In the fetal human hippocampus, Cajal–Retzius (CR) cells coexpress p73, a p53-family member involved in cell survival and apoptosis, and the glycoprotein reelin, crucial for radial migration. We distinguish two populations of putative CR cells. (1) p73/reelin expressing cells appear around 10 gestational weeks (GW) at the cortico-choroid border in the temporal horn of the lateral ventricle (the ventral cortical hem) and occupy the marginal zone (MZ) overlying the ammonic and dentate primordia. (2) Additional p73-positive cells appear from 14 GW onward in the neuroepithelium near the dentate–fimbrial boundary and spread toward the pial surface, flanking the migrating secondary dentate matrix. From 13 to 17 GW, large parts of the dentate gyrus are almost devoid of CR cells. p73/Reelin-positive CR cells appear in the MZ of the suprapyramidal blade at 16 GW and around 21 GW in the infrapyramidal blade. The p73-positive cells of the dentate–fimbrial boundary express reelin when they are close to the pial surface, suggesting that they differentiate into CR cells of the infrapyramidal blade. Reelin-positive, p73-negative interneurons are prominent in the prospective strata lacunoso-molecular and radiatum of cornu ammonis as early as 14 GW; in the dentate molecular layer and hilus they appear around midgestation.

We propose that CR cells of the human hippocampal formation belong to two distinct cell populations: an early one derived from the ventral cortical hem and mainly related to migration of the ammonic and dentate plates and a later appearing one derived from the dentate–fimbrial neuroepithelium, which may be related to the protracted neurogenesis and migration of dentate granule cells, particularly of the infrapyramidal blade.

Keywords: choroid plexus, cortical hem, dentate gyrus, immunocytochemistry, Ki-67

Introduction

Cajal–Retzius (CR) cells are the dominant cell population in the developing marginal zone (MZ), the future molecular layer, of neocortex and archicortex (Marin-Padilla, 1978; Del Rio et al., 1997; Meyer et al., 1999). They secrete the extracellular matrix protein reelin, which is critically involved in the control of radial migration (D’Arcangelo et al., 1995, 1997; Ogawa et al., 1995) through a signaling pathway that includes the glycoprotein reelin. We recently showed that CR cells and reelin in the pathogenesis of certain mental disorders.

In the present study, we analyze the development of CR cells, defined by coexpression of p73 and reelin, in the human CA and dentate gyrus. We propose that distinct populations of CR cells are involved in the development of these two components of the human hippocampal formation. Furthermore, our results point to the ventral cortical hem as an important site of origin of human hippocampal CR cells.

Materials and Methods

Immunocytochemistry was carried out in 38 embryonic and fetal human brains obtained after spontaneous and legal abortions, following national guidelines in Spain and supervised by the Ethical Committee of the University of La Laguna. These cases were used also in previous papers (Meyer et al., 2000, 2002).

Fetal brains aged 7–27 gestational weeks (GW: 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 17.5, 18, 19, 20, 21, 22, 23, 25 and 27) and blocks from newborn brains were fixed in Bouin or Carnoy, embedded in paraffin and cut into 10 µm thick serial sections. For immunohistochemistry, sections were deparaffinized, rehydrated and washed in 0.05 M Tris-buffer saline (TBS; pH 7.6). To detect p73 and Ki-67, the
sections were boiled in citrate buffer (pH 6.00) for 15 min, washed in TBS and incubated with the primary antiserum overnight in a humid chamber at room temperature. Following incubation with the biotinylated secondary antibody diluted in TBS (1:150; DAKO, Glostrup, Denmark) for 30 min and with avidin–biotin–peroxidase complex 1:150 (ABC; DAKO), sections were immersed into a TBS solution (pH 7.6) containing 0.04% 3′-diamino-benzidine (DAB; Sigma), 0.04% nickel sulfate (Sigma) and 0.003% hydrogen peroxide.

In addition, double staining of p73/reelin, p73/Ki-67, p73/GFAP, p73/vimentin and Ki-67/GFAP was carried out (p73 and Ki-67 stain the nucleus, reelin, GFAP and vimentin the cytoplasm). Sections were incubated with the first primary antibody (p73 or Ki-67) overnight in a humid chamber at room temperature. After incubation with the biotinylated secondary antibody diluted in TBS (1:150), and with the ABC (1:150) for 30 min each, visualization was done using a solution containing DAB, nickel sulfate and hydrogen peroxide, which yielded a black reaction product. The sections were then washed and incubated with the second primary antibody (reelin, Ki-67, GFAP or vimentin) overnight. Following incubation with the biotinylated secondary antibody and ABC, they were immersed in TBS containing 0.03% DAB and 0.003% hydrogen peroxide. In this case, the reaction product was brown.

Sections were dehydrated, cleared with xylene and covered with Eukitt (O.Kindler GmbH, Freiburg, Germany).

The following primary antibodies were used: mouse monoclonal anti-reelin antibody 142 (1:350; de Bergeyck et al., 1998); rabbit polyclonal anti-p73α (1:150; Kaghad et al., 1997); mouse monoclonal anti-GFAP (1:250) and mouse monoclonal anti-vimentin (1:250; (Neomarkers, Fremont, CA); rabbit polyclonal anti-Ki-67 (1:200) (DAKO); and rabbit polyclonal anti-calretinin (1:1500) and anti-calbindin (1:2000; Swant, Bellinzona, Switzerland).

Results

**p73/Reelin-expressing Cells at the Ventral Cortical Hem (7–11 GW)**

The human hippocampal anlage is an annular structure along the midline of the hemisphere; its border with the choroid plexus epithelium forms the limbus or hem of the cerebral cortex (Broca, 1878). The dorsal extension of the hippocampus is transient and recedes as the corpus callosum develops (Stephan, 1975; Berger and Alvarez, 1996). The ventral hippocampus, located in the temporal lobe, develops into the adult hippocampal formation.

In our early fetal material, from 7 to 11 GW, coronal sections through the telencephalon revealed an almost symmetrical arrangement of the dorsal and ventral extensions of the cortical hem (Fig. 1). In the following, we refer exclusively to the ventral cortical hem and the development of the definitive human hippocampal formation.

At 7 GW, the MZ continuous with the ventral cortical hem contained a few p73-immunoreactive (ir) cells (Fig. 1A), most of which expressed also reelin (not shown). At 9/10 GW (Fig. 1B–D), the MZ had widened and a slight curve marked the site of the emerging hippocampal fissure (open arrow) and prospective hippocampal formation, although a condensed ammonic plate was not yet recognizable. The MZ of the hippocampal fissure narrowed as it blended with the MZ of the adjacent temporal cortex.

Figure 1. The cortical hem at 7 and 10 GW. (A) At 7 GW; dorsal and ventral cortical hem (arrowheads) are continuous with the choroid plexus anlage (Chp). The dorsal hippocampal primordium is slightly more advanced and its MZ contains numerous p73-ir cells. (B–D) At 10 GW; p73 (B), Ki-67 (C) and reelin (D) in adjacent sections showing dorsal and ventral cortical hem (arrowheads). The connection with the choroid plexus is not visible in B and C. p73- and reelin-ir cells in the MZ of the prospective hippocampal fissure (open arrow in B) have the same distribution, but cells in the hem express only p73. The open arrow in B marks the area where colocalization of p73 and reelin is shown in Figure 7A. The boxed areas in B and C are shown at higher magnification in Figure 2A, C. VZ, ventricular zone. Scale bars: A, 125 µm; B–D, 250 µm.
At 10 GW, the ventral cortical hem was populated by numerous p73-expressing cells; their presence in the VZ suggested that they were born at this site. Immunocytochemistry for the cell-proliferation marker Ki-67 and for p73 revealed a complementary distribution (Figs 1B, C and 2A, C): Ki-67 (Fig. 2A) was expressed in cells throughout the VZ but was almost absent in the MZ, while p73-expression (Fig. 2C) was faint in the VZ and strongest in the MZ, suggesting that cells initiated p73-expression after exiting the cell cycle.

To know whether the p73-ir cells in the ventral cortical hem were CR cells, we examined coexpression of p73 and reelin which defines this cell type. Virtually all cells in the MZ of the ventral cortical hem coexpressed p73 and reelin (Fig. 2D), whereas Ki-67 positive cells were reelin-negative (Fig. 2B). This finding indicated that the postmitotic derivatives of the cortical hem matched the neurochemical profile of CR cells.

We next asked whether the ventral cortical hem displayed distinctive features which set it apart from the adjacent hippocampal anlage. Vimentin immunostaining showed a differential distribution of radial glia fibers: in the hippocampal fissure, vimentin-ir fibers were radially oriented and their endfeet terminated at the pial surface (Fig. 2F); in the ventral cortical hem, radial orientation and pial endfeet of vimentin-ir fibers were not recognizable (Fig. 2E). This finding pointed to a different relationship between p73/reelin-ir cells and radial glia in ventral cortical hem and in future hippocampus.

**p73/Reelin-expressing Cells in Early Cornu Ammonis and Dentate Anlage (11–16 GW)**

From 11 to 16 GW, the components of the hippocampal formation acquired their distinctive locations at the dorsal (dentate gyrus and CA) and ventral (subiculum) aspects of the hippo-

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**Figure 2.** Ki-67, p73 and reelin in the ventral cortical hem at 10 GW. (A) Ki-67 positive cells are present throughout the VZ, but usually not in the MZ. (B) Double-staining Ki-67 (black)/reelin (brown) shows that reelin-ir cells in the MZ are not proliferative. (C) Strongest p73 expression is in cells in the MZ, weaker expression in the upper ventricular zone (VZ). (D) Double-staining p73 (black)/reelin (brown) in cells below the pial surface. (E) Double-staining p73 (black)/vimentin (brown) in the cortical hem and (F) in the hippocampal fissure. In the hem (E), vimentin-ir fibers do not extend radially toward the pia, while in the hippocampal fissure (F) radial glia fibers and pial endfeet are clearly observed. Scale bars: A, C, 30 μm; B, D, F, 20 μm; E, 50 μm.
campal fissure, which was progressively deepening (see also Humphrey, 1967). The major developmental steps of CA and dentate gyrus are shown in Figures 3 and 4, to illustrate the complex changes in the topographical relationships of CR cells with the underlying structures. The progressive deepening of the hippocampal fissure and its relationship with adjacent structures are shown at 12GW (Fig. 4A), 14GW (Fig. 4B) and 21 GW (Fig. 4C), the last representing an almost adult-like stage.

At 12 GW, the dentate anlage was visible with calretinin-immunostaining (Fig. 3B), while the CA3-field of the ammonic plate was calbindin-positive (Fig. 3A; see also Berger and Alvarez, 1996). At this stage, the dentate anlage did not contain Ki-67 ir cells (not shown) and thus the calretinin-ir cells did not represent a dentate matrix zone, but rather an early appearing ‘dentate plate’. The dentate anlage was further characterized by the strictly radial orientation of vimentin-ir fibers extending from the VZ to the pial surface and by a lack of a recognizable MZ and CR cells (Fig. 3C). By contrast, in the CA fields and subiculum, the MZ was wide and densely populated by CR cells (Fig. 3C). The MZ of CA was subdivided into an inner calbindin-positive MZ and an outer calbindin-negative MZ (Fig. 3A); CR cells were present in both, but more numerous in the outer MZ (Fig. 3C). The fimbria appeared at the site of the ventral cortical hem and contained calretinin-ir fibers (Fig. 3B), but no p73-ir cells.

From 14 GW onward, we identified the expanding dentate anlage by the presence of Ki-67 ir cells, which clearly marked the territory of granule precursor cells forming the secondary

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**Figure 3.** Cornu ammonis and dentate area from 12 to 14 GW. (A) At 12 GW, calbindin marks the end of the ammonic plate. (B) Calretinin marks a cell aggregate in the dentate area (DA) that may represent a dentate plate. The fimbria (F) occupies now the site of the former cortical hem. (C) Double-staining reelin (black)/vimentin (brown) at 13 GW; this section is at a more rostral level than A and B. The dentate area is almost devoid of reelin-ir cells. This is in contrast to the MZ of cornu ammonis (CA), which is densely populated by CR cells. (D) Reelin, (E) Ki-67 and (F) p73 at 14 GW. The dentate area is occupied by the proliferative cells of the secondary dentate matrix (E), but lacks a proper MZ and reelin-ir (D) and p73-ir (F) CR cells. The asterisks mark the deepest point of the hippocampal fissure, which corresponds to MZ of the CA fields and adjacent part of the dentate anlage. ChP, choroid plexus; imz, inner marginal zone; omz, outer marginal zone. Scale bars: A–C, 125 µm; D–F, 250 µm.
dentate matrix (Fig. 3E). p73-ir cells were no longer present at the former cortical hem but concentrated in the hippocampal fissure (asterisk in Fig. 3F), where they also expressed reelin (Fig. 3D). At 14 GW, a cluster of p73-ir cells appeared in the VZ near the dentate–fimbrial boundary (Fig. 3F). The surface of the proliferating dentate anlage was almost devoid of p73- and reelin-ir cells, except for a small part bordering with CA3 (Fig. 3D–F).

At 16/17 GW, CA and dentate gyrus had folded into the adult-like C-shape (Fig. 5A). The hippocampal fissure was partially fused between the MZ of CA and the suprapyramidal blade of the dentate gyrus. The highest number of CR cells was in the MZ of CA, but they extended also into the adjacent dentate gyrus, where the suprapyramidal blade began to develop a MZ. Around midgestation, also the infrapyramidal blade was abundantly populated by CR cells (Fig. 5G, H).

**p73-expressing Cells of the Dentate–Fimbrial Boundary (14–25 GW)**

As described above, the dentate anlage is particularly poor in p73- and reelin-expressing cells during early development; in contrast, the postnatal and adult dentate gyrus contains numerous p73/reelin-ir CR cells (Abraham and Meyer, 2003). We thus searched for possible origins and migration routes of the later-appearing CR cells.

In parallel with the intense cell proliferation in the dentate gyrus from 14 GW onward (Fig. 6), we identified a population of p73-positive cells which originated in the neuroepithelium near the dentate–fimbrial boundary, often marked by an indentation of the ventricle, and spread along the fimbria toward the pial surface near the infrapyramidal blade of the dentate gyrus (Fig. 5A,B). In the dentate area, they assumed a subpial position (Fig. 5C). On the whole, the territory of these p73-ir cells was more medial than the secondary dentate matrix, identified by Ki-67 immunoreactivity (Figs 5B and 6), although there was a certain overlap. Along the dentate–fimbrial border, reelin-staining was rather faint (Fig. 5D), and high reelin expression, characteristic of CR cells, was visible only in cells close to the pial surface.

We reconstructed the distribution of p73-positive cells at different ages from serial sections stained for p73, reelin, and Ki-67, to establish their possible relationship with the developmental kinetics of the dentate gyrus (Fig. 6). We observed that the subpial secondary matrix reached its maximum extent at 16 GW and receded at 21 GW, when the tertiary matrix became established in the hilus and infragranular layer. p73-ir cells flanked the secondary matrix at 16 GW and were most numerous at 21 GW along the dentate–fimbrial boundary and near the subpial end of the infrapyramidal blade. In turn, p73-reelin-ir cells in the dentate MZ increased in number from 16 to 21 GW (Figs 5G, H and 6), suggesting that p73-ir cells were indeed differentiating into CR cells. From 21 to 25 GW, the overall number of Ki-67-ir cells decreased in the hippocampal VZ as well as in the dentate matrix zones; in parallel, p73-ir cells along the dentate–fimbrial boundary also decreased in number.

To further characterize the p73-ir cells, we performed double-staining GFAP/p73 and vimentin/p73 at 16 GW. The dentate–fimbrial p73-ir cells did not express glial markers and GFAP-positive cells in the VZ (Fig. 5E) and vimentin-positive cells along the dentate–fimbrial boundary (Fig. 5F) had p73-negative cell nuclei.

**p73 in the Hippocampal Ependyma (16–40 GW)**

Until 15 GW, p73-immunoreactivity in VZ was conspicuous but concentrated around the dentate–fimbrial border (Figs. 3F). From 16/17 GW onward, p73-ir cells in the VZ became more numerous and gradually extended laterally (Fig. 5A), until after midgestation the entire hippocampal and adjacent temporal ventricular surface was lined by p73-ir ependymal cells (Fig. 6). p73-ir ependymal cells did not express reelin.
Reelin-expressing Interneurons of the Hippocampal Formation

In the initial stages of hippocampal development, virtually all reelin-ir cells were also p73-positive (Fig. 7A). The first reelin-positive, p73-negative neurons appeared at 14 GW in the inner MZ overlying the ammonic plate (Fig. 7B, arrows), while p73/reelin-ir cells predominated in the outer MZ. From 16GW onward, reelin-ir neurons were found widely scattered throughout the strata lacunosum-moleculare and radiatum of CA (Fig. 7C) and also intermixed with the p73/reelin-positive CR cells along the hippocampal fissure. These cells displayed the variable orientations and morphologies described for hippocampal interneurons (Alcántara et al., 1998; Abraham and Meyer, 2003).

The time course of reelin expression in the dentate gyrus was different. The late appearance of CR cells in the dentate molecular layer was most evident in the infrapyramidal blade [cf. Fig. 5A (17 GW) and G (21 GW)]. Similarly, small reelin-positive, p73-negative neurons appeared in the dentate molecular layer and hilus only during the second half of gestation (Fig. 7D, arrowheads). In the adult, p73/reelin-ir CR cells are rare, whereas reelin-positive interneurons are numerous in both CA and dentate gyrus (Abraham and Meyer, 2003).

Discussion

CR Cells in the Developing Human Hippocampus

CR cells are thought to be important for cell migration and positioning in the developing neocortex and archicortex. They secrete reelin, a glycoprotein necessary for the normal inside-out migration gradient of neocortex and CA (D’Arcangelo et al., 1995, 1997). In keeping with this essential role, reelin-
Figure 6. Distribution of Ki 67-ir cells (small black dots), p73-ir cells (orange dots) and p73/reelin double stained CR cells (blue dots) at 16, 21 and 25 GW. At 16 GW, the secondary dentate matrix extends from the VZ to the pial surface; the dentate MZ contains few p73/reelin-ir cells. A trail of p73-ir cells lies medial to the secondary matrix; some cells coexpress reelin. At 21 GW, the tertiary dentate matrix has appeared in the hilus; p73-ir cells along the dentate–fimbria boundary have increased in number. The dentate molecular layer has widened, and contains more p73/reelin-ir CR cells. At 25 GW, proliferative cells in the hilus and p73-ir cells of the dentate–fimbria boundary are reduced in number compared to 21 GW. At 21 and 25 GW, the ependyma is p73-ir (orange line). Asterisks mark the hippocampal fissure. DG, dentate gyrus; E, ependyma; F, fimbria; h, hilus; i, infrapyramidal blade; IZ, intermediate zone; MZ, marginal zone; s, suprapyramidal blade; SUB, subiculum, VZ, ventricular zone. The white arrow marks the ventricular indentation at the dentate–fimbria boundary. Scale bars: 300 µm.
expressing CR cells are present from the earliest moments of corticogenesis; they are complemented by later-appearing reelin-ir cells as migration proceeds and the cortical surface expands (Meyer and Goffinet, 1998; Zecevic et al., 1999; Meyer et al., 2000, 2002; Zecevic and Rakic, 2001). However, reelin is widely distributed throughout the CNS (Schiffmann et al., 1997); in neocortex and hippocampus, it is also present in a variety of interneurons (Alcántara et al., 1998; Drakew et al., 1998; Pesold et al., 1998, 1999; Pérez-Garcia et al., 2001; Abraham and Meyer, 2003). Conversely, developmental expression of p73 is restricted to CR cells, a few cells in the hypothalamus and the choroid plexus epithelium (Yang et al., 2000; Meyer et al., 2002; Tissir et al., 2003), so that p73 is more specific for CR cells than reelin. The possible activities of p73 in the developing brain are as yet unclear. Deficiency of p73 leads to postnatal neuronal death (Pozniak et al., 2002), whereas prenatal cortical development is not significantly disturbed (Yang et al., 2000; Meyer et al., 2002). Expression of p73 may be related to survival of CR cells under developmental stress (Meyer et al., 2002).

The neurochemical phenotype of neocortical and archicortical CR cells is similar in that both coexpress reelin and p73. However, on morphological grounds, human archicortical CR cells differ from CR cells in the neocortex: they do not display the exuberant processes and variable soma orientations characteristic of human neocortical CR cells (Meyer et al., 1999) and their axonal plexus is not as sharply defined. In this aspect, they rather resemble CR cells of the rodent neocortex, described as horizontal bipolar or fusiform neurons (Derer and Derer, 1990; Soriano et al., 1994). In addition, hippocampal CR

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**Figure 7.** Cajal-Retzius cells and reelin-ir interneurons. (A) At 10 GW, p73 (black) and reelin (brown) are almost completely colocalized in cells of the prospective hippocampal fissure. (B) At 14 GW, MZ of CA; p73 and reelin colocalize in the outer MZ, while in the deeper MZ there are also reelin-positive, p73-negative cells (arrowheads). (C) At 21 GW; p73 and reelin are coexpressed in CR cells in the hippocampal fissure (asterisk). Reelin-positive, p73-negative interneurons (arrowheads) are distributed throughout the strata radiatum (r) and lacunosum-moleculare (lm). (D) At 27 GW, in addition to CR cells in the hippocampal fissure (asterisk), reelin-ir cells appear also in the deep molecular layer (arrowheads) of dentate gyrus and in the hilus (h). gl, granule cell layer; ml DG, molecular layer of the dentate gyrus. Scale bars: A, 20 µm; B–D, 40 µm.
cells may survive in small numbers throughout adult life (Abraham and Meyer, 2003), whereas most neocortical CR cells die during the last months of fetal life (Meyer and Gonzalez-Hernandez, 1993; Meyer et al., 2002).

The Ventral Cortical Hem as a Possible Origin of Early Hippocampal Cajal–Retzius Cells

The cortical hem represents the interface of choroid plexus epithelium and proliferative cortical neuroepithelium. Studies in rodent brain showed that the dorsal cortical hem is defined by the graded expression of a variety of genes, most prominently of the Wnt and BMP families, and proposed that it represents a putative signaling center during embryonic development (Grove et al., 1998). The cortical hem is adjacent to the roof plate, and cell migrations from this site may influence the size of the future cortical territory (Monuki et al., 2001). We previously described a subset of p73/reelin expressing CR cells which migrated tangentially from the dorsal hem into the neocortical MZ and were undetectable in p73-deficient mice (Meyer et al., 2002). The cortical hem, defined as a developmental territory characterized by Wnt and p73 expression, is transient and declines with the appearance of the fimbria (Grove et al., 1998; Meyer et al., 2002).

We noted that early in development the ventral cortical hem resembled a mirror image of the dorsal cortical hem. In analogy with our previous results in the dorsal cortical hem of the mouse, we propose here that the p73-ir cells of the ventral cortical hem differentiate into p73/reelin expressing CR cells of the hippocampal fissure. According to this model, the ventral cortical hem provides early CR cells for the initial steps in hippocampal development, which comprise the establishment of the radial glia scaffold in the hippocampal fissure and the migration of the ammonic and dentate plates. The early presence of CR cells is in line with the observation that reelin regulates the development of Dab1-positive radial glia in the hippocampal primordium, which provide a template for radially migrating neurons ( Förster et al., 2002; Frotscher et al., 2003). CR cells are thus needed for the most basic steps in archicortical development and are already in place at the onset of radial migration.

Neurogenesis of CA begins early in fetal life and proceeds rapidly; major cytoarchitectonic fields are established by 15 GW (Arnold and Trojanowski, 1996) and a significant number of synapses exist in MZ and in subplate (Kostovic et al., 1989). During the migration period of CA pyramidal cells, the MZ of CA contains numerous p73/reelin-ir CR cells to control correct lamination. The severe malformations of the ammonic plate in the absence of a functional reelin–Dab1 pathway (Stanfield and Cowan, 1979; Caviness, 1982; Nakajima et al., 1997; Deller et al., 1999) support the critical role of reelin signaling in radial migration of Ammon’s horn and emphasize the importance of early-appearing CR cells.

It should be noted that a ventral cortical hem has not been described in rodents. We may hypothesize that a ventral hem is particularly highly evolved in the primate or human brain, because the definitive hippocampus is restricted to the temporal horn of the lateral ventricle. It is possible that a ventral hem as a source of CR cells may not be evident in species which are characterized by a prominent dorsal hippocampus.

Cajal–Retzius Cells of the Developing Dentate Gyrus

One of our most striking findings is that the distribution of reelin-expressing cells along the dentate surface undergoes continuous changes, which accompany the complex unfolding of the secondary and tertiary matrix zones. In the initial phase of hippocampal development, p73/reelin-positive CR cells extend over the entire hippocampal primordium, including a distinct non-proliferating ‘dentate plate’ within the radial glia scaffold. A dentate plate was previously reported in fetal monkey hippocampus (Eckenhoff and Rakic, 1984), but seems to have no counterpart in the rodent brain. The presence of reelin in the earliest stage of dentate formation concurs with the establishment of the primary radial glia scaffold, a process in which the reelin–Dab1 signaling pathway plays a crucial role (Fürst et al., 2002; Frotscher et al., 2003; Weiss et al., 2003).

The second phase of dentate development begins around 14 GW, when a massive stream of proliferative cells toward the pial surface marks the appearance of the secondary matrix. Most noticeably, this process takes place in the practical absence of CR cells in the dentate gyrus, which is in contrast with the ubiquitous presence of CR cells in the cortical mantle. The paucity of reelin at this stage may be related to the outside-in migration gradient of the dentate, which is opposite to the generalized inside-out gradient of neocortex and Ammon’s horn (Angevine, 1965; Bayer, 1980; Rakic and Nowakowski, 1981; Altman and Bayer, 1990a,b), where radial migration is controlled by the reelin–Dab1 signaling pathway. Our data suggest that the migration of the proliferative neuroblasts of the secondary matrix is independent of reelin.

The massive appearance of dentate CR cells concurs with the establishment of the tertiary dentate matrix in the infragranular zone and hilus (Novakowski and Rakic, 1981; Altman and Bayer, 1990a,b). CR cells populate first the suprapyramidal blade, and later also the infrapyramidal blade, following the neurogenetic gradient of granule cells (Rakic and Nowakowski, 1981; Altman and Bayer, 1990a,b). At this stage, the reelin–Dab1 pathway may be needed for the establishment of the secondary radial glia scaffold extending between the hilar zone and the granule cell layer (Rickmann et al., 1987; Förster et al., 2002). In reeler, scrambler and double VLDLR/ApoER2 mutant mice, late-born granule cells are not clearly segregated from the hilus and less densely packed compared to wildtype (Stanfield and Cowan, 1979; Drakew et al., 2002; Förster et al., 2002; Weiss et al., 2003). Similarly, in adult human brain, reelin in CR cells seems to be important for the normal compact arrangement of the granule cell layer (Haas et al., 2002) and alterations of the number of CR cells and levels of reelin expression are associated with granule cell dispersion and temporal lobe epilepsy (Haas et al., 2002; Thom et al., 2002; Frotscher et al., 2003).

The Dentate–Fimbrial Cell Population

Our observation that CR cells appear relatively late in the molecular layer of the dentate gyrus raises the question of their origin and migration route. We identified a possible source in a distinct sector of the neuroepithelium near the dentate-fimbrial border, where p73-positive cells leave the VZ and reach the pial surface near the medial end of the infrapyramidal blade.

Our evidence that these p73-ir cells develop into CR cells is indirect, because reelin was expressed mostly in cells near the pial surface and only initially (15 GW) in cells along the dentate-fimbrial border. However, several arguments support...
our hypothesis: dentate CR cells are p73-positive and there were no other visible sources of p73-ir cells in the telencephalon; conversely, no other cell types in the hippocampus expressed the protein. p73 expression was associated only with reelin, not with other markers such as vimentin or GFAP, which would suggest a glial phenotype. However, it is possible that not all p73-ir cells are destined to become CR cells, but remain in an undifferentiated state in the dentate-fimbrial area, or downregulate protein-expression.

In any case, the phenotype of the p73 knock-out mouse indicates that the formation of the infrapyramidal blade depends critically on the presence of CR cells (Yang et al., 2000). We suggest here that p73-ir cells of the dentate–fimbrial boundary may develop into late-appearing infrapyramidal dentate CR cells; however, a similar cell system has as yet not been identified in the mouse. Our current studies of p73-deficient mice suggest that CR cells may act on hippocampal development through reelin-independent mechanisms (Meyer et al., 2003). Regardless of the possible molecular pathways involved, the human dentate gyrus develops and expands during a protracted period of pre- and postnatal life (Eriksson et al., 1998; Seress et al., 2001) and may require regulatory cell systems that are rudimentary or unrecognizable in the mouse brain.

The Reelin-positive Interneurons of CA and Dentate Gyrus

Reelin is expressed by a variety of cell classes, including CR cells and interneurons. The presence of reelin-positive interneurons in adult life (Pesold et al., 1998; Pérez-García et al., 2001; Abraham and Meyer, 2003) indicates that reelin fulfills multiple functions, from a role in the establishment of the radial glia scaffold at the onset of corticogenesis (Förster et al., 2002) and in the extension of growth cones during migration (Beffert et al., 2002), to ongoing brain plasticity in the adult (Pesold et al., 1999; Guidotti et al., 2000; Rodriguez et al., 2000).

Reelin-expressing interneurons are GABAergic (Pesold et al., 1998; Guidotti et al., 2000); in human hippocampus, most of them express also calretinin, a few are calbindin-positive, while others do not express calcium-binding proteins (Abraham and Meyer, 2003). Our main criterion to distinguish CR cells from interneurons was the selective expression of p73 in CR cells and its absence in interneurons (Meyer et al., 2002; Abraham and Meyer, 2003). In fact, functional inactivation of the p73 gene leads to a loss of CR cells, while reelin-positive interneurons are not affected (Yang et al., 2000). Large areas of the neocortex of p73-deficient mice develop normally (Meyer et al., 2003), possibly because the abundance of reelin in subcortex and early-appearing interneurons may compensate for the absence of CR cells. By contrast, the dentate gyrus may be particularly vulnerable to a loss of CR cells since its reelin-positive interneurons appear late in development.

While we identified the cortical hem and dentate/fimbrial VZ as possible birth places of CR cells, we were unable to determine the origin and migration routes of reelin-positive interneurons. Recent rodent studies indicated that interneurons are born in ganglionic eminences and migrate tangentially to the hippocampus (Pleasure et al., 2000). The migratory mechanisms of the reelin-expressing cell populations of the human hippocampus require further studies, the more so since changes in the number of reelin-expressing cells have been reported in human pathological conditions, particularly in temporal lobe epilepsy (Haas et al., 2002; Thom et al., 2002; Frotscher et al., 2003).

Notes

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References


Förster E, Tielsch A, Baumbach KH, Johannsen C, Graus-Porta D (2002) Reelin, disabled 1, and beta integrins are required for the formation of the radial glia scaffold in the hippocampus. Proc Natl Acad Sci USA 99:13178–13183.


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