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The transcription factors Emx1 and Emx2 suppress choroid plexus development and promote neuroepithelial cell fate

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Abstract

The transcription factors Emx1 and Emx2 exert important functions during development of the cerebral cortex, including its arealization. Here, we addressed their role in development of the derivatives of the midline region in the telencephalon. The center of the midline region differentiates into the choroid plexus, but little is known about its molecular specification. As we noted a lack of Emx1 or 2 expression in the midline region early in development, we interfered by misexpressing Emx1 and/or Emx2 in this region of the chick telencephalon. Ectopic expression of either Emx1 or Emx2 prior to HH 13 instructed a neuroepithelial identity in the previous midline region instead of a choroidal fate. Thus, Gli3 and Lhx2 normally restricted to the neuroepithelium expanded into the Emx misexpressing region. This was accompanied by down-regulation of Otx2 and BMP7, which implicates that these factors are essential for choroid plexus specification and differentiation. Interestingly, the region next to the ectopic Emx-misexpression then acquired a hybrid identity with some choroidal features such as Bmp7, Otx2 and Ttr gene expression, as well as some neuroepithelial features. These observations indicate that the expression levels of Emx1 and/or Emx2 restrict the prospective choroid plexus territory, a novel role of these transcription factors.

Keywords: Telencephalon; Cortical hem; Midline; Roof plate; Patterning; Emx1; Emx2

Introduction

Patterning mechanisms are crucial to establish regional differences within the neuroepithelium, yet less is known about the mechanisms delineating neuroepithelial cells from the non-neural choroid plexus (ChP) epithelium, a secretory epithelium that differentiates from the roof of the neural tube in all brain ventricles. In the telencephalon, the roof plate undergoes a dramatic morphological change at midneurogenesis stages in rodents and birds: it invaginates and becomes buried between the two cerebral hemispheres. Concomitant with this invagination, the roof plate differentiates into the non-neural secretory ChP (Dziegielewska et al., 2001; Sturrock, 1979; Zaki, 1981). This differentiation is first detectable by the cessation of proliferation and the expression of Transthyretin (Ttr), an indicator of secretory epithelial differentiation (Currle et al., 2005; Furuta et al., 1997).

Between the ChP and the hippocampal region of the cortex, an intermediate multilayered epithelium forms a Wnt-rich signaling center, known as the cortical hem (Grove et al., 1998), that may partially derive from the roof plate (Currle et al., 2005). Due to its position as a signaling center between ChP and hippocampal anlage, the cortical hem might influence both sides. Indeed, mice lacking the region of the hem, usually also lack the ChP and hippocampus, which is the case in Emx1 and Emx2-double knockout mice (Shinozaki et al., 2004), Gli3-mutants (Grove et al., 1998; Theil et al., 1999) and the Lhx5−/− mice (Zhao et al., 1999). Mice displaying partial defects in the hem-region like Emx2−/− (Tole et al., 2000a; Yoshida et al., 1997) and Wnt3a−/− (Lee et al., 2000) also exhibit reductions in the ChP and hippocampal regions. Conversely, deletion of the Lhx2 gene leads to a significant enlargement of the cortical hem region accompanied by an enlargement of the ChP (Bulchand et

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al., 2001; Monuki et al., 2001). These dates suggest that Wnt-signaling from the cortical hem is important for the development of the ChP and seems to regulate its extension.

Members of the bone morphogenic proteins (Bmps) are crucial for ChP differentiation as conditional deletion of the Bmp receptor1a (BmpR1a) in the telencephalon interrupts ChP differentiation (Hebert et al., 2002). High levels of Bmps are expressed in the telencephalic roof plate region (Hebert et al., 2003; Jones et al., 1991) and contribute to the characteristic thin morphology of the differentiating ChP epithelium by instructing apoptosis and differentiation (Panchision et al., 2001; Solloway and Robertson, 1999). Indeed, Bmps seemingly act by an autocrine mechanism as the BMP-expressing cells themselves differentiate into the ChP as recently demonstrated in elegant fate mapping as well as cytotoxic deletion experiments (Currle et al., 2005; Monuki et al., 2001).

While the importance of Wnt and Bmp signaling for ChP specification and differentiation has been demonstrated, it is not known how the border between the neuroepithelium and the secretory epithelium is established and maintained. Wnt signaling affects both development of the neuroepithelial hippocampus as well as the secretory epithelium of the ChP. The molecular cues that instruct the hippocampal anlage to become or remain neuroepithelial tissue upon Wnt signaling, while the region medially adjacent to the hem differentiates into secretory epithelium upon Wnt signaling remain unknown. Here, we examined whether the transcription factors Emx1 and Emx2 may exert such a role, as they are expressed at particularly high levels in the medial telencephalon (hippocampal anlage and hem), but are absent from the roof plate and ChP region in the developing telencephalon of both mice and chick (Fernandez et al., 1998; Mallamaci et al., 1998; Muzio and Mallamaci, 2003, Shinozaki et al., 2004). In contrast, other transcription factors present in the neuroepithelium, such as Pax6, are also expressed in the chick ChP (data not shown). Here, we examined the role of the gap of Emx1 and Emx2 expression in the roof plate and ChP region by ectopic expression of these transcription factors in early development in the chick telencephalon (HH 9–14). Our results demonstrate a key role for Emx1 and Emx2 in instructing neuroepithelial identity, as misexpression of Emx1 and Emx2 was sufficient to convert non-neuronal ChP tissue into neuroepithelium. These data therefore imply that the limits of Emx1 and Emx2 expression determine the border between the neural and secretory epithelium.

Materials and methods

Electroporation

Fertilized eggs were windowed with scissors at embryonic day (E) 2. Electroporation was performed between HH 9 and HH 14, staged according to Hamburger and Hamilton (1951). The control plasmids pCAG containing either GFP or mRFP (kind gift of J. Guilford) and pCAX containing GFP, or the pMES plasmid (Swartz et al., 2001) containing either IRES-EGFP only, Emx1-IRES-EGFP or Emx2-IRES-EGFP (2–5 μg/μl) were injected into the forebrain and electroporated into the tissue (1–5 pulses, 50 ms, 20–25 V) with electrodes placed left and right of the forebrain. 70 μl of Penicillin/Streptomycin (GIBCO) was applied onto the embryo and the egg was sealed with tape. The embryos were harvested at E4 or E6 and the heads were fixed for 2–4 h at 4°C in 4% paraformaldehyde in phosphate buffered saline (PBS) and cryoprotected in 20% sucrose in PBS at 4°C over night. Coronal cryostat sections (20 μm) were collected on Superfrost Plus slides (Menzel).

Plasmid constructs for electroporation

Emx1 and Emx2 mouse cDNAs (kind gift of Antonio Simeone) containing parts of the 3′ and 5′ UTR were cloned into the multiple cloning site of the expression plasmid pMES containing an IRES-EGFP sequence that allows reliable coexpression of EGFP and the gene of interest (gift of Cathrin Krull) (Swartz et al., 2001). The expression of ectopic mouse Emx1 and Emx2 was confirmed by in situ hybridization (Supplementary Fig. 1) with a mouse-specific antisense mRNA probe (Emx1, Emx2) (Simeone et al., 1992a,b) and by immunostaining for Emx1 (rabbit, 1:1000, G. Corte) or Emx2 (rabbit, 1:4000, O. Hatano) and GFP (Supplementary Fig. 1).

Immunohistochemistry and RNA in situ hybridization

Sections were stained as described previously (Hartfuss et al., 2001), using primary antibodies against the phosphorylated form of Histon H3 (PH3, rabbit, Biomol, 1:200), MAP2 (mouse IgG1, 1:500, Sigma), Pax6 (rabbit, 1:300, Babco or Chemicon) and EGFP (rabbit, 1:500, RDI; or mouse IgG1, 1:300, Chemicon). Secondary antibodies were purchased from Jackson ImmunoResearch and Southern Biotechnology Associates. DAPI (4′,6-diamidino-2-phenylindole, Pierce) was used as a nuclear stain. Plasmid templates were used to generate digoxygenin antisense riboprobes: Otx2 (Wassef et al., 1987), Wnt7b (J. McMahon) (Hollyday et al., 1995), Bmp7 (A. Graham) (Begbie et al., 1999), Gli3 (C. Tabin) (Schweitzer et al., 2000), Ttr (M. Wassef) (Duan et al., 1991), Emx1, Emx2 (E. Bell) (Bell et al., 2001), Lhx2a (S. Richter) and in situ hybridization was performed as previously described (Chapouton et al., 2001).

Analysis of tissue thickness and proliferation

Cell proliferation (PH3+ cells) was quantified in single optical sections as the percentage of GFP-positive cells at E4 (electroporated with the control plasmid pMES or the pMES-Emx1 and/or Emx2-EGFP plasmid). This analysis was performed in a defined square, covering the entire neuroepithelium from the ventricular zone to the pial surface. Alternatively, quantification was performed per area in the electroporated region mostly at later stages (E6), since the GFP-signal was starting to weaken and individual GFP-positive cells were sometimes difficult to discern. Quantification was performed separately in the ChP region, the hem and the dorsal telencephalon in a radial stripe covering 200 μm of the ventricular surface (about 50 cell diameters). In these regions, we also counted the number of DAPI-positive nuclei located in a radial stripe from the ventricular to the pial surface to assess the radial thickness of the respective tissue. In cases, in which the identity of the tissue was not easily discernible by morphology any more (after ectopic expression of Emx1 and/or Emx2), the ‘midline’ of the dorsal telencephalon was defined as located at half the extension of the dorsal telencephalon. Error bars indicate the standard error of the mean (SEM) and the unpaired Student’s *t* test was used to test for significance.

Fig. 1. Gene expression changes upon ectopic expression of Emx1 and/or Emx2 at E4. In situ hybridization was performed in frontal sections for the genes indicated in the panels. Filled arrowheads show the extension of expression of the indicated genes, while the open arrowheads in panel D indicate also the region lacking expression around the dorsal midline. (A–F) Expression patterns in the telencephalon at E4 indicate the regional specification of the ChP (A–C), the cortical hem (D) and dorsal pallium (E, F). (G) Schematic summary of the region-specific expression pattern. (H–M) Frontal sections of E4 telencephali electroporated either with control plasmid (H–J) or the Emx1- and/or Emx2-containing plasmids (K–M). (H′, K′) GFP immunostaining indicates the electroporated region, the extent of which is indicted by arrows in panels H–M. Note the expression of Otx2, Bmp7 and Wnt7b in the midline region of control electroporated brains (H–J), while these mRNAs are down-regulated, respectively shifted laterally to the region of ectopic Emx1 and/or Emx2 expression (K–M). NE, neuroepithelium; RP, roof plate. Scale bar: 250 μm.