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Hes genes and neurogenin regulate non-neural versus neural fate specification in the dorsal telencephalic midline

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The choroid plexus in the brain is unique because it is a non-neural secretory tissue. It secretes the cerebrospinal fluid and functions as a blood-brain barrier, but the precise mechanism of specification of this non-neural tissue has not yet been determined. Using mouse embryos and lineage-tracing analysis, we found that the prospective choroid plexus region initially gives rise to Cajal-Retzius cells, specialized neurons that guide neuronal migration. Inactivation of the bHLH repressor genes *Hes1*, *Hes3* and *Hes5* upregulated expression of the proneural gene neurogenin 2 (*Ngn2*) and prematurely depleted Bmp-expressing progenitor cells, leading to enhanced formation of Cajal-Retzius cells and complete loss of choroid plexus epithelial cells. Overexpression of *Ngn2* had similar effects. These data indicate that Hes genes promote specification of the fate of choroid plexus epithelial cells rather than the fate of Cajal-Retzius cells by antagonizing *Ngn2* in the dorsal telencephalic midline region, and thus this study has identified a novel role for bHLH genes in the process of deciding which cells will have a non-neural versus a neural fate.

KEY WORDS: Cajal-Retzius cells, Choroid plexus, Hes1, Hes5, Neurogenin, Mouse

INTRODUCTION

The telencephalic hemispheres are formed by bilateral evagination of the anterior end of the neural tube. The dorsal telencephalon is further subdivided along the medial-lateral axis into three regions. The most lateral region becomes cortical neuroepithelium, which later gives rise to neurons and glial cells of the cerebral cortex. The medial region (the dorsal telencephalic midline region) is divided into the most medial part, the choroid plexus epithelium, and an intermediate part, the cortical hem, which is a major source of Cajal-Retzius cells of the neocortex (Grove et al., 1998; Meyer et al., 2002; Takiguchi-Hayashi et al., 2004; Yoshida et al., 2005). Cajal-Retzius cells are distributed in the neocortex and guide neuronal migration. It has been shown that this medial-lateral patterning of the dorsal telencephalon is regulated by a combination of transcription factors and secreted signaling factors. For example, the homeodomain transcription factors Msx1/2 and Lhx2/Foxg1 (Bf1) are involved in the development of the choroid plexus and the cortical neuroepithelium, respectively, whereas secreted factors such as Bmps regulate specification of the choroid plexus epithelium by inducing *Msx1* and repressing *Lhx2*/*Foxg1* expression (Bach et al., 2003; Xuan et al., 1995; Furuta et al., 1997; Porter et al., 1997; Monuki et al., 2001; Panchision et al., 2001; Hébert et al., 2002; Fernandes et al., 2007).

The choroid plexus is unique in the brain, because it is a non-neural secretory tissue. It produces the cerebrospinal fluid and functions as a blood-brain barrier. The choroid plexus derives from both epithelial and mesenchymal components, with the epithelium facing the ventricular lumen. The choroid plexus epithelial cells are generated from neuroepithelial cells like other cell types of the central nervous system, such as neurons, astrocytes and oligodendrocytes (Sturrock, 1979; Thomas and Dziadek, 1993; Awatramani et al., 2003; Currle et al., 2005; Hunter and Dymecki, 2007). The role of Bmp signaling in the development of the choroid plexus has been intensively analyzed. It has been shown that misexpression of the constitutively active form of Bmp receptors results in an expansion of the choroid plexus at the expense of the cortical neuroepithelium (Panchision et al., 2001), whereas inactivation of the Bmp receptor results in defects of specification of choroid plexus epithelial cells (Hébert et al., 2002; Fernandes et al., 2007). Bmp signaling induces expression of the homeodomain factors Msx1/2, which are involved in the development of the dorsal midline region (Bach et al., 2003; Hébert et al., 2002). However, the precise mechanism underlying generation of this non-neural tissue during the development of the nervous system is, as yet, undetermined.

It is well known that in *Drosophila*, the basic helix-loop-helix (bHLH) repressor genes of *hairy* and *Enhancer of split* [*E*(*spl*)] regulate non-neural versus neural fate specification in the ectoderm (Campos-Ortega and Jan, 1991). Cells expressing proneural bHLH genes at higher levels, such as the *achaete-scute* complex, adopt the neural fate and express Delta, which then activates Notch signaling of neighboring cells. Activation of Notch signaling leads to upregulation of *E*(*spl*) genes, which promote non-neural fate specification by repressing proneural genes (lateral inhibition). Thus, proneural and *E*(*spl*) genes antagonistically regulate neural versus non-neural cell fate specification. These results raise the possibility that the bHLH repressor genes such as Hes genes, mammalian *hairy* and *E*(*spl*) homologues (Kageyama et al., 2007), are likewise involved in the formation of non-neural tissues in the developing mammalian brain. Although it has been shown that Hes genes repress proneural gene expression (Ishibashi et al., 1995; Chen et al., 1997; Hatakeyama et al., 2004), no previous analyses have shown that Hes genes regulate non-neural versus neural fate specification in the mammalian brain. Hes genes have been shown to maintain neural progenitors or to promote gliogenesis (Ross et al., 2003; Kageyama et al., 2007; Miller and Gauthier, 2007).

In this study, we found that the prospective choroid plexus epithelium of the telencephalon expresses both the proneural bHLH gene neurogenin 2 (*Ngn2*) and the repressor genes *Hes1* and *Hes5*, and gives rise to two cell lineages: choroid plexus epithelial cells and

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Cajal-Retzius cells. Furthermore, *Hes1*-, *Hes3*- and *Hes5*-null mutations lead to the upregulation of *Ngn2*, to a lack of choroid plexus epithelial cells and to the promotion of Cajal-Retzius cell differentiation. Overexpression of *Ngn2* had similar effects. These results suggest that *Hes* and *Ngn2* genes antagonistically regulate the specification of non-neural (choroid plexus) versus neural (Cajal-Retzius cell) fate in the mouse brain.

MATERIALS AND METHODS

In utero microelectroporation

Mouse *Ngn2* cDNA in pEF-BOS was introduced into the developing brain by microelectroporation, as described previously (Fukuchi-Shimogori and Grove, 2001; Fukuchi-Shimogori and Grove, 2003). Transfected cells were monitored by co-electroporation of pEF-EGFP.

Generation of *Hes1* floxed mice

The floxed *Hes1* targeting vector (see Fig. 4A) was linearized with *Not*I and transfected into TT2 cells, and G418-resistant clones were selected. Genomic DNA was digested with *Hin*dIII and analyzed by Southern blot using a 0.6 kb *Hin*dIII-*Bam*HI fragment as a 5′-probe. Neomycin selection cassette was removed by transient Cre expression in the targeted TT2 cells. Genotypes were determined by PCR using the following primers: 5′-CAGCCAGTGTCAACACGACACCGGACAAAC-3′ and 5′-TGCCCT-TCGCCTCTTCTCCATGATA-3′. The sizes of PCR products for floxed and wild-type alleles are 272 bp and 224 bp, respectively.

Mice breeding

Hes1 conditional knockout (cKO) mice were obtained by crossing homozygous *Hes1* floxed mice with Emx1-Cre (Iwasato et al., 2000);*Hes1*$^{+/–}$ (Ishibashi et al., 1995) mice. *Hes1*;*Hes3*;*Hes5* cKO mice were acquired by crossing *Hes1*(floxed/floxed);*Hes3*$^{–/–}$;*Hes5*$^{–/–}$ with Emx1-Cre;*Hes1*$^{+/–}$;*Hes3*$^{–/–}$;*Hes5*$^{–/–}$. Emx1-Cre;*Hes1*(floxed/+);*Hes3*$^{–/–}$;*Hes5*$^{–/–}$ embryos were normal and used as control. Nes-CreER^{T2};*Hes1*;*Hes3*;*Hes5* cKO mice were acquired by crossing *Hes1*(floxed/floxed);*Hes3*$^{–/–}$;*Hes5*$^{–/–}$ with Nes-CreER^{T2} line5-1;*Hes1*$^{+/–}$;*Hes3*$^{–/–}$;*Hes5*$^{–/–}$. Nes-CreER^{T2} line5-1;*Hes1*(floxed/+);*Hes3*$^{–/–}$;*Hes5*$^{–/–}$ embryos were normal and were used as control. Tamoxifen (6 mg/35 g body weight) was administered by oral gavage to the pregnant mice at E9.5. *Rbpj* cKO mice were obtained by crossing Emx1-Cre;*Rbp-j*$^{+/–}$ with homozygous *Rbp-j* floxed mice. These mice were maintained on ICR or C57BL/6;ICR mixed background.

Generation of pMsx1-EGFP mice

A transgene containing 5 kb upstream fragment of *Msx1* gene (MacKenzie et al., 1997; Takahashi et al., 1997), EGFP cDNA and SV40 poly-adenylation sequence was used to generate pMsx1-EGFP mice. Mice were analyzed at E10.0 (*n*=5), E10.5 (*n*=4) and E11.5 (*n*=4).

Histochemistry and in situ hybridization

X-gal staining was performed as described previously (Imayoshi et al., 2006). Immunohistochemistry was performed as described previously (Imayoshi et al., 2006) with primary antibodies against β-tubulin III/Tuj1 (Covance), GFP (Molecular Probes), reelin (Calbiochem), Msx1/2 (DSHB, The University of Iowa, Department of Biological Sciences), Ngn2 (Santacruz), doublecortin (DCX) (Santacruz), p73 (Neomarkers), Calretinin (Swant) and Hes1 (aa86-278), which was produced as previously described (Baek et al., 2006). Goat or donkey anti-species IgG conjugated with Alexa 488 or Alexa 594 (Molecular Probes) were used as secondary antibodies. Sections were analyzed with LSM510 confocal microscopy. In situ hybridization was carried out as described previously (Ohsawa et al., 2005) using mouse reelin, *p73*, *Math2*, *Slit1*, *Robo1*, *Er81* (GenBank Accession Number, BI901885; IMAGE, 5663418), *Cux2*, *Rorb* (GenBank Accession Number; IMAGE, 6490704), *Prox1*, *Steel*, *Ka1*, *Nt3*, *Big1* (Shinozaki et al., 2004), *Hey1*, *Hey2*, *Bmpr1a*, *Lmx1a*, *Ttr*, *Wnt3a*, *Mash1*, *Egfp*, *Msx1*, *Wnt3a, Wnt2b* (GenBank Accession Number, AI893147; IMAGE, 353765), *Bmp4* (GenBank Accession Number, AA473799; IMAGE, 873328), *Hes1*, *Hes5, Msx2, Foxg1* (GenBank Accession Number, AI893944; IMAGE, 388688), *Lhx2*, *Lhx5*, *Ngn2* and *Ngn1* probes.

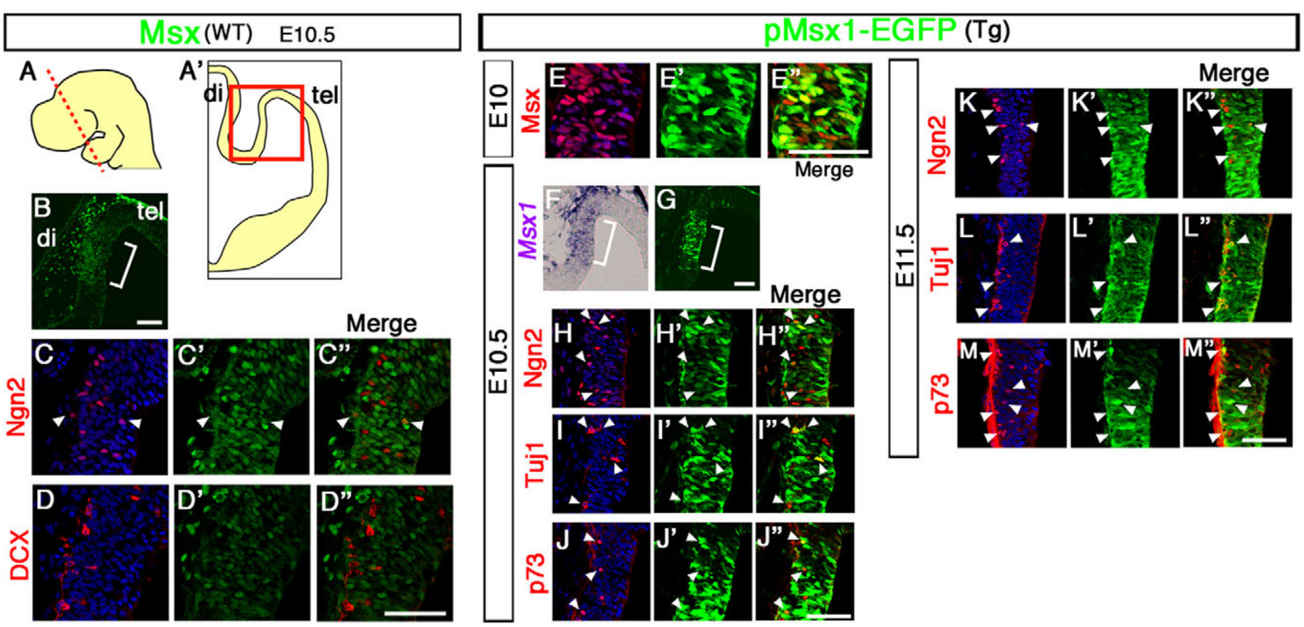

Fig. 1. Formation of Cajal-Retzius cells from Msx1⁺ domain of the dorsal telencephalic midline. (**A,A′**) Orientation of sections. (**B-D″**) Coronal sections of E10.5 embryos. Msx1 was expressed in the prospective choroid plexus region (B). Some Msx1⁺ cells expressed Ngn2 (C-C″, arrowheads) but not DCX (D-D″). (**E-M″**) Transgenic mice carrying the *Msx1* promoter-driven EGFP reporter were examined for lineage tracing of Msx1⁺ cells. At E10.0, EGFP was specifically expressed by Msx1⁺ cells (E-E″). At E10.5, EGFP was expressed in the prospective choroid plexus region (G) like the endogenous *Msx1* (F). Subsets of EGFP⁺ cells expressed Ngn2 (H-H″,K-K″, arrowheads), Tuj1 (I-I″,L-L″, arrowheads) and p73 (J-J″,M-M″, arrowheads) at both E10.5 and E11.5, suggesting that some Msx1⁺ cells differentiated into Cajal-Retzius cells. di, diencephalon; tel, telencephalon. Scale bars: 50 μm.

RESULTS

Lineage analysis of the prospective choroid plexus region

To determine the cell lineage of the prospective choroid plexus epithelium of the telencephalon, we first examined whether or not neurogenesis occurs in this region. The homeodomain factor Msx1 is expressed in this region of mouse embryos from E10.0 to E11.5 and in the choroid plexus epithelium at E12.5 (Fig. 1B,E,F; see Fig. 3I,Q). At E10.5, subsets of Msx1$^+$ cells expressed the proneual factor Ngn2 (Fig. 1C-C″, arrowheads), suggesting that neurogenesis occurs in the prospective choroid plexus region. In agreement with this notion, some differentiating neurons (DCX$^+$) were found in this region (Fig. 1D-D″), although they did not express Msx1, suggesting that Msx1 is downregulated when Ngn2$^+$ cells start neuronal differentiation. To trace the lineage of Msx1$^+$ cells, we generated transgenic mice carrying the *Msx1* promoter-driven EGFP reporter. Because EGFP is relatively stable, it can be used as a short-term lineage tracer that detects cells expressing *Msx1* both currently and previously. As expected, EGFP was specifically expressed in the prospective choroid plexus region of transgenic mice from E10.0 to E11.5 (Fig. 1E-M″). At E10.0, 96.6±3.8% of EGFP$^+$ cells expressed Msx1, indicating that the EGFP expression occurred specifically in Msx1$^+$ cells (Fig. 1E-E″). At E10.5 and E11.5, subsets of EGFP$^+$ cells expressed Ngn2 (Fig. 1H-H″,K-K″, arrowheads) and the neuronal marker Tuj1 (Fig. 1I-I″,L-L″, arrowheads), indicating that some Msx1$^+$ cells indeed differentiated into neurons. Interestingly, p73, a marker of Cajal-Retzius cells, was also expressed (Fig. 1J-J″,M-M″, arrowheads), suggesting that neurons formed in the Msx1$^+$ region are Cajal-Retzius cells.

To further analyze the cell lineage of the prospective choroid plexus region, we introduced pEF-EGFP, which directs EGFP expression under the control of the elongation factor 1α promoter, into the prospective choroid plexus region at E9.5 by using an in utero microelectroporation method (Fukuchi-Shimogori and Grove, 2001; Fukuchi-Shimogori and Grove, 2003). At E10.5, all EGFP$^+$ cells resided in the Msx1$^+$ prospective choroid plexus region of the dorsal telencephalic midline (*n*=6) (Fig. 2A-C). However, at E11.5, many EGFP$^+$ cells migrated tangentially into the cortical neuroepitheium, and some of them had already reached the marginal zone of the piriform cortex (*n*=6) (Fig. 2D-F). At E12.5, most of the cells that migrated laterally seemed to have reached the piriform cortex (Fig. 2G, arrow), and only two regions, the choroid plexus epithelium (the origin, Fig. 2G, asterisk) and the piriform cortex (the destination, Fig. 2G, arrow), were labeled with EGFP (*n*=5). This finding suggests that the electroporated region gives rise to migrating cells around E11.5 and ceases the formation by E12.5. It has been reported that Cajal-Retzius cells in the piriform cortex expressed *Lhx5* (Yamazaki et al., 2004), and the EGFP$^+$ cells in this region seemed to express this marker (Fig. 2J,K). Furthermore, many of the EGFP$^+$ cells expressed Tuj1 (Fig. 2L,L′) and reelin (63.5%, *n*=148) (Fig. 2M,M′, arrowheads) but not calretinin (11.4%, *n*=210) (Fig. 2N,N′). These results suggest that the electroporated region gives rise to Cajal-Retzius cells destined for the piriform cortex around E11.5. In this experiment, EGFP was not expressed in the hem (*Wnt2b*$^+$, Fig. 2H,I), a known source of Cajal-Retzius cells. Furthermore, cell migration from the electroporated region ceased by E12.5, although the hem is known to generate migrating Cajal-Retzius cells even after E13.5 (Takiguchi-Hayashi et al., 2004). These results support the notion that these Cajal-Retzius cells do not derive from the cortical hem but from the prospective choroid plexus region. At E12.5, the cells remaining at the origin expressed the choroid plexus-specific marker transthyretin (*Ttr*) (Fig. 2O,P) but

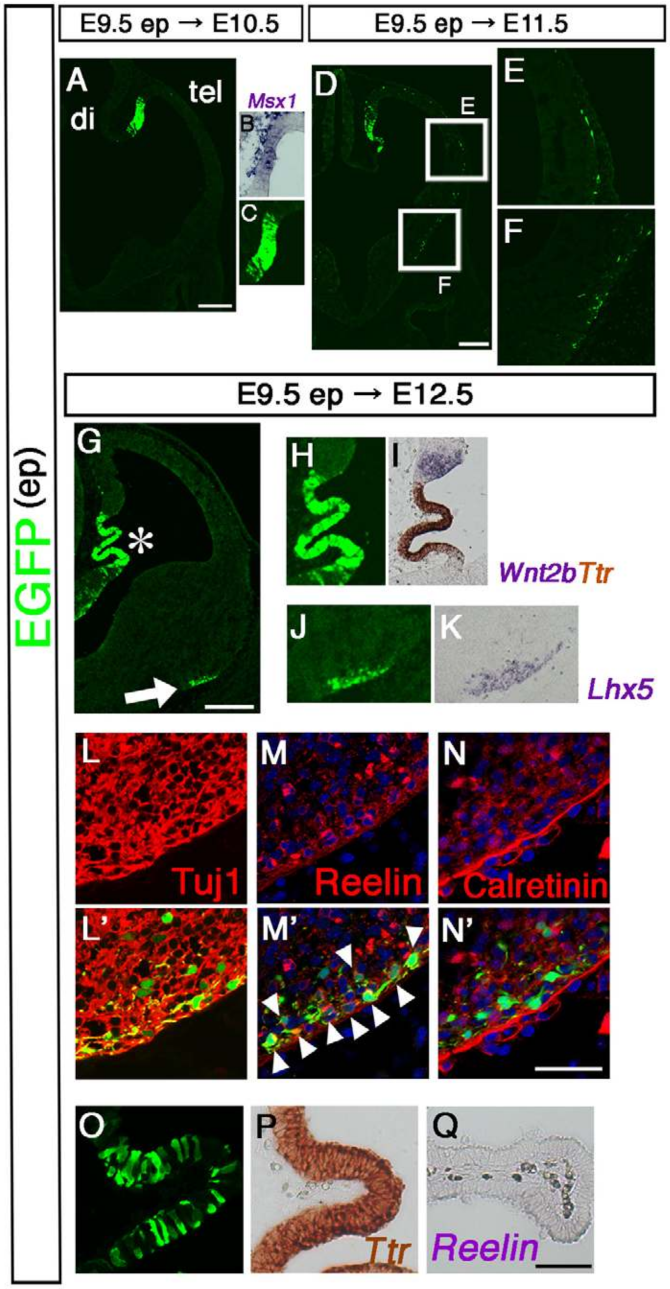

Fig. 2. Formation of Cajal-Retzius cells and choroid plexus epithelial cells in the dorsal telencephalic midline. pEF-EGFP was introduced into the dorsal telencephalic midline at E9.5 by in utero microelectroporation, and the fate of EGFP$^+$ cells was examined at the indicated time points. For the orientation of the planes, see Fig. 1A,A′. (**A-F**) At E10.5, all EGFP$^+$ cells resided in the *Msx1*$^+$ region (A-C). At E11.5, many EGFP$^+$ cells migrated tangentially into the cortical neuroepitheium (D,E), and some of them had already reached the marginal zone of the piriform cortex (D,F). (**G-Q**) At E12.5, all cells that migrated laterally seemed to have reached the piriform cortex (G, arrow). Only two regions, the choroid plexus epithelium (the origin, asterisk) and the piriform cortex (the destination, arrow) were labeled with EGFP (G). The cells that remained at the origin expressed *transthyretin* (*Ttr*) (H,I,O,P) but not reelin (Q). The cells that migrated into the piriform cortex expressed *Lhx5* (J,K, adjacent sections), Tuj1 (L,L′) and reelin (M,M′, arrowheads) but not calretinin (N,N′). di, diencephalon; tel, telencephalon. Scale bars: 150 μm in A,D; 500 μm in G; 50 μm in L-Q.

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not reelin (Fig. 2Q). These results suggest that the prospective choroid plexus region (*Msx1*$^{+}$*Wnt2b*$^{-}$) gives rise to two distinct cell types: Cajal-Retzius cells (neural) and the choroid plexus epithelium (non-neural) around E10.5 to E11.5.

Expression of *Hes1* and *Hes5* in the developing dorsal telencephalic midline region

To reveal the molecular mechanism of the fate choice in the dorsal midline region, we examined expression of *Hes1* and *Hes5* from E10.5 to E12.5. The telencephalic choroid plexus forms bilaterally at the dorsomedial edge of the telencephalon (Fig. 3A). At E10.5, the epithelium of the dorsal telencephalic midline region expressed *Bmp4* and the homeodomain gene *Lmx1a*, which regulates development of the choroid plexus and the cortical hem (Fig. 3C,D) (Millonig et al., 2000; Kuwamura et al., 2005). Likewise, *Hes1* was expressed in this region, as well as in the neighboring diencephalic and telencephalic neuroepithelium, while *Hes5* was expressed at a lower level in this region than in the telencephalic neuroepithelium at E10.5 (Fig. 3E,F). At E11.5, the *Bmp4* and *Lmx1a* expression domain was elongated (Fig. 3G,H) and gradually divided into two regions, the choroid plexus epithelium (*Msx1*$^{+}$,*Ttr*$^{+}$) and the cortical hem (*Wnt2b*$^{+}$) (Fig. 3I-K,N). At this stage, *Hes1* and *Hes5* expression continued but was gradually downregulated in *Ttr*$^{+}$ cells of the prospective choroid plexus region (Fig. 3L,M). At E12.5, the choroid plexus epithelial cells became thin and cuboidal (*Ttr*$^{+}$), whereas cells in the cortical hem were still pseudostratified (Fig. 3R). *Bmp4* and *Lmx1a* were expressed in both regions, whereas *Msx1* and *Wnt2b* expression occurred in the choroid plexus epithelium and in the cortical hem, respectively (Fig. 3O-R). At this stage, *Hes1* and *Hes5* expression occurred at a high level in the cortical hem but was almost completely repressed in the differentiated choroid plexus epithelium (Fig. 3S,T). These results show that *Hes1* and *Hes5* expression occurs in the prospective choroid plexus region at E10.5 to E11.5, when fate choice between

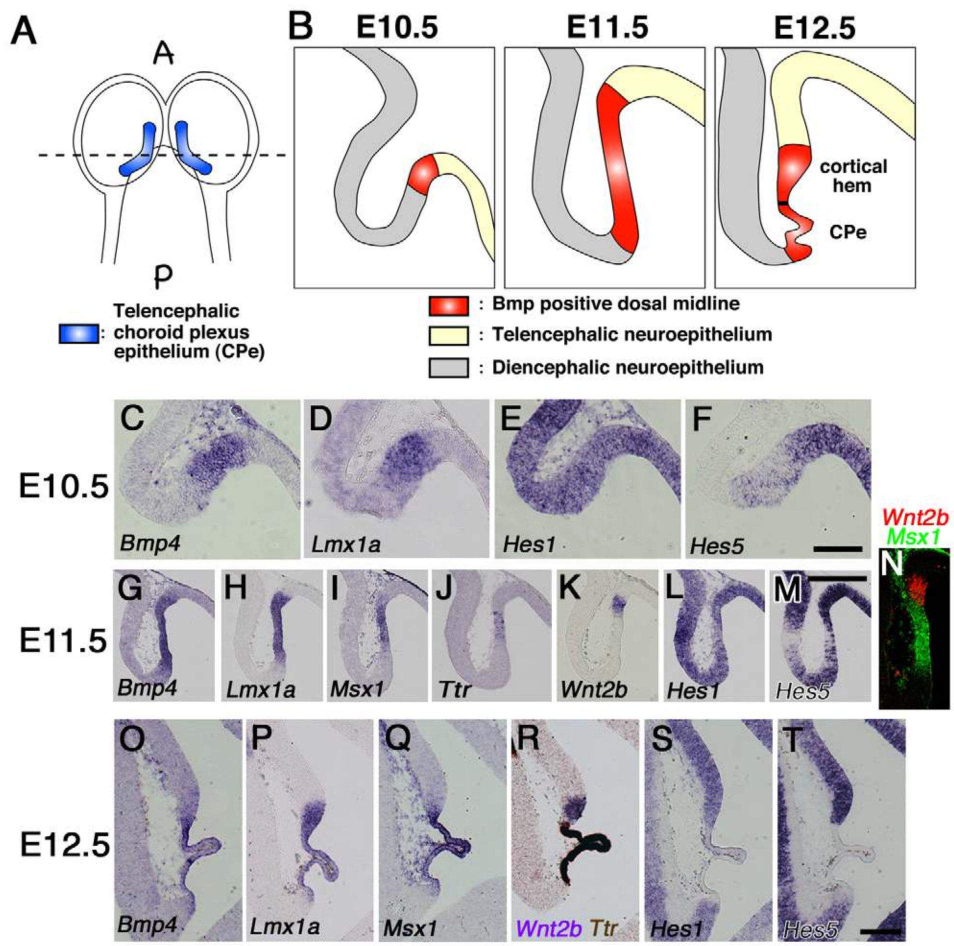

Fig. 3. Expression of *Hes1* and *Hes5* in the dorsal telencephalic midline. (**A**) Scheme of a dorsal view of E12.5 forebrain. Coronal sections are made along the broken line. (**B**) Schemes of the coronal sections of the dorsomedial telencephalon at E10.5-E12.5. (**C-F**) At E10.5, the dorsal telencephalic midline expressed *Bmp4* and *Lmx1a* (C,D). *Hes1* was likewise expressed in this region as well as in the neighboring diencephalic and telencephalic neuroepithelium (E). *Hes5* was expressed at a lower level in this region than in the telencephalic neuroepithelium (F). (**G-N**) At E11.5, the *Bmp4* and *Lmx1a* expression domain was elongated (G,H). *Msx1* and *Ttr* were expressed in differentiating choroid plexus epithelium (I,J), whereas *Wnt2b* was expressed in the prospective cortical hem (K). *Hes1* expression was gradually downregulated in *Ttr*$^{+}$ cells (L). Although *Hes5* expression was upregulated in the telencephalon and the diencephalon, it was also downregulated in *Ttr*$^{+}$ cells (M). Wnt2b^{+} and Msx1^{+} domains were clearly separated at this stage (N). (**O-T**) At E12.5, the choroid plexus epithelial cells became thin and cuboidal (R, *Ttr*$^{+}$), whereas the cortical hem was still pseudostratified. *Bmp4* and *Lmx1a* were expressed in both regions (O,P). *Msx1* expression occurred mainly in the choroid plexus epithelium (Q), whereas *Wnt2b* expression occurred in the cortical hem (R). *Hes1* and *Hes5* expression was almost completely repressed in the choroid plexus epithelium (S,T). Scale bars: 100 μm in C-F; 200 μm in G-T.

choroid plexus epithelial cells and Cajal-Retzius cells takes place, and is downregulated at E12.5, when the cell fate is completely specified.

Generation of conditional *Hes*-null mice

The above results suggest that both *Hes1* and *Hes5* are expressed in the prospective choroid plexus epithelium when neural versus non-neural cell fate specification occurs. To reveal the role of Hes genes in this region, we decided to examine Hes-null mice. However, *Hes1*;*Hes5* double-null embryos die by E11 before the establishment of the telencephalon (Hatakeyama et al., 2004), and thus they were not suitable for analysis. To overcome this problem, we generated *Hes1* floxed mice, in which the region containing exons 2 to 4 was deleted by Cre recombinase (Fig. 4A,B). These mice were crossed with Emx1-Cre mice, which had previously been shown to efficiently result in the recombination of floxed alleles in the dorsal telencephalon (Iwasato et al., 2000). It has been reported that expression of *Emx1* starts at E9.5 (Yoshida et al., 1997). To monitor the Cre-mediated recombination, we crossed Emx1-Cre mice with R26R reporter mice (Soriano, 1999). Recombination occurred efficiently in the dorsal telencephalic neuroepithelium, including progenitors to the choroid plexus epithelium at E10.5 to E12.5 (Fig. 4C-E). We generated the *Hes1* conditional knock-out (cKO) mice by crossing *Hes1* floxed mice and Emx1-Cre mice. In *Hes1* cKO mice, *Hes1* expression in the dorsal telencephalon was downregulated around E10.5 (Fig. 4J, asterisk) and was lost by E11.5 (Fig. 4O, asterisk). Thus, compared with the control, where *Hes1* expression was lost by E12.5, downregulation of *Hes1* occurred 1-2 days earlier in *Hes1* cKO mice. No apparent defect was observed in the developing telencephalon of *Hes1* cKO mice (data not shown), probably owing to compensation by other members of the Hes family such as *Hes5*, which was upregulated in *Hes1* cKO mice (Fig. 4K, compare with 4H). Additionally, *Hes3* could be upregulated in the absence of *Hes1* and *Hes5*, and we decided to make mice lacking *Hes1*, *Hes3* and *Hes5*. Because *Hes3*;*Hes5* double-null mice are apparently normal (Hatakeyama et al., 2004), we generated *Hes1* cKO mice on a *Hes3*;*Hes5* double-null background (*Hes1*;*Hes3*;*Hes5* cKO).

Although it has previously been shown that the midbrain, the hindbrain and the spinal cord can develop severe defects such as premature depletion of neural progenitors and disruption of the neural tube structures in conventional *Hes1*;*Hes3*;*Hes5* KO mice (Hatakeyama et al., 2004; Baek et al., 2006), it was surprising that the cortical hem and the cortical neuroepithelium were only mildly affected in *Hes1*;*Hes3*;*Hes5* cKO mice. *Hes1*;*Hes3*;*Hes5* cKO mice showed accelerated differentiation of neurons, including Cajal-Retzius cells in the dorsal telencephalon (see Fig. S1 in the supplementary material), but there were many neural progenitors, and the laminar structures of the neocortex and the hippocampus were not affected (see Fig. S2 in the supplementary material). Hes-related genes *Hey1* and *Hey2* were found to be expressed in the cortical hem and the cortical neuroepithelium (see Fig. S3 in the supplementary material), and *Hey1* expression was upregulated in *Hes1*;*Hes3*;*Hes5* cKO mice (see Fig. S3A,B in the supplementary material). It has previously been shown that both *Hey1* and *Hey2* inhibit neuronal differentiation and promote maintenance of neural progenitors, such as Hes genes (Sakamoto et al., 2003). Therefore, such mild phenotypes of the dorsal telencephalon of *Hes1*;*Hes3*;*Hes5* cKO mice were probably due to compensation by *Hey1* and *Hey2*. However, *Hey1* and *Hey2* were not expressed in the prospective choroid plexus epithelium (see Fig. S3 in the supplementary material and data not shown), and *Hes1*;*Hes3*;*Hes5* cKO mice displayed a severe defect of the choroid plexus, as described below.

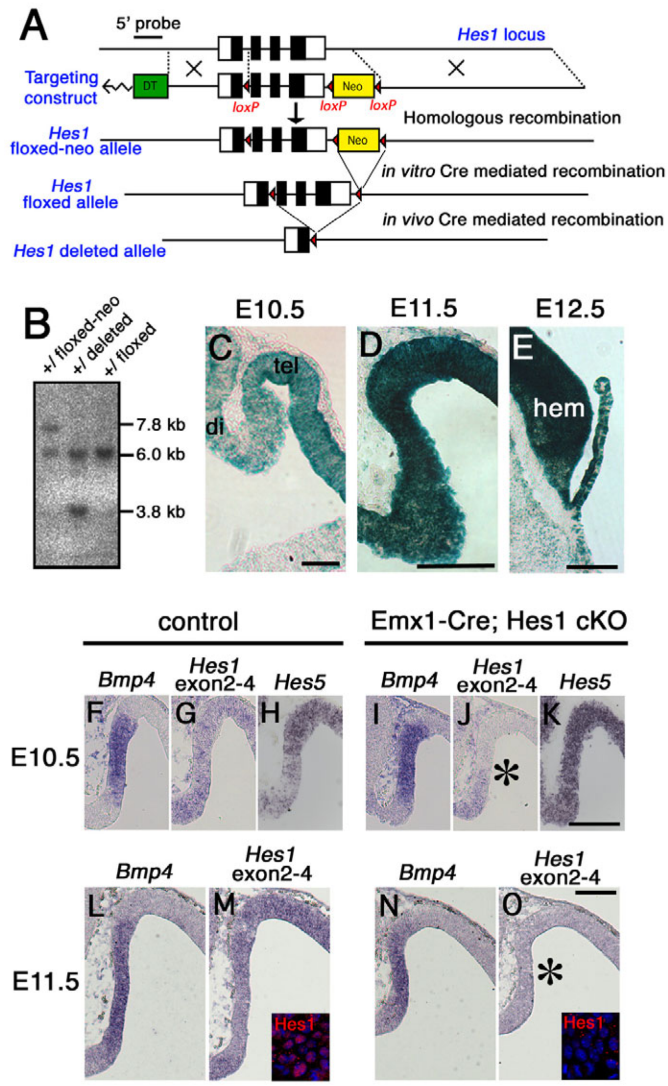

Fig. 4. Generation of *Hes1* cKO mice. (**A**) Strategy for generation of *Hes1* cKO mice. (**B**) Genomic DNA from drug-resistant cells was digested with *Hin*dIII and analyzed by Southern blot using a 0.6-kb *Hin*dIII-*Bam*HI fragment as a 5′-probe, which detected wild-type and floxed fragments (6.0 kb), floxed-neo (7.8 kb) and deleted fragments (3.8 kb). (**C-E**) β-Galactosidase activity in the forebrain of Emx1-Cre;R26R mice at E10.5-E12.5. (**F-O**) *Hes1* and *Hes5* were expressed in the dorsal telencephalon of the control (*Bmp4*$^+$, F,L) at E10.5 and E11.5 (G,H,M). In *Hes1* cKO mice, *Hes1* expression was downregulated around E10.5 (J, asterisk) and was lost by E11.5 (O, asterisk). Insets of M and O show immunohistochemistry for Hes1. Hes1 protein expression was lost in *Hes1* cKO mice by E11.5. *Hes5* expression was upregulated in *Hes1* cKO mice (K). di, diencephalon; tel, telencephalon; hem, cortical hem. Scale bars: 100 μm in C,D,F-O; 200 μm in E.

Defect of the choroid plexus and increase of Cajal-Retzius cell formation in *Hes1*;*Hes3*;*Hes5* cKO mice

In the control mice, the neuroepithelial cells at the midline became flattened from E11.5 to E12.5 (Fig. 5A,A′,B,B′), and the thin cuboidal epithelium protruded into the lateral ventricles around E12.5

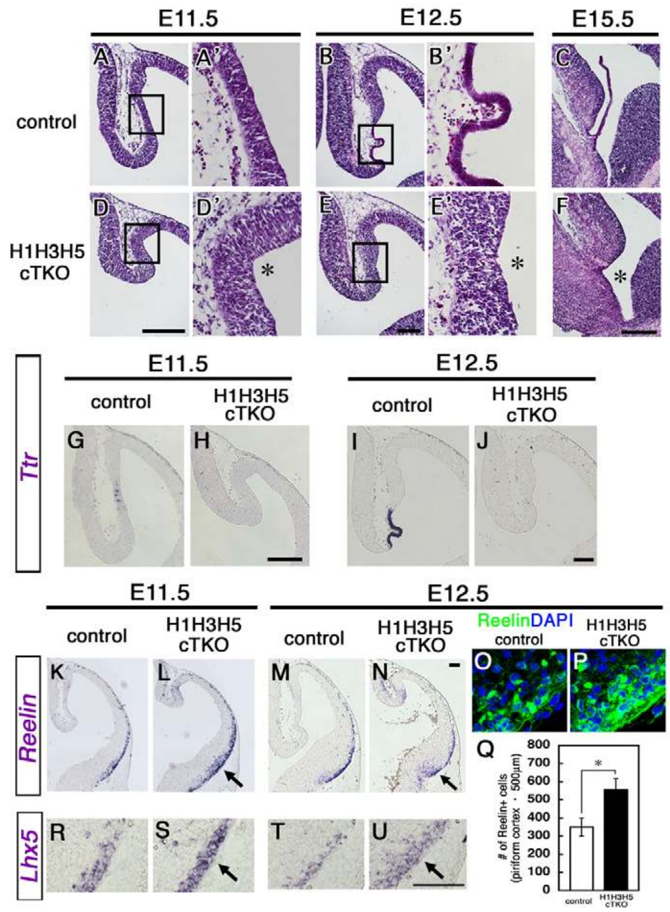

Fig. 5. Defect of the choroid plexus and increase of Cajal-Retzius cell formation in *Hes1*;*Hes3*;*Hes5* cKO mice. (**A-F**) HE staining of the dorsal telencephalic midline. In *Hes1*;*Hes3*;*Hes5* cKO mice, the dorsal midline cells were not flattened but remained pseudostratified at E11.5 and E12.5 (D,D′,E,E′, asterisks). Even at E15.5, no choroid plexus was formed in *Hes1*;*Hes3*;*Hes5* cKO mice (F, asterisk). (**G-J**) *Ttr* was expressed in the control at E11.5 and E12.5 (G,I) but not in *Hes1*;*Hes3*;*Hes5* cKO mice (H,J). (**K-N**,**R-U**) Cajal-Retzius cells (reelin$^+$,*Lhx5*$^+$) of the mutant piriform cortex were increased in number (L,N,S,U arrows), compared with the control (K,M,R,T) at both E11.5 and E12.5. (**O-Q**) The number of reelin$^+$ cells was quantified by counting DAPI$^+$ cells on every five sections from four independent embryos for each genotype. *P<0.01, t-test. Scale bars: 100 μm in A,D; 200 μm in B,E,G-N; 250 μm in C,F; 50 μm in R-U.

to E15.5 (Fig. 5C). By contrast, in *Hes1*;*Hes3*;*Hes5* cKO mice, the dorsal midline cells were not flattened but remained pseudostratified at E11.5 and E12.5 (Fig. 5D,D′,E,E′, asterisks). This region was morphologically very similar to the neighboring cortical and diencephalic neuroepithelium. Even at E15.5, no choroid plexus was formed in *Hes1*;*Hes3*;*Hes5* cKO mice (Fig. 5F, asterisk). Furthermore, expression of the choroid plexus epithelium-specific gene *Ttr* was not detectable in the mutant mice at E11.5 or E12.5 (Fig. 5H,J), although it had already occurred in the control (Fig. 5G,I). These results indicate that the choroid plexus is completely missing in *Hes1*;*Hes3*;*Hes5* cKO mice. Similarly, the choroid plexus in the fourth ventricle was severely affected in *Hes1*;*Hes5* conventional KO mice at E10.5 (see Fig. S4 in the supplementary material), although it was not yet formed in the telencephalon of both the wild-type and *Hes1*;*Hes5* conventional KO mice at this stage.

We then examined the lineage of Cajal-Retzius cells which originate from the prospective choroid plexus region and migrate into the piriform cortex. We found that there were more Cajal-Retzius cells (reelin$^+$,Lhx5$^+$) in the marginal zone of the piriform cortex of *Hes1*;*Hes3*;*Hes5* cKO mice than in the control mice at both E11.5 (Fig. 5K,L,R,S, arrows) and E12.5 (Fig. 5M-Q,T,U, arrows). This finding suggests that Cajal-Retzius cell development is enhanced in the absence of Hes genes. Although significant defects were not observed in the cortical development (see Fig. S2 in the supplementary material), it is possible that overall acceleration of cortical neurogenesis is involved in enhancement of Cajal-Retzius cell formation in the piriform cortex of *Hes1*;*Hes3*;*Hes5* cKO mice. However, Cajal-Retzius cell formation was not significantly affected in the pallial-subpallial boundary region of the mutant mice (see Fig. S5 in the supplementary material). Furthermore, we generated *Hes1*;*Hes3*;*Hes5* cKO mice by using Nes-CreER^{T2} mice (Imayoshi et al., 2006), in which *Hes1* was knocked out in the cortical neuroepithelium and the hem but not in the choroid plexus region (see Fig. S6A-F′ in the supplementary material), which developed normally (see Fig. S6I,L in the supplementary material). In these mice, Cajal-Retzius cell formation was not significantly affected in the piriform cortex (see Fig. S6M-P′ in the supplementary material). These results suggest that inactivation of Hes genes in the prospective choroid plexus region mainly contributes to enhancement of Cajal-Retzius cell formation in the piriform cortex, although the possibility of contribution by overall accelerated neurogenesis is not totally excluded.

Bmp signaling and homeodomain gene expression are affected in *Hes1*;*Hes3*;*Hes5* cKO mice

It was previously shown that the telencephalic choroid plexus is missing in the absence of the Bmp receptor gene *Bmpr1a* (Hébert et al., 2002). We therefore examined expression of Bmp signaling and related molecules in *Hes1*;*Hes3*;*Hes5* cKO mice. At E11.5, in these mutant mice, the expression domain of *Bmp4* and *Lmx1a* was reduced in size (Fig. 6A.A′,B,B′), and the expression of the downstream homeodomain genes *Msx1* and *Msx2* was severely downregulated compared with the control (Fig. 6C,C′,D,D′). Thus, Bmp signaling was attenuated in the absence of Hes genes. However, expression of the Bmp receptor *Bmpr1a* (see Fig. S7A,B in the supplementary material) and of *Noggin*, an antagonist of Bmp (data not shown), as well as its responsiveness to Bmp (see Fig. S7C-F in the supplementary material) were not affected in *Hes1*;*Hes3*;*Hes5* cKO mice. The expression domain of *Wnt3a* was also reduced in size at this stage (Fig. 6E,E′), although expression of *Foxg1* and *Lhx2*, which are required for cortical development (Xuan et al., 1995; Porter et al., 1997; Monuki et al., 2001), was not significantly affected (Fig. 6F,F′,G,G′). In *Hes1*;*Hes3*;*Hes5* cKO mice, the dorsal telencephalic midline was reduced in size, but the cortical neuroepithelium did not expand. Cell death and proliferation were not responsible for the reduction in size of the dorsal telencephalic midline (see Fig. S7G-L in the supplementary material).

At E12.5, in the control mice, the telencephalic midline region was clearly separated into the choroid plexus epithelium and the cortical hem, while *Bmp4* and *Lmx1a* were expressed in both regions (Fig. 6H,I). In *Hes1*;*Hes3*;*Hes5* cKO mice, the prospective choroid plexus region remained pseudostratified, and the *Bmp4* and *Lmx1a* expression domain became smaller (Fig. 6H′,I′). In the control, *Msx1* was expressed at a high level in the choroid plexus epithelium and at a low level in the ventral part of the cortical hem (Fig. 6J),

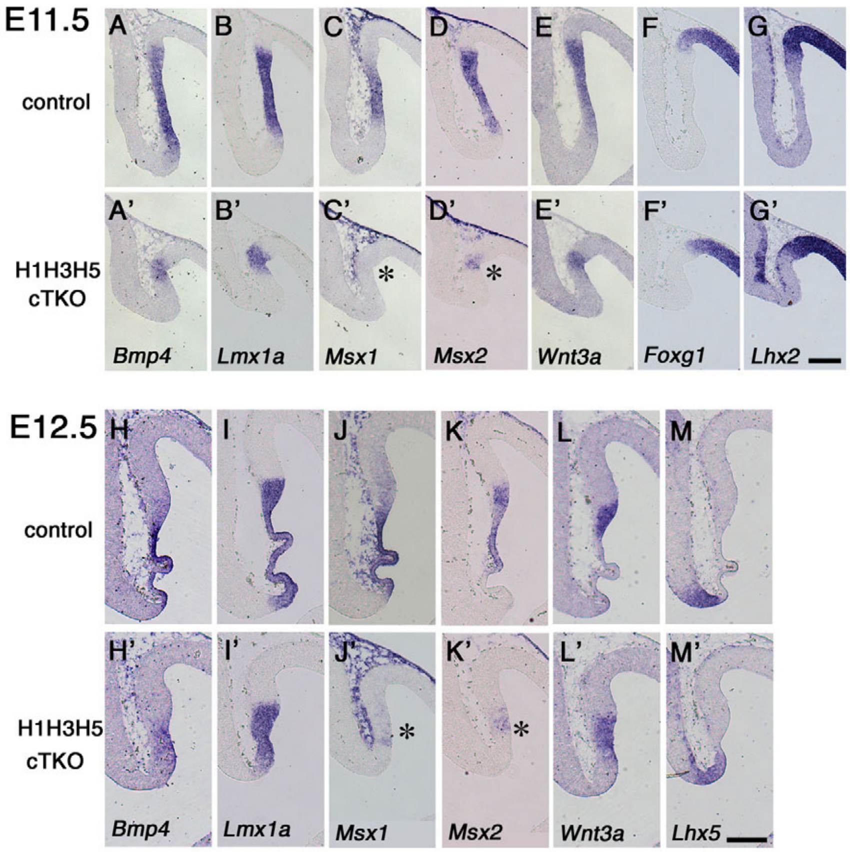

Fig. 6. Bmp signaling and homeodomain gene expression in *Hes1*;*Hes3*;*Hes5* cKO mice. (**A-G′**) At E11.5, in *Hes1*;*Hes3*;*Hes5* cKO mice, the expression domains of *Bmp4*, *Lmx1a*, *Msx1*, *Msx2* and *Wnt3a* were reduced in size, compared with the control, although *Foxg1* and *Lhx2* expression (cortex) was not significantly affected. (**H-M′**) At E12.5, in the control, the telencephalic midline region was clearly separated into the choroid plexus epithelium and the cortical hem, and *Bmp4* and *Lmx1a* were expressed in both regions (H,I). In *Hes1*;*Hes3*;*Hes5* cKO mice, the prospective choroid plexus region remained pseudostratified, and *Bmp4* and *Lmx1a* expression domain was smaller in size (H′,I′). *Msx1* and *Msx2* expression domains were also reduced in size (J′,K′, asterisks), whereas expression of *Wnt3a* (cortical hem) and *Lhx5* (eminentia thalami) was not significantly affected (L′,M′). Scale bars: 100 μm in A-G′; 200 μm in H-M′.

while *Msx2* was expressed in both the choroid plexus epithelium and the cortical hem (Fig. 6K). In *Hes1*;*Hes3*;*Hes5* cKO mice, both *Msx1* and *Msx2* were expressed at very low levels (Fig. 6J′,K′, asterisks). However, the *Wnt3a* expression domain was not significantly changed between control and *Hes1*;*Hes3*;*Hes5* cKO mice at this stage (Fig. 6L,L′). Expression of the homeodomain gene *Lhx5* in the eminentia thalami, which physically links the telencephalic choroid plexus to the diencephalon (Hébert et al., 2002), was not significantly affected in *Hes1*;*Hes3*;*Hes5* cKO mice, indicating that the diencephalon is not expanded in the absence of Hes genes (Fig. 6M,M′). This finding reveals that inactivation of Hes genes leads to attenuation of Bmp signaling and lack of the choroid plexus epithelium with no expansion of the cortical and diencephalic neuroepithelium.

Upregulation of proneural genes in the dorsal telencephalic midline of *Hes1;Hes3;Hes5* cKO mice

In *Hes1*;*Hes3*;*Hes5* cKO mice, Cajal-Retzius cells increased in number in the piriform cortex at E12.5. Furthermore, neurogenesis was accelerated in the dorsal telencephalic midline region of *Hes1*;*Hes3*;*Hes5* cKO mice at E10.5 and E11.5 (Fig. 7B,D, asterisks) compared with the control mice (Fig. 7A,C). We then sought to determine the mechanism for this enhanced Cajal-Retzius cell formation in *Hes1*;*Hes3*;*Hes5* cKO mice. In the dorsal telencephalic midline (*Lmx1a*$^+$) of wild-type embryos, *Ngn1* and *Ngn2* were expressed at E10.5 and E11.5 (Fig. 7G-K). Interestingly, at E10.5, Hes1 and Ngn2 were co-expressed by many cells (Fig. 7E, arrowheads), but the expression became mostly segregated at E11.5 (Fig. 7F, arrows), suggesting that Hes1$^+$Ngn2$^+$ cells gradually become either Hes1$^+$ or Ngn2$^+$ cells during this period. In *Hes1*;*Hes3*;*Hes5* cKO mice, *Ngn1* and *Ngn2* expression were highly upregulated at both E10.5 and E11.5 (Fig. 7L-P, asterisks, 7R,R′,S) compared with the control (Fig. 7G-K,Q,Q′,S). These results suggest that inactivation of Hes genes leads to upregulation of *Ngn1* and *Ngn2* expression, which contributes to enhanced Cajal-Retzius cell formation.

To further clarify the role of *Ngn2* in Cajal-Retzius cell formation, we next examined *Ngn2*-null mice (Fode et al., 2000). The number of Cajal-Retzius cells (reelin$^+$, *p73*$^+$), which are derived from the dorsal telencephalic midline, was reduced in *Ngn2*-null mice compared with the control mice (see Fig. S8 in the supplementary material), indicating that *Ngn2* indeed contributes to Cajal-Retzius cell formation. Nevertheless, there was no significant difference in the number of Cajal-Retzius cells in the piriform cortex (data not shown). It is partly because Cajal-Retzius cells in this region come from other regions in addition to the choroid plexus region (Bielle et al., 2005). Furthermore, the dorsal telencephalic midline region developed normally in *Ngn2*-null mice (see Fig. S9 in the supplementary material), suggesting that *Ngn1* compensates *Ngn2* to some extent.

Upregulated expression of *Ngn2* inhibits choroid plexus formation and enhances formation of Cajal-Retzius cells derived from the dorsal telencephalic midline

We found that in the absence of Hes genes, *Ngn2* expression was upregulated and that Cajal-Retzius cells in the piriform cortex increased in number at the expense of the choroid plexus cell fate. We then examined whether misexpression of *Ngn2* in the dorsal telencephalic midline promotes formation of Cajal-Retzius cells at the expense of the choroid plexus. Misexpression of *Ngn2* in the dorsal telencephalic midline at E9.5 inhibited the development of the choroid plexus (Fig. 8D′,E′) (n=4). Furthermore, this misexpression of *Ngn2* generated more Cajal-Retzius cells (reelin$^+$) in the piriform cortex (Fig. 8F,G). These results suggest that misexpression of *Ngn2* in the dorsal telencephalic midline at E9.5 promotes formation of Cajal-Retzius cells at the expense of the choroid plexus.

To examine the plasticity of the differentiation competency at a later stage, we electroporated the *Ngn2* vector at E11.5 (this procedure should induce the ectopic expression around E12). Misexpression of *Ngn2* increased Cajal-Retzius cell formation (reelin$^+$) from the cortical hem (*Wnt2b*$^+$, Fig. 8H,I) but did not affect choroid plexus formation (*Ttr*$^+$) at E12.5 (Fig. 8J,K). These results suggest that the choroid plexus epithelial region loses competency to produce Cajal-Retzius cells by E12.5.

The above results indicate that *Hes*-expressing cells and *Ngn2*-expressing cells are segregated in the dorsal telencephalic midline region around E10.5 to E11.5, and that *Hes*-expressing cells adopt the choroid plexus fate, whereas *Ngn2*-expressing cells adopt Cajal-Retzius cell fate. We then sought to determine the mechanism responsible for this segregation. The most likely mechanism is Notch-mediated lateral inhibition: proneural genes such as *Ngn2* induce expression of the Notch ligand, leading to activation of the Notch pathway and to the induction of *Hes1*/*Hes5* expression in neighboring cells (Kageyama et al., 2007). We thus examined mice mutant for *Rbpj*, an essential effector of Notch signaling (Tanigaki and Honjo, 2007). However, because conventional *Rbpj*-null mice die very early (Oka et al., 1995), we generated *Rbpj* cKO mice by crossing floxed *Rbpj* mice (Han et al., 2002) with Emx1-Cre mice. In these cKO mice, although *Hes5* expression was downregulated, *Hes1* was still expressed (see Fig. S10C,D,K,L in the supplementary material), and *Ttr* expression occurred normally (see Fig. S10E,M in the supplementary material). These results indicate that the Notch-Rbpj pathway is not involved in segregation of *Hes*- and *Ngn2*-expressing cells.

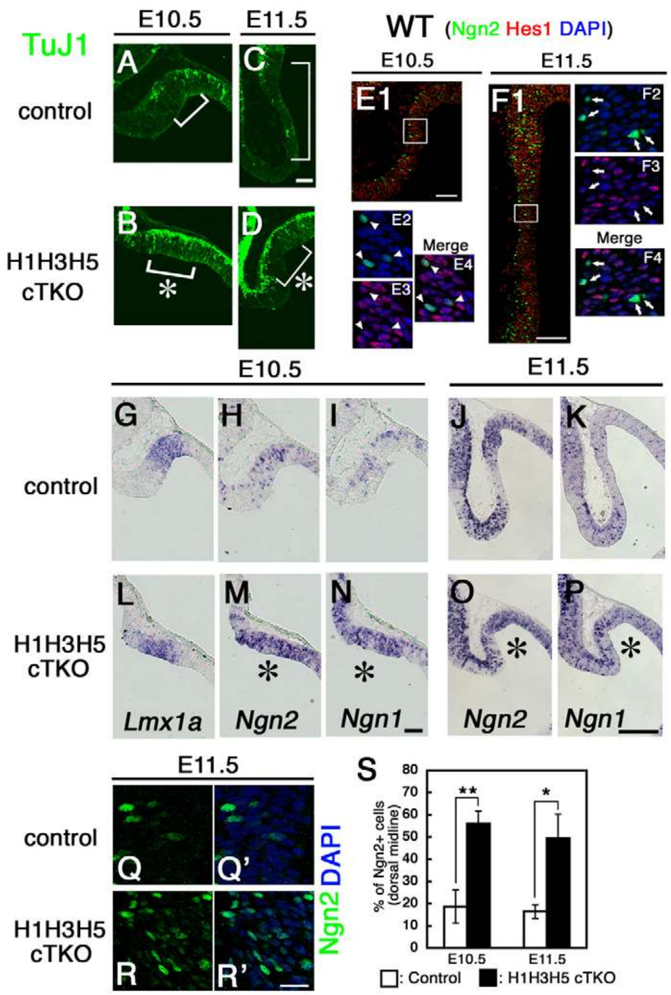

Fig. 7. Upregulation of proneural genes in the dorsal telencephalic midline of *Hes1*;*Hes3*;*Hes5* cKO mice. (**A-D**) Neurogenesis (Tuj1$^+$) was enhanced in the dorsal telencephalic midline (brackets) of *Hes1*;*Hes3*;*Hes5* cKO mice at E10.5 and E11.5 (B,D, asterisks), compared with the control (A,C). (**E**,**F**) Double immunostaining for Ngn2 and Hes1 in wild-type embryos. Many cells co-expressed Ngn2 and Hes1 at E10.5 (arrowheads), but the expression was mostly segregated at E11.5 (arrows). (**G-S**) In the dorsal telencephalic midline region (*Lmx1a*$^+$) of control mice, *Ngn1* and *Ngn2* expression occurred at low levels in subsets of cells at E10.5 (G-I) and was down-regulated at E11.5 (J,K,Q,Q′). By contrast, *Ngn1* and *Ngn2* expression was highly upregulated in *Hes1*;*Hes3*;*Hes5* cKO mice at both E10.5 and E11.5 (L-P, asterisks; R,R′,S). *P<0.01; **P<0.001, t-test. Scale bars: 50 μm in A-D,E1,F1,G-I,L-N; 100 μm in J,K,O,P; 20 μm in Q-R′.

DISCUSSION

Hes and *Ngn* antagonistically regulate the non-neural versus neural fate specification

It has been shown that Cajal-Retzius cells are born at multiple places in the developing telencephalon, such as the cortical hem, the septum and the pallial-subpallial boundary (Takiguchi-Hayashi et al., 2004; Yoshida et al., 2005; Bielle et al., 2005). By lineage-tracing analysis, we found that cells in the prospective choroid plexus region have potential to give rise to Cajal-Retzius cells first, and later differentiate into choroid plexus epithelial cells. Thus, neural (Cajal-Retzius) and non-neural (choroid plexus epithelial) cells are sequentially born in the dorsal telencephalic midline region. We further showed that Hes1$^+$Ngn2$^+$ cells gradually become either Hes1$^+$ or Ngn2$^+$ cells, and that inactivation of *Hes1*, *Hes3* and *Hes5* upregulated expression of *Ngn2*, accelerating Cajal-Retzius cell formation at the expense of the choroid plexus (Fig. 9A). In these mutant mice, it is likely that almost all cells in the prospective choroid plexus epithelium adopted Cajal-Retzius cell fate and migrated into the piriform cortex, because the choroid plexus epithelium is completely lacking. However, it is also possible that some cells remain as pseudostratified epithelial cells, and further experiments will be required to resolve this issue. Similarly, overexpression of *Ngn2* enhanced formation of Cajal-Retzius cells and inhibited differentiation of the choroid plexus. These results suggest that Hes1$^+$Ngn2$^+$ cells are bi-potent and become segregated into Hes1-expressing cells that adopt the choroid plexus fate and Ngn2-expressing cells that adopt Cajal-Retzius cell fate, and that *Hes* and *Ngn2* antagonistically regulate the non-neural versus neural fate decision in the dorsal telencephalic midline region (Fig. 9B). However, it is also possible that these two cell types derive from two different types of progenitor cells rather than from bi-potent cells. We were not able to resolve this issue decisively, because it is technically difficult to perform a clonal dissociation culture of the E10.5 dorsal telencephalic midline.

It is surprising that the prospective choroid plexus region initially give rise to Cajal-Retzius cells before differentiating into the choroid plexus epithelium. Because the hem, a well known source of Cajal-Retzius cells, is located next to the prospective choroid plexus

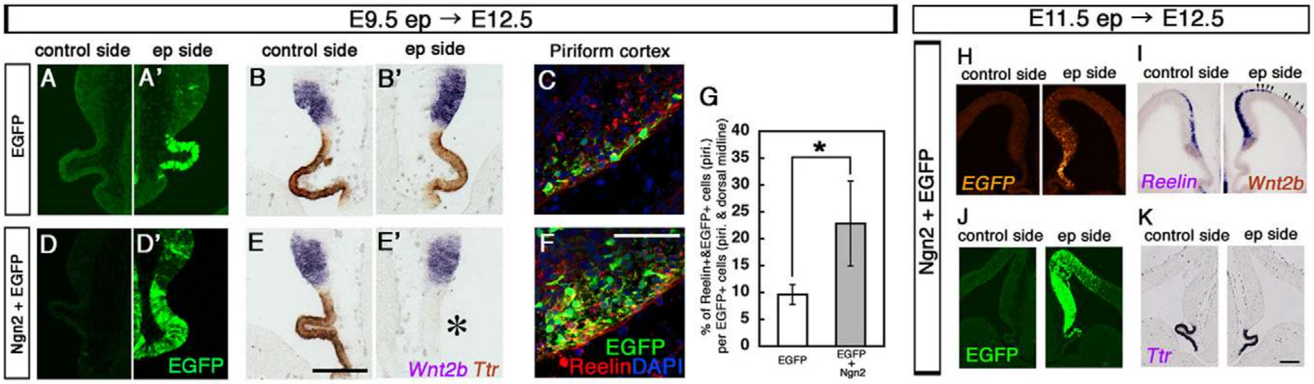

Fig. 8. Misexpression of *Ngn2* inhibits choroid plexus formation and enhances Cajal-Retzius cell formation in the dorsal telencephalic midline. (**A-G**) pEF-EGFP alone (A-C) or the *Ngn2* expression vector together with pEF-EGFP (D-F) was introduced into the dorsal telencephalic midline at E9.5 by in utero microelectroporation, and the region was analyzed at E12.5. Misexpression of *Ngn2* inhibited formation of the choroid plexus (E′, asterisk) and increased the number of Cajal-Retzius cells in the piriform cortex (F,G). (**H-K**) *Ngn2* was overexpressed in the prospective choroid plexus and cortical hem regions by electroporation at E11.5, and the coronal sections were examined at E12.5. Forced expression of *Ngn2* at E11.5 increased Cajal-Retzius cell (reelin$^+$) formation in the cortical hem (H,I, arrows) but did not affect the choroid plexus development (J,K). *P<0.05, t-test. Scale bars: 200 μm in A,B,D,E,H-K; 50 μm in C,F.

region, it is possible that these two regions are not clearly separated at early stages and thus some cells in the boundary region could contribute to Cajal-Retzius cell formation. However, around E10.5 to E11.5, neurogenesis occurs widely in the prospective choroid plexus region and is not restricted to the boundary to the prospective hem region (Fig. 1). Furthermore, Cajal-Retzius cell migration from the prospective choroid plexus region ceases by E12.5 (Fig. 2G), although that from the hem continues even after E13.5 (Takiguchi-Hayashi et al., 2004). These data support the notion that these Cajal-Retzius cells derive from the prospective choroid plexus region.

Differentiation competency of the dorsal telencephalic midline cells

In the dorsal telencephalic midline of wild-type mice, *Hes1* and *Hes5* expression occurred at high levels until E11.5 but was then downregulated in the choroid plexus epithelium at E12.5. In our *Hes1*;*Hes3*;*Hes5* cKO mice, *Hes1* expression occurred at a lower level at E10.5 and was lost around E11.5. Thus, in *Hes1*;*Hes3*;*Hes5* cKO mice, *Hes1* expression was repressed only 1 or 2 days earlier than in the control. Nevertheless, we found profound defects (loss of the choroid plexus), suggesting that *Hes* expression around E10.5 to E11.5 has a crucial role in the specification of the choroid plexus. At this stage, *Hes*-expressing cells and *Ngn2*-expressing cells seem to be segregated in the dorsal telencephalic midline region, but after this stage, the cell fates seem to be determined and are unchangeable. In accordance with this notion, development of the choroid plexus was severely affected by electroporation of the *Ngn2* vector at E9.5 (expression occurs around E10) but not at E11.5 (expression occurs around E12). These results suggest that the differentiation competency becomes unchangeable soon after E11.5 in the dorsal telencephalic midline. The mechanism underlying how segregation of choroid plexus epithelial cells and

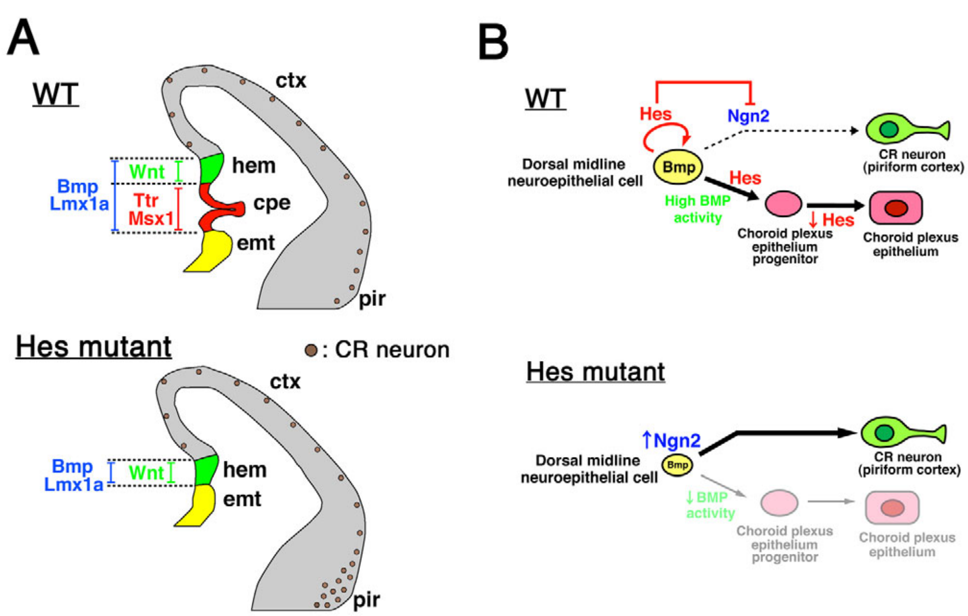

Fig. 9. Summary of developmental defects of the dorsal telencephalic midline region of *Hes1*;*Hes3*;*Hes5* cKO mice. (**A**) In *Hes1*;*Hes3*;*Hes5* cKO mice, the choroid plexus epithelium (cpe) is lacking, and Cajal-Retzius (CR) cell formation in the piriform cortex (pir) is enhanced. (**B**) *Ngn2* promotes Cajal-Retzius cell formation, whereas Hes genes regulate specification of the choroid plexus epithelium by antagonizing *Ngn2*. In *Hes1*;*Hes3*;*Hes5* cKO mice, *Ngn2* expression is upregulated and Cajal-Retzius cell formation is enhanced, whereas choroid plexus epithelial cells are lacking. ctx, neocortex; hem, cortical hem; emt, eminentia thalami.

DEVELOPMENT

Cajal-Retzius cells is regulated is not known. Lateral inhibition mediated by Notch signaling is a possible mechanism. However, inactivation of *Rbpj*, an essential mediator of Notch signaling, neither abolishes *Hes1* expression nor significantly affects the choroid plexus development, thus suggesting that Notch signaling is not involved in this process.

Although the choroid plexus was completely missing in *Hes1*;*Hes3*;*Hes5* cKO mice, the boundary between the prospective choroid plexus epithelium and the diencephalon was not affected. Thus, it is likely that none of the cells in the prospective choroid plexus epithelium adopted the diencephalic cell fate. This finding suggests that these prospective choroid plexus epithelial cells do not have the competency to become cell types other than choroid plexus and Cajal-Retzius cells.

The role of Bmp signaling in the non-neural versus neural cell fate specification

Our finding that *Hes1* expression is not regulated by the Notch-Rbpj pathway raised another important question: which factors regulate *Hes1* expression in the dorsal telencephalic midline? One of the candidates is Bmp signaling, because previous studies have shown that activation of Bmp signaling induces *Hes1* expression in cultured cells (Dahlqvist et al., 2003). Additionally, our preliminary study also showed that treatment with Bmp leads to increased *Hes1* expression in neural progenitor cultures (I.I., T.S., T.O. and R.K., unpublished). Furthermore, Bmp genes are expressed at high levels in the dorsal telencephalic midline. Thus, Bmp signaling seems to be important for *Hes1* expression in this region. Conversely, Hes genes are required for maintenance of Bmp signaling, because expression of *Bmp* and of its downstream genes is severely downregulated in *Hes1*;*Hes3*;*Hes5* cKO mice. Apparently, Cajal-Retzius cells do not express *Bmp*, so premature differentiation of these cells may lead to loss of *Bmp* expression. We speculate that Hes genes maintain *Bmp*-expressing cells by inhibiting Cajal-Retzius cell formation rather than directly activating *Bmp* expression.

It has been shown that Bmp signaling is required locally for the development of the dorsal telencephalic midline but not for the medial-lateral patterning of the dorsal telencephalon (Hébert et al., 2002). Regions where Bmp signaling is inactive seem to become the neural cells (cortical hem and cortical neuroepithelium), whereas regions with a high Bmp activity become the non-neural cells (the choroid plexus). This effect of Bmp signaling is reminiscent of the epidermal versus neural fate specification of *Xenopus*. In early *Xenopus* embryos, Bmp signaling induces naïve ectoderm to adopt the epidermal fate, whereas anti-Bmp factors such as noggin and chordin inhibit Bmp signaling and promote the neural fate specification (Sasai and De Robertis, 1997). It is likely that the Bmp-Hes pathway regulates the choroid plexus fate, whereas the Bmp antagonist-Ngn pathway regulates the Cajal-Retzius cell fate (Fig. 9B). A full understanding of this process, however, will require further analysis, including the functional interaction between bHLH and homeodomain factors that are required for the choroid plexus formation.

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Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/135/15/2531/DC1

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