in primates. *Neuroimage* 53, 1103–1108. doi: 10.1016/j.neuroimage.2010.02.020

Rupaimooore, R., and Slack, F. J. (2017). MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nat. Rev. Drug Discov.* 16, 203–222. doi: 10.1038/nrd.2016.446

Saliminejad, K., Khorram Khorshid, H. R., Soleymani Fard, S., and Ghaffari, S. H. (2019). An overview of microRNAs: biology, functions, therapeutics and analysis methods. *J. Cell. Physiol.* 234, 5451–5465. doi: 10.1002/jcp.27486

Salmen, F., Stahl, P. L., Mollbrink, A., Navarro, J. F., Vickovic, S., Frisen, J., et al. (2018). Barcoded solid-phase RNA capture for spatial transcriptomics profiling in mammalian tissue sections. *Nat. Protoc.* 13, 2501–2534. doi: 10.1038/s41596-018-0045-2

Sattija, R., Farrell, J. A., Gennert, D., Schier, A. F., and Regev, A. (2015). Spatial reconstruction of single-cell gene expression data. *Nat. Biotechnol.* 33, 495–502. doi: 10.1038/nbt.3192

Schrott, G. (2009). Fine-tuning neural gene expression with microRNAs. *Curr. Opin. Neurobiol.* 19, 213–219. doi: 10.1016/j.conb.2009.05.015

Sherwood, C. C., Holloway, L. R., Erwin, J. M., and Hof, P. R. (2004). Cortical orofacial motor representation in Old World monkeys, great apes and humans: II. Stereologic analysis of architectonich. *Brain Behav. Evol.* 63, 82–106. doi: 10.1159/000075673

Shi, Y., Chichung Lie, D., Taupin, P., Nakashima, K., Ray, J., Yu, R. T., et al. (2004). Expression and function of orphan nuclear receptor TLX in adult neural stem cells. *Nat. Neurosci.* 7, 427, 78–83. doi: 10.1038/nn1002

Shi, Y., Kirwan, P., Smith, J., Robinson, H. P., and Livesey, F. J. (2012). Human neocortical development from pluripotent stem cells to functional excitatory synapses. *Nat. Neurosci.* 15, 477–486. doi: 10.1038/nn.3041
Silbereis, J. C., Pochareddy, S., Zhu, Y., Li, M., and Sestan, N. (2016). The cellular and molecular landscapes of the developing human central nervous system. *Neuron* 89, 248–268. doi: 10.1016/j.neuron.2015.12.008

Smart, I. H., Dehay, C., Giroud, P., Berland, M., and Kennedy, H. (2002). Evolution of the human nervous system function, structure and development. *Annu. Rev. Cell Dev. Biol.* 18, 33–53. doi: 10.1146/annurev.cellbio.18.012801.093915

Tu, J., Cao, D., Li, L., Cheung, H. H., and Chan, W. Y. (2018). MicroRNA profiling during directed differentiation of cortical interneurons from human-induced pluripotent stem cells. *FEBS Open Bio* 8, 502–512. doi: 10.1002/2211-5463.12377

van Rooij, E., Purcell, A. L., and Levin, A. A. (2012). Developing microRNA therapeutics. *Curr. Opin. Pharmacol.* 12, 496–507. doi: 10.1016/j.coph.2012.04.012

Wang, B., Yin, L., Zou, X., Ye, M., Liu, Y., He, T., et al. (2015). A subtype of inhibitory interneuron with intrinsic persistent activity in human and monkey neocortex. *Cell Rep.* 10, 1450–1458. doi: 10.1016/j.celrep.2015.02.018

Winter, J., and Diederichs, S. (2011). Argonaute proteins regulate microRNA stability: increased microRNA abundance by Argonaute proteins is due to microRNA stabilization. *RNA* 8, 1149–1157. doi: 10.4161/rna.8.6.17665

Winter, J., Jung, S., Keller, S., Gregory, R. I., and Diederichs, S. (2009). Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat. Cell Biol.* 11, 228–234. doi: 10.1038/nclb03092-228

Wood, H. (2018). FDA approves patisiran to treat hereditary transthyretin amyloidosis. *Nat. Rev. Neurol.* 14,570. doi: 10.1038/s41585-018-0065-0

Xiang, Y., Tanaka, Y., Cakir, B., Patterson, B., Kim, K. Y., Sun, P., et al. (2019). hESC-derived thalamic organoids form reciprocal projections when fused with cortical organoids. *Cell Stem Cell* 24, 487.e7–497.e7. doi: 10.1016/j.stem.2018.12.015

Xiang, Y., Tanaka, Y., Patterson, B., Kang, Y. J., Voidainia, G., Roselaar, N., et al. (2017). Fusion of regionally specified hPSC-derived organoids models human brain development and interneuron migration. *Cell Stem Cell* 21, 383.e7–398.e7. doi: 10.1016/j.stem.2017.07.007

Yang, L., Yang, Y., Yuan, J., Sun, Y., Dai, J., and Su, B. (2019). Transcriptomic landscape of von economo neurons in human anterior cingulate cortex revealed by microdissected-cell RNA sequencing. *Cereb. Cortex* 29, 838–851. doi: 10.1093/cercor/bhy286

Yao, A. S., Stahl, B. T., Shen, L., and Crabtree, G. R. (2009). MicroRNA-mediated switching of chromatin-remodelling complexes in neural development. *Nature* 460, 642–646. doi: 10.1038/nature08139

Zhang, C., Lu, J., Liu, B., Cui, Q., and Wang, Y. (2016). Primate-specific miR-603 is implicated in the risk and pathogenesis of Alzheimer’s disease. *Aging* 8, 272–290. doi: 10.18632/aging.100887

Zhang, C. L., Zou, Y., He, W., Gage, F. H., and Evans, R. M. (2008). A role for adult TLX-positive neural stem cells in learning and behaviour. *Nature* 451, 1004–1007. doi: 10.1038/nature06562

Zhao, C., Sun, G., Li, S., and Shi, Y. (2009). A feedback regulatory loop involving microRNA-9 and nuclear receptor TLX in neural stem cell fate determination. *Nat. Struct. Mol. Biol.* 16, 365–371. doi: 10.1038/nsmb.1576

Zhao, X., Wu, J., Zheng, M., Gao, F., and Ju, G. (2012). Specification and maintenance of oligodendrocyte precursor cells from neural progenitor cells: involvement of microRNA-7a. *Mol. Biol. Cell* 23, 2867–2878. doi: 10.1091/mbc.e12-04-0270

Zhong, S., Zhang, S., Fan, X., Wu, Q., Yan, L., Dong, J., et al. (2018). A single-cell RNA-seq survey of the developmental landscape of the human prefrontal cortex. *Nature* 555, 524–528. doi: 10.1038/nature25980

Zhu, Y., Sousa, A. M. M., Gao, T., Skarica, M., Li, M., Santpere, G., et al. (2018). microRNA-9 and nuclear receptor TLX in neural stem cell fate determination. *Nat. Struct. Mol. Biol.* 15, 365–371. doi: 10.1038/nsmb.3370

Ziats, M. N., and Rennert, O. M. (2014). Identification of differentially expressed microRNAs across the developing human brain. *PLoS One* 9:e1001214. doi: 10.1371/journal.pbio.1001214

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.