



Recent advances in the involvement of long non-coding RNAs in neural stem cell biology and brain pathophysiology

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INTRODUCTION

A long standing question in biological sciences is how cell diversity, specification patterns, and tissue complexity are generated during organ development. The mammalian brain is the most complex organ of any living organism. The question of how this enormous complexity is generated is still open. Such complexity is the result of millions of years of evolution that have equipped neural stem/progenitor cells (NSCs/NPCs) in the embryo with the ability to generate every neuron and glial cell in the brain via a combined action of extrinsic morphogenetic cues and intrinsic gene regulatory networks. For long time, it was thought that these networks are mainly based on cross-regulatory interactions between transcription factors (TFs) (Jessell, 2000; Politis et al., 2008; Kaltezioti et al., 2010; Martynoga et al., 2012; Stergiopoulos and Politis, 2013). However, the recent advent of sequencing

Exploration of non-coding genome has recently uncovered a growing list of formerly unknown regulatory long non-coding RNAs (lncRNAs) with important functions in stem cell pluripotency, development and homeostasis of several tissues. Although thousands of lncRNAs are expressed in mammalian brain in a highly patterned manner, their roles in brain development have just begun to emerge. Recent data suggest key roles for these molecules in gene regulatory networks controlling neuronal and glial cell differentiation. Analysis of the genomic distribution of genes encoding for lncRNAs indicates a physical association of these regulatory RNAs with transcription factors (TFs) with well-established roles in neural differentiation, suggesting that lncRNAs and TFs may form coherent regulatory networks with important functions in neural stem cells (NSCs). Additionally, many studies show that lncRNAs are involved in the pathophysiology of brain-related diseases/disorders. Here we discuss these observations and investigate the links between lncRNAs, brain development and brain-related diseases. Understanding the functions of lncRNAs in NSCs and brain organogenesis could revolutionize the basic principles of developmental biology and neuroscience.

Keywords: non-coding genome, regulatory RNAs, gene regulatory networks, neural differentiation, neurogenesis, gliogenesis, brain-related diseases

methodologies and experimental data from large-scale consortia focused on characterizing functional genomic elements such as ENCODE and FANTOM, have revolutionized our view for organization, activity, and regulation of the mammalian genome (Carninci et al., 2005; Katayama et al., 2005; Birney et al., 2007). Surprisingly, the vast majority of the genome is transcribed producing not only protein-coding RNAs but also a vast number of different kinds of newly-identified classes of non-coding RNAs, including microRNAs (miRNAs-small non-coding RNAs) and long non-coding RNAs (lncRNAs). miRNAs have been extensively studied while the role of lncRNAs in cell biology together with novel roles of miRNAs in directly regulating lncRNAs have just begun to emerge (Amodio et al., 2012a,b; Rossi et al., 2013; Tay et al., 2014). lncRNAs are transcribed by RNA polymerase II, defined as endogenous cellular RNAs longer than 200 nt in length that lack an ORF, can be post-transcriptional processed by 5' capping, polyadenylation, splicing, RNA editing, and exhibit specific sub-cellular localization (Qureshi and Mehler, 2012). Most importantly, it was recently shown that lncRNAs participate in the gene regulatory networks controlling embryonic stem cell (ESC) pluripotency and metastasis of cancer cells, as well as development and function of various tissues (Guttman et al., 2011; Gutschner and Diederichs, 2012; Qureshi and Mehler, 2012; Yang et al., 2013). Although lncRNAs are the most abundant classes of RNAs (Katayama et al., 2005; Birney et al., 2007; Kapranov et al., 2007b; Kapranov and St Laurent, 2012), they remain poorly characterized and their roles in brain development have just

Abbreviations: lncRNA, long non-coding RNA; lincRNA, large intergenic non-coding RNA; miRNA, microRNA; NSCs, neural stem cells; ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; CNS, central nervous system; SVZ, sub-ventricular zone; ChIP, chromatin immunoprecipitation; RBP, RNA binding protein; RNA-Seq, RNA sequencing; RIP-Chip, RBP immunoprecipitation-microarray (Chip); ChIRP, chromatin isolation by RNA precipitation; CHART, capture hybridization analysis of RNA targets; CLIP, crosslinking-immunoprecipitation; UCSC, University of California Santa Cruz; ORFs, open reading frames; TFs, transcription factors; HAR1, human accelerated region 1; AD, Alzheimer's disease; HD, Huntington's disease; *Dlx1AS*, distal-less homeobox 1 antisense; *Miat*, myocardial infarction associated transcript; *Rmst*, rhabdomyosarcoma 2 associated transcript; *Eyf2*, embryonic ventral forebrain 2; *UtNgn1*, untranslated neurogenin1; *NEAT*, non-coding nuclear enriched abundant transcript.

begun to arise. Notably, it has been proposed that the number of lncRNAs far exceeds the number of protein-coding mRNAs in the mammalian transcriptome (Carninci et al., 2005; Kapranov et al., 2010) and the vast majority of them appears to be expressed in adult brain in a highly specific manner (Qureshi and Mehler, 2012).

In the last few years, many groups have focused their efforts toward understanding the actions of lncRNAs in brain function and evolution. In this mini-review, we highlight the emerging evidence of the involvement of this new class of RNA molecules in NSC biology during development and adulthood, in health and disease. In particular, we summarize recent progress regarding the participation of lncRNAs in the regulatory networks that control the fine balance between proliferation and differentiation decisions of NSCs. The ability of lncRNAs to interact with both genomic loci and protein products of TF genes appears to endow them with a remarkable capacity to control NSC maintenance and differentiation, suggesting an undoubtedly important influence on the pathophysiology of several neurodegenerative disorders (Kapranov et al., 2007a,b; Guttman et al., 2009; Khalil et al., 2009; Orom et al., 2010; Bian and Sun, 2011; Qureshi and Mehler, 2012; Spadaro and Bredy, 2012; Ng et al., 2013).

LncRNAs: NEW PLAYERS IN CELL BIOLOGY

LncRNAs can be classified as bidirectional, intronic, intergenic, sense, antisense or 3'-UTR (untranslated region) transcripts with respect to nearby protein-coding genes (Mercer et al., 2009; Derrien et al., 2012). Some lncRNAs are exported from nucleus and perform important functions in the cytoplasm, but more often they are found in nucleus, particularly associated with chromatin (Saxena and Carninci, 2011). In more detail, these transcripts may be bound by proteins, other RNA molecules or even fulfil intrinsic catalytic functions (Fedor and Williamson, 2005; Ye, 2007; Guttman and Rinn, 2012). The vast majority of these transcripts are not translated, supporting a role as non-coding RNAs rather than protein precursors (Banfai et al., 2012; Derrien et al., 2012). Furthermore, lncRNAs are developmentally regulated, expressed in specific cell types, associated with chromatin signatures indicating transcriptional regulation, are under evolutionary constraint and associated with carcinogenesis and other diseases; all of which support meaningful roles of lncRNAs (Qureshi and Mehler, 2012; Fatica and Bozzoni, 2014). LncRNAs also participate in a striking diversity of cellular processes including transcriptional regulation, genomic imprinting, alternative splicing, mRNA stability, translational control, DNA damage response, cell cycle regulation and organelle biogenesis (Rinn and Chang, 2012). Interestingly, it seems that knock-down or over-expression of many of these lncRNAs produce phenotypes that are very well correlated with their aberrant expression in disease states (Qureshi et al., 2010; Gutschner and Diederichs, 2012; Qureshi and Mehler, 2012). Therefore, we believe that lncRNA transcripts would eventually occupy a central place in our understanding of the molecular mechanisms underlying human diseases.

Systematical profiling and comprehensive annotation of lncRNAs in various cell types can shed light on the functionalities of these novel regulators. LncRNA-protein interactions can be

currently predicted, approached and analyzed by various research advanced tools and strategies apart from bioinformatics, including high-throughput analysis of lncRNA expression [microarrays, RNA sequencing [RNA-Seq] (Mortazavi et al., 2008; Wang et al., 2009; Ilott and Ponting, 2013), RNA CaptureSeq, iSeeRNA [support vector machine (SVM)-based classifier] (Sun et al., 2013), BRIC-Seq [50-bromo-uridine (BrU) immunoprecipitation chase-deep sequencing analysis] (Imamachi et al., 2013)], RIP [RNA-binding protein (RBP) immunoprecipitation] coupled with microarray and high-throughput sequencing (RIP-Chip/Seq respectively) (Zhao et al., 2010; Jain et al., 2011), ChIRP (chromatin isolation by RNA purification) and CHART (capture hybridization analysis of RNA targets—High-throughput finding of RBPs and DNAs) as well as CLIP (crosslinking-immunoprecipitation)-Seq, which provides transcriptome-wide coverage for mapping RBP-binding sites (Khalil et al., 2009; Wu et al., 2010; Chu et al., 2011; Zhu et al., 2013).

LncRNAs IN NEURAL STEM CELLS

These technologies have greatly helped the efforts to elucidate the role of lncRNAs in NSCs, neural differentiation, migration and maturation. Toward this goal, Ramos and colleagues identified and predicted regulatory roles for more than 12,000 novel lncRNAs (2,265 lncRNAs had proximal protein-coding gene neighbors) in the subventricular zone (SVZ) of adult mice. In FACS-sorted NSCs, they found a unique lncRNA expression pattern for each of the three stages of neurogenesis analyzed. Moreover, by using ChIP-Seq (chromatin immunoprecipitation-sequencing), lncRNAs were shown to be transcriptionally regulated in a manner analogous to mRNAs, enabling the prediction of lncRNAs that may function in the glial-neuronal lineage specification of multipotent adult NSCs (Ramos et al., 2013; Wang et al., 2013). Further transcriptome-next-generation-sequencing revealed that lncRNA-mediated alternative splicing of cell fate determinants controls stem-cell commitment during neurogenesis. Specifically, Aprea and co-workers identified several genic and intergenic lncRNAs that are functionally involved in neurogenic commitment, including *Cosl1*, *Btg2-AS1*, *Gm17566*, *Miat* (Myocardial infarction associated transcript-*Gomafu*), *Rmst* (Rhabdomyosarcoma 2 associated transcript), *Gm17566*, *Gm14207*, *Gm16758*, *2610307P16Rik*, *AC102815.1*, *C230034O21Rik* and *9930014A18Rik* (Aprea et al., 2013). Accordingly, subsequent studies using high-throughput transcriptomic data (microarray platform) to examine lncRNA differential expression in NSCs, GABAergic neurons and oligodendrocytes, led to the identification of lncRNAs that are dynamically regulated during neural lineage specification, neuronal-glia fate switching and oligodendrocyte maturation [i.e., *Dlx1AS* (Distal-less homeobox 1 antisense), *Evf2* (Embryonic ventral forebrain 2), *Rmst*, *utNgn1* (untranslated Neurogenin1), *MALAT1* (metastasis-associated lung adenocarcinoma transcript 1; also named *NEAT2*)] (Mercer et al., 2010; Qureshi and Mehler, 2012; Ng et al., 2013). ChIP-Seq approaches were also developed to generate and investigate genome-wide chromatin-state maps. These functional analyses predicted hypothetical roles for 150 mammalian lncRNAs (long intergenic non-coding RNAs) in a “guilt-by-association” manner in NSCs.

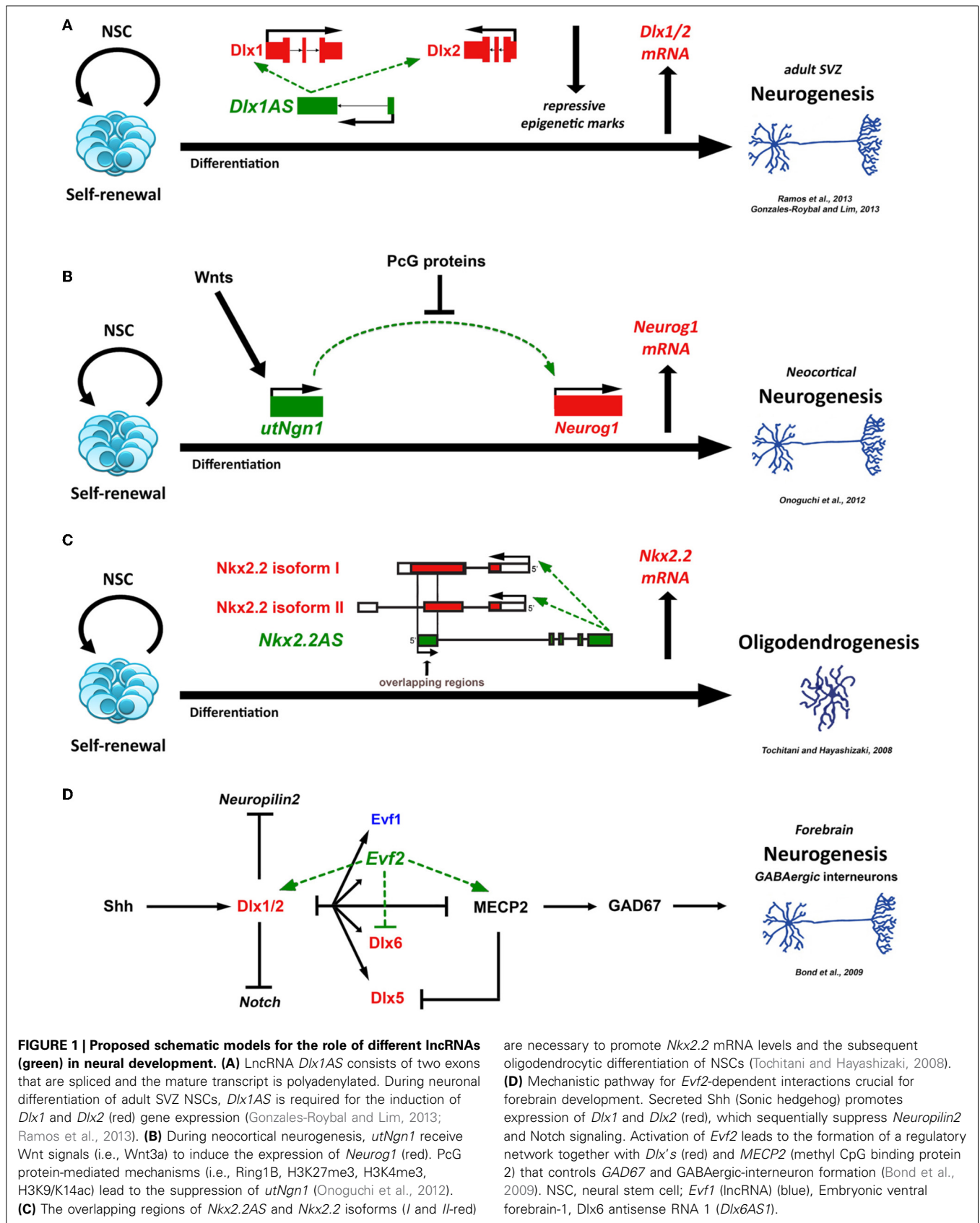


FIGURE 1 | Proposed schematic models for the role of different lncRNAs (green) in neural development. (A) LncRNA *Dlx1AS* consists of two exons that are spliced and the mature transcript is polyadenylated. During neuronal differentiation of adult SVZ NSCs, *Dlx1AS* is required for the induction of *Dlx1* and *Dlx2* (red) gene expression (Gonzales-Roybal and Lim, 2013; Ramos et al., 2013). **(B)** During neocortical neurogenesis, *utNgn1* receive Wnt signals (i.e., Wnt3a) to induce the expression of *Neurog1* (red). PcG protein-mediated mechanisms (i.e., Ring1B, H3K27me3, H3K4me3, H3K9/K14ac) lead to the suppression of *utNgn1* (Onoguchi et al., 2012). **(C)** The overlapping regions of *Nkx2.2AS* and *Nkx2.2* isoforms (I and II-red)

are necessary to promote *Nkx2.2* mRNA levels and the subsequent oligodendrocytic differentiation of NSCs (Tochitani and Hayashizaki, 2008). **(D)** Mechanistic pathway for *Evf2*-dependent interactions crucial for forebrain development. Secreted Shh (Sonic hedgehog) promotes expression of *Dlx1* and *Dlx2* (red), which sequentially suppress *Neuropilin2* and Notch signaling. Activation of *Evf2* leads to the formation of a regulatory network together with *Dlx*'s (red) and *MECP2* (methyl CpG binding protein 2) that controls *GAD67* and GABAergic-interneuron formation (Bond et al., 2009). NSC, neural stem cell; *Evf1* (lncRNA) (blue), Embryonic ventral forebrain-1, *Dlx6* antisense RNA 1 (*Dlx6AS1*).

In particular, specific lincRNAs appeared to localize near genes encoding key TFs (such as *Sox2*, *Klf4*, *Myc*, *p53*, *NFkB* and *Brn1*) that are involved in processes ranging from hippocampal development to neuronal and oligodendrocyte maturation (Guttman et al., 2009; Qureshi and Mehler, 2012).

More concisely, Ramos et al. have also performed *in vitro* knock-down studies and observed that *Dlx1AS*, a lincRNA encoded from a bigene *Dlx1/2* cluster, is associated with fate determination of adult SVZ NSCs via positive regulation of *Dlx1* and *Dlx2* gene expression. Mechanistically, enhanced transcription of *Dlx1AS* occurs during neurogenesis when H3K27me3 (trimethylation of histone H3 Lys-27) repression is decreased. The H3K27me3-specific demethylase JMJD3 was also found to be enriched at the *Dlx1AS* locus (**Figure 1A**) (Gonzales-Roybal and Lim, 2013; Ramos et al., 2013). Recently, a comprehensive study came up with the finding that *UtNgn1* is a non-coding RNA transcribed from an enhancer region of the *Neurogenin1* (*Neurog1*) locus. This non-coding transcript seems to positively regulate *Neurog1* transcription during neuronal differentiation of NSCs. In detail, during the late stage of neocortical NSC development, *utNgn1* expression is up-regulated via involvement of Wnt signaling whereas it is down-regulated by PcG (polycomb group) proteins (**Figure 1B**) (Onoguchi et al., 2012). Additionally, two lincRNAs, nuclear enriched abundant transcripts *NEAT1* and *NEAT2* (*MALAT1*) are up-regulated in neuronal and glial progeny, and are associated with neuronal activity, growth and branching (Mercer et al., 2010; Ng et al., 2013). *Miat* (also called *Gomafu*) is one of the known non-coding RNAs that participates in neuronal subtype-specific determination and is also highly expressed in oligodendrocyte lineage specification (Aprea et al., 2013). In the same lineage, another lincRNA, *Nkx2.2AS*, an antisense RNA of the known homeobox TF *Nkx2.2*, appears to lead NSCs into oligodendrocytic fate through modest induction of *Nkx2.2* gene. By using deletion mutants, Tochitani and Hayashizaki showed that the overlapping regions of *Nkx2.2AS* and *Nkx2.2* isoforms are required for promoting *Nkx2.2* mRNA levels and the subsequent oligodendrocytic differentiation of NSCs (**Figure 1C**) (Tochitani and Hayashizaki, 2008). *Evf2* is predominantly detected in the developing forebrain, both in human and mouse, and is critical for GABAergic-interneuron formation. Like other lincRNAs, *Evf2* seems to control the expression of specific genes that are necessary during brain development, such as *Dlx5*, *Dlx6* and *Gad1*, through distinct *trans*- and *cis*-acting mechanisms (**Figure 1D**) (Bond et al., 2009). Interestingly, *Rmst* is presented to be a novel marker for dopaminergic neurons during NSC differentiation, where it is co-expressed with the midbrain-specific TF *Lmx1a* (Uhde et al., 2010).

Considering the tight connection between pluripotency of human ESCs and their differentiation into NSCs (ESC-derived NSCs) and subsequently into neurons, Ng and co-workers extensively showed that diverse lincRNAs are essential for ESC proliferation and neurogenesis through physical interaction with master TFs (e.g., *Sox2*). In detail, microarray data analysis led to the identification of 35 neuronal lincRNAs with important roles in neuronal differentiation, e.g., *Rmst* (*AK056164*, *AF429305* and *AF429306*), *lincRNA_N1* (*AK124684*), *lincRNA_N2* (*AK091713*),

Table 1 | List of genes encoding TFs with critical roles in brain development that also contain lincRNA genes in close proximity to their genomic loci (<3,000 bp).

Genes encoding for TFs	LncRNAs	Position
Ascl4	AK018959	Sense
Atoh7	AK005214	Sense
Crebbp	4930455F16Rik	Intergenic
Crx	CrxOS	Bidirectional
Ctnnb1	4930593C16Rik	Bidirectional
Cux2	AK006762	Sense
Cux2	AK187608	Bidirectional
Dlx1	Dlx1AS	Antisense
Dlx4	A730090H04Rik	Bidirectional
Dlx6	Dlx6AS1	Antisense
Dlx6	Dlx6AS2	Intronic
Emx2	Emx2OS	Bidirectional
Evx1	5730457N03Rik	Bidirectional
Fezf1	Fezf1-AS1	Bidirectional
Fezf1	AK086573	Intergenic
FoxA2	AK156045	Antisense
FoxG1	AK158887	Intergenic
FoxG1	3110039M20Rik	Sense
Gata1	S57880	Sense
Gata2	AK137172	Sense
Gata3	4930412013R	Bidirectional
Gata4	AK031341	Intronic
Gata6	AK033147	Bidirectional
Gata6	AK003136	Bidirectional
Gbx2	D130058E05Rik	Bidirectional
Gli1	AK157048	Sense
Gli2	AK054469	Intronic
Gli3	AK135998	Intergenic
Hmx1	E130018O15Rik	Bidirectional
Irx2	Gm20554	Bidirectional
Lef1	Lef1-AS1	Bidirectional
Lhx1	Lhx1OS	Bidirectional
Lhx3	AK035055	Bidirectional
Lhx8	AI606473	Bidirectional
Lmx1b	C130021I20	Bidirectional
Lxrb (NR1H2)	AK184603	3'-UTR
Meis1	AK144295	Antisense
Meis2	AK012325	Sense
Meis2	AK144367	Bidirectional
Meis2	AK144485	Bidirectional
Msx1	Msx1AS	Antisense
MycN	MYCNOS	Bidirectional
Myt1L	AK138505	Antisense
NFATc1	AK155068	3'-UTR
NFATc4	AK014164	Sense
NFIA	E130114P18Rik	Bidirectional
NFIB	AK081607	Intronic
NFIX	AK168184	Antisense
NFKB2	AK029443	Bidirectional
Ngn1	AK016084	Intergenic

(Continued)

Table 1 | Continued

Genes encoding for TFs	LncRNAs	Position
Nkx2.2	Nkx2.2AS	Antisense
Notch1	AK075572	Antisense
NR2F1	AK051417	Intergenic
NR2F1	A830082K12Rik	Bidirectional
NR2F2	AK135306	Intronic
NR3C2	Gm10649	Bidirectional
NR4A2	BB557941	Intergenic
NR5A2 (Irh-1)	AK178198	Antisense
NR5A2 (Irh-1)	AK145521	Intronic
Otx2	Otx2OS	Bidirectional
Pax2	AK006641	Antisense
Pax6	AK044354	Antisense
Pbx3	AK138624	Intronic
Pou4F1	AK084042	Intergenic
PPARd	AK033897	Intronic
PPARd	AK007468	Intronic
Prox1	AK142161	Bidirectional
Ptf1a	AK053418	Antisense
RARa	AK031732	Intronic
RARb	AK052306	3'-UTR
RBPJK	AK164362	Intronic
Runx1	AK131747	Intergenic
SATB2	9130024F11Rik	Bidirectional
Six1	AK035085	3'-UTR
Six3	Six3OS1	Bidirectional
Six6	4930447C04Rik	Bidirectional
Sox1	Gm5607	Sense
Sox10	Gm10863	Bidirectional
Sox2	Sox2OT	Sense
Sox21	AK039417	Bidirectional
Sox8	AK079380	Bidirectional
Sox9	BC006965	Bidirectional
STAT5b	AK088966	Intronic
Tgif2	5430405H02Rik	3'-UTR
THRA	AK165172	Intronic
THRB	AK088911	Antisense
WT1	AK033304	Intronic
WT1	AI314831	Bidirectional
Zeb1	AK041408	Intronic
Zeb1	Gm10125	Bidirectional
Zeb2	Zeb2OS	Bidirectional

Some of these TFs contain more than one lncRNA near to their genes (duplicates). The position of each lncRNA relatively to its nearby protein-coding gene is indicated at the third column of the table.

lncRNA_N3 (AK055040, etc.). They also suggested that these lncRNAs are required for generation of neurons and suppression of gliogenesis by association with chromatin modifiers and nuclear proteins (Ng et al., 2012). In agreement, another study that aimed at identifying functional features of lncRNAs during ESC differentiation to adult cerebellum by utilizing RNA-Seq and ChIP-Seq technologies, demonstrated that filtered novel lncRNAs are located close to protein-coding genes involved in functions such as neuronal differentiation and transcriptional

regulation (Guttman et al., 2009; Lv et al., 2013). Particular studies have been successful in identifying *anti-NOS2A* as an antisense transcript of the *NOS2A* (Nitric oxide synthase 2 enzyme) gene, an isoform of the NOS protein which induces hESC differentiation into neurogenic precursors (Korneev et al., 2008). Specific expression pattern was also detected for *Otx2c* (Orthodenticle homeobox 2 c), an alternative splicing variant of the pre-mRNA *Otx2* with a possible role in neural differentiation of hESCs (Liu et al., 2013). Another lncRNA that is correlated with the proliferation state of ESCs is *lincRNA-Sox2*, which is located at the promoter of *Sox2* locus and is regulated by *Sox2* and *Oct4* (Guttman et al., 2009). Finally, a thorough targeted RNA-Seq analysis carried out using neurons derived from patient-specific induced pluripotent stem cells (iPSCs) showed that more than 1,500 lncRNAs are dynamically regulated during differentiation of iPSCs toward glutamatergic neurons. Particularly, the expression of 1,622 non-coding genes (lncRNAs/lincRNAs) was dramatically affected during conversion from iPSCs to differentiating neurons, while alternative splicing occurred. Significant alterations were also observed in the expression patterns of non-coding genes involved in neuropsychiatric disorders (Lin et al., 2011; Qureshi and Mehler, 2012; Akula et al., 2014).

LncRNAs IN BRAIN FUNCTION, EVOLUTION AND NEUROLOGICAL DISEASES

The importance of lncRNAs in the brain is explicitly highlighted by the observation that most of them are expressed in the adult mammalian brain in highly regional-, cellular- and sub-cellular compartment-specific and neuronal activity dependent profiles (Mercer et al., 2008; Ponjavic et al., 2009; Qureshi and Mehler, 2012). In fact, it seems that lncRNAs have played important roles in the evolution of the form and function of human brain, since the fastest evolving regions of the primate genome are non-coding sequences that generate lncRNAs implicated in the modulation of neuro-developmental genes (Pollard et al., 2006; Qureshi and Mehler, 2012). The most dramatic of these regions, called *HAR1* (human accelerated region 1), is part of a novel lncRNA gene (*HAR1F*) that is expressed specifically in Cajal–Retzius neurons in the developing human neocortex from 7 to 19 gestational weeks, a crucial period for cortical neuron-specification and migration. Additionally, analysis of highly conserved lncRNAs in birds, marsupials and eutherian mammals revealed a remarkable similarity in spatiotemporal expression profiles of orthologous lncRNAs, suggesting ancient roles during brain development (Chodroff et al., 2010). Moreover, lncRNAs directly modulate synaptic processes (*MALAT1*), protein synthesis (*BC1*), learning and memory (*Tsx*), and are associated with neuronal activity in mouse cortex (Qureshi and Mehler, 2012). In agreement with these effects on neurons, lncRNAs have also been implicated in the pathophysiology of neuro-developmental, neurodegenerative, neuro-immunological and neuro-oncological diseases/disorders. For instance, high levels of *BC1/BC200* and *BACE1-AS* have been implicated in Alzheimer's disease (AD) while *NEAT1*'s in Huntington's disease (HD). *BC1/BC200* normally seems to selectively regulate local protein synthesis in post-synaptic dendritic compartments, by repressing translation via an *eIF4-A*-dependent mechanism (Qureshi et al., 2010; Niland et al., 2012).

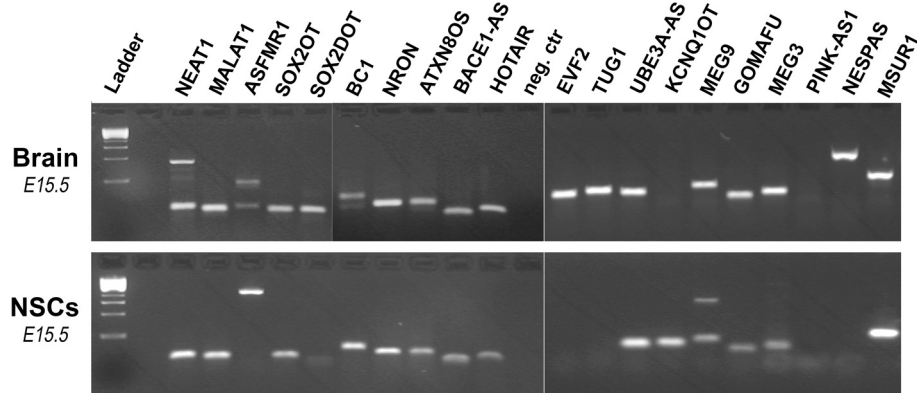


FIGURE 2 | RT-PCR-based detection of various lncRNAs associated with CNS-related diseases or disorders. Experiments were performed using cDNA samples derived from embryonic mouse brain (*E15.5*) or NSCs isolated

from embryonic mouse telencephalon and cultured *ex vivo*, as indicated. In some cases alternative splicing isoforms are also evident. RT-PCR primer sequences are available upon request.

De-regulation of *BACE1-AS* has been shown to be responsible for feed-forward induction of *BACE1* (β -secretase); thus leading to amyloid β ($A\beta$) increased production and possible AD pathogenesis (Qureshi et al., 2010). Additionally, *NEAT1* is a lncRNA known to be involved in cell death mechanisms. In particular, *NEAT1* controls target gene transcription by protein sequestration into paraspeckles (stress-responsive sub-nuclear structures), a potentially dysfunctional pathway in HD progression. In accordance, Hirose and co-workers have very recently shown that *NEAT1* transcriptional up-regulation leads to the enlargement of these structures after proteasome inhibition (Johnson, 2012; Hirose et al., 2014). Various lncRNAs have also been correlated with Parkinson's disease (*PINK-AS1*, *UCHL1-AS1*), amyotrophic lateral sclerosis (*MSUR1*), Down's syndrome (*NRON*) as well as with tumor progression (*ANRIL*, *MALAT1*, *HOTAIR*, *NOS3AS*) (Gutschner and Diederichs, 2012). A common theme emerging from all these cases is that lncRNAs control the expression of nearby protein-coding genes *in cis* and de-regulation of this relationship could lead to nervous system-related diseases. In agreement, brain-expressed lncRNAs are preferentially located adjacent to protein-coding genes that are also expressed in the brain and involved in transcriptional regulation and/or nervous system development (Mercer et al., 2008; Ponjavic et al., 2009). Many of these pairs often exhibited coordinated expression during developmental transitions. Therefore, it was suggested that these lncRNAs may influence the expression of the associated protein-coding genes similarly to previously characterized examples such as *Nkx2.2AS*, *HOTAIR*, *p15AS*, *p21AS* and *Evf2*.

PERSPECTIVE LINKS BETWEEN lncRNAs AND BRAIN DEVELOPMENT

Over recent years, it has become more and more evident that lncRNAs play key roles in gene regulatory networks controlling nervous system development. To initially investigate this link, we bioinformatically screened numerous mouse genes encoding TFs with well-established roles in CNS (central nervous system) development for close proximity with lncRNA genes (based on publicly available data from UCSC genome-browser).

Accordingly, we managed to identify 79 such TF genes encompassing 91 transcriptional units for lncRNA genes in close proximity (<3,000 base pairs-bp) (Table 1). In particular, 40.65% of these lncRNAs are bidirectional, 16.48% intronic, 9.89% intergenic, 12.08% sense, 15.38% antisense and 5.49% 3'-UTR. The majority of these lncRNAs have only been identified in genome-wide expression screens, are expressed in nervous system, and intriguingly, their functions are totally unknown. Some of them have been reported to interact with TFs or chromatin-associated complexes (Wang and Chang, 2011; Guttman and Rinn, 2012; Rinn and Chang, 2012), indicating potential contribution to the combinatorial transcriptional codes involved in NSC maintenance, sub-type/lineage specification and terminal differentiation. Based on these preliminary evidence, we postulate that lncRNAs and TFs with regulatory role in neurogenesis are interconnected and may form coherent cross-regulatory networks that are associated with CNS development. However, this hypothesis needs to be extensively interrogated in NSCs. Moreover, considering the fact that some TFs are used as reprogramming tools for the production of iPSCs, induced neurons, induced NSCs, cardiomyocytes, etc. (Yang et al., 2011), these lncRNAs may be utilized for the production of clinically useful cell types in the near future, revolutionizing the basic principles of developmental/cell biology as well as neuroscience.

To further examine the links between lncRNAs, brain development and neurological disorders, we tested whether lncRNAs, associated with nervous system-related diseases in humans, are expressed in embryonic mouse brain (*E15.5*) or *ex vivo* cultured NSCs (isolated from mouse telencephalon, *E15.5*). The rationale for these experiments was based on observations showing many neurological diseases to be initiated very early during brain maturation and development (Bian and Sun, 2011; Qureshi and Mehler, 2012). Specifically, we first identified *in silico* and then tested for expression 20 such lncRNAs with clear conservation in mouse genome. Interestingly, RT-PCR (reverse transcription-PCR) analysis revealed that most lncRNAs are expressed in both brain and NSCs (Figure 2), suggesting potential involvement in brain development. Moreover, a possible function of these

lncRNAs in NSCs may contribute to their roles in neurological diseases. Nevertheless, further investigation and initiation of new experimental studies are needed to uncover the exact function of lncRNAs in NSCs and nervous system in general.

CONCLUSIONS

LncRNAs represent a new exciting frontier in molecular biology with major roles in NSC fate decisions, specification and commitment. Full understanding of how these non-coding transcripts regulate the expression of protein-coding genes and participate in gene regulatory circuitries could lead to the discovery of novel cellular/molecular mechanisms and signaling pathways involved in neural development; thus having potential implications for the treatment of nervous system-related diseases and traumas.

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