Development 130, 93-102

doi:10.1242/dev.00184

Fgfr3 expression by astrocytes and their precursors: evidence that astrocytes and oligodendrocytes originate in distinct neuroepithelial domains

Nigel P. Pringle[1], Wei-Ping Yu[2], Marisa Howell[1], Jennifer S. Colvin[3], David M. Ornitz[3] and William D. Richardson[1,*]

[1]Wolfson Institute for Biomedical Research and Department of Biology, University College London, Gower Street, London WC1E 6BT, UK
[2]Institute of Molecular and Cell Biology, 30 Medical Drive, Singapore 117609
[3]Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110, USA
*Author for correspondence (e-mail: w.richardson@ucl.ac.uk)

Accepted 3 October 2002

SUMMARY

The postnatal central nervous system (CNS) contains many scattered cells that express fibroblast growth factor receptor 3 transcripts (*Fgfr3*). They first appear in the ventricular zone (VZ) of the embryonic spinal cord in mid-gestation and then distribute into both grey and white matter – suggesting that they are glial cells, not neurones. The *Fgfr3*$^+$ cells are interspersed with but distinct from platelet-derived growth factor receptor α (*Pdgfra*)-positive oligodendrocyte progenitors. This fits with the observation that *Fgfr3* expression is preferentially excluded from the pMN domain of the ventral VZ where *Pdgfra*$^+$ oligodendrocyte progenitors – and motoneurones – originate. Many glial fibrillary acidic protein (Gfap)-positive astrocytes co-express *Fgfr3* in vitro and in vivo. *Fgfr3*$^+$ cells within and outside the VZ also express the astroglial marker glutamine synthetase (*Gln*s). We conclude that (1) *Fgfr3* marks astrocytes and their neuroepithelial precursors in the developing CNS and (2) astrocytes and oligodendrocytes originate in complementary domains of the VZ. Production of astrocytes from cultured neuroepithelial cells is hedgehog independent, whereas oligodendrocyte development requires hedgehog signalling, adding further support to the idea that astrocytes and oligodendrocytes can develop independently. In addition, we found that mice with a targeted deletion in the *Fgfr3* locus strongly upregulate Gfap in grey matter (protoplasmic) astrocytes, implying that signalling through Fgfr3 normally represses Gfap expression in vivo.

Key words: Fgfr3, Targeted deletion, Astrocyte, Reactive gliosis, CNS, Neuroepithelium

INTRODUCTION

In the embryonic CNS, neurones and glia develop from the neuroepithelial cells of the ventricular zone (VZ) that surrounds the ventricles of the brain and the lumen of the spinal cord. Different domains of the VZ express different gene products and generate different subsets of neurones and/or glia. For example, the ventral half of the spinal cord VZ is subdivided into five regions labelled (from ventral to dorsal) p3, pMN, p2, p1 and p0. These five domains express different combinations of homeodomain (HD) and basic helix-loop-helix (bHLH) transcription factors and generate distinct classes of spinal neurones; pMN gives rise to somatic motoneurones, whereas p0-p3 give rise to four classes of ventral interneurones (V0-V3 respectively) (reviewed by Briscoe and Ericson, 1999; Jessell, 2001). In the brainstem, p3 also gives rise to visceral motoneurones (Ericson et al., 1997).

After neurones, the VZ switches to producing glial cells. Oligodendrocytes, the myelinating glial cells of the CNS, develop from the ventral VZ. Small numbers of oligodendrocyte progenitors (OLPs), which express the platelet-derived growth factor receptor-α (Pdgfra), first appear at the ventricular surface on embryonic day 12.5 (E12.5) in the mouse, then proliferate and migrate away into the grey and white matter before starting to differentiate into myelin-forming oligodendrocytes (Miller, 1996; Rogister et al., 1999; Richardson et al., 2000; Spassky et al., 2000). In rodents, OLPs are generated from the same part of the neuroepithelium as somatic motoneurones (MNs) but not until after MN production has ceased (Sun et al., 1998; Lu et al., 2000) (for a review, see Rowitch et al., 2002). This prompted us to suggest that there is a pool of shared neuroglial precursors that first generates MNs, then switches to OLPs (Richardson et al., 1997; Richardson et al., 2000). This idea has been supported recently by the finding that the bHLH proteins Olig1 and Olig2 are expressed and required in pMN for production of both motoneurones and OLPs (Lu et al., 2002; Zhou and Anderson, 2002; Takebayashi et al., 2002) (reviewed by Rowitch et al., 2002).

Where do astrocytes, the other major class of CNS glia, originate in the neuroepithelium? It is believed that at least some astrocytes are generated by transdifferentiation of radial

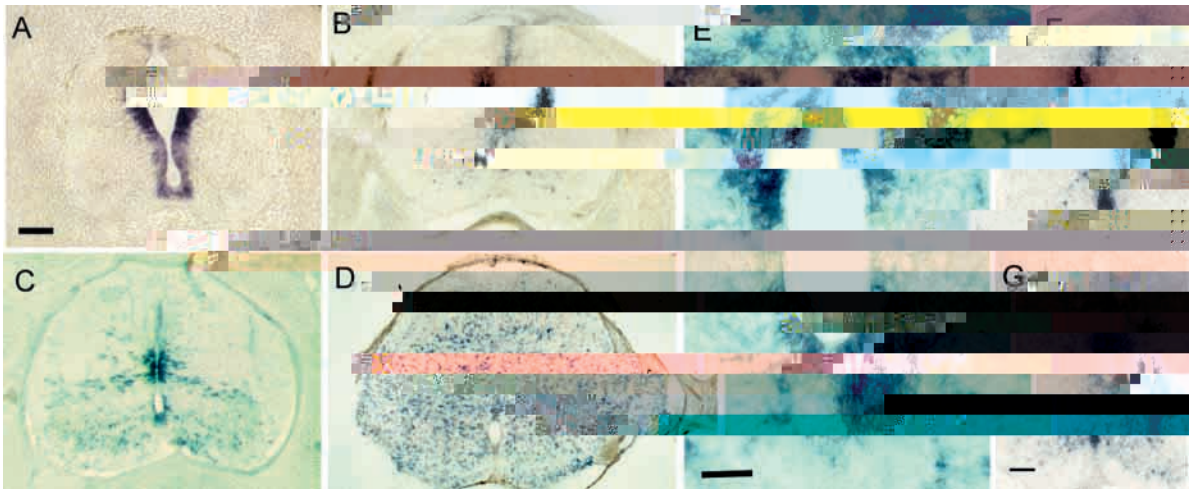

Fig. 1. *Fgfr3* expression in transverse sections of embryonic chick and mouse cervical spinal cords. (A) Chick stage 22-24 (E3.5-4); (B) chick stage 34 (E8); (C) chick stage 35 (E9); (D) chick stage 37 (E11); (E) chick stage 35 (E9); (F) mouse E13.5; and (G) mouse E14.5. Initially, *Fgfr3* is expressed in the floor plate and the ventral two-thirds of the VZ (A) and is later downregulated in part of the ventral VZ (B). Starting around stage 34 (E8) *Fgfr3*$^+$ cells are visible in the parenchyma of the cord. By stage 37 (E11) the floor plate and VZ no longer express *Fgfr3* but scattered *Fgfr3*$^+$ cells are present throughout most of the cross-section of the cord, including both grey and white matter (D). (E) A magnified image of the ventral VZ from a stage 35 (E9) cord, showing the two spatially separated domains of *Fgfr3* expression. A similar progression occurs in mouse (F,G). However, the ventral 'gap' is not so pronounced in mouse (arrow in G). Scale bars: 200 μm (A-D), 100 μm (F,G), 50 μm (E).

For double in situ hybridisation, two probes – one FITC labelled and the other DIG labelled – were applied to sections simultaneously. The FITC signal was visualised with alkaline phosphatase (AP)-conjugated anti-FITC Fab_2 fragments before developing in p-iodonitrotetrazolium violet (INT) and 5-bromo-4-chloro-3-indolyl phosphate (toluidine salt) (BCIP), which produces a magenta/brown reaction product. The sections were photographed, then the AP was inactivated by heating at 65°C for 30 minutes followed by incubating in 0.2 M glycine (pH 2) for 30 minutes at room temperature. The INT-BCIP reaction product was removed by dehydration through graded alcohols, concluding with 100% ethanol for 10 minutes at room temperature. The DIG signal was then visualised with AP-conjugated anti-DIG Fab_2 fragments and a mixture of nitroblue tetrazolium (NBT) and BCIP (all reagents from Roche Molecular Biochemicals) and the sections re-photographed. No labelling with NBT/BCIP was observed when we omitted either the DIG labelled probe or the anti-DIG antibody (data not shown).

For the *Fgfr3-Pdgfra* double in situ hybridisation of Fig. 4 we visualised the FITC (*Pdgfra*) signal with horseradish peroxidase (HRP)-conjugated anti-FITC Fab_2 fragments (Roche) before developing in fluorescein-tyramide reagent (NEN^{TM} Life Science Products, Boston) according to the manufacturer's instructions. The HRP-conjugate was inactivated by incubating in 2% (v/v) hydrogen peroxide for 30 minutes at room temperature. The DIG (*Fgfr3*) signal was then visualised with HRP-conjugated anti-DIG Fab_2 fragments followed by rhodamine-tyramide, and the sections photographed under fluorescence optics. As specificity controls we omitted either the FITC-labelled *Pdgfra* probe or the HRP-conjugated anti-FITC antibody, which gave no staining other than for *Fgfr3* (not shown).

Combined immunolabelling and in situ hybridisation

For the experiment of Fig. 7, cultured cells were first subjected to in situ hybridisation with a [^{35}S]-labelled RNA probe against *Fgfr3* then immunolabeled with anti-Gfap and biotinylated goat-anti-mouse Ig. The Gfap signal was developed with DAB and the slides dehydrated through ascending alcohols, dipped in nuclear emulsion (Ilford K5), exposed in the dark for several days and developed in Kodak D19.

RESULTS

Fgfr3 expression in the embryonic spinal cord

We examined *Fgfr3* expression in the embryonic chick spinal cord by in situ hybridisation. At stage 22-24 (corresponding to ~E4), *Fgfr3* expression was confined to the floor plate and the ventral two-thirds of the VZ (Fig. 1A). By stage 34 (E8) *Fgfr3* expression had been extinguished in part of the ventral VZ so that a gap developed in the expression pattern (e.g. Fig. 1B).

Individual *Fgfr3*$^+$ cells were also present outside the VZ

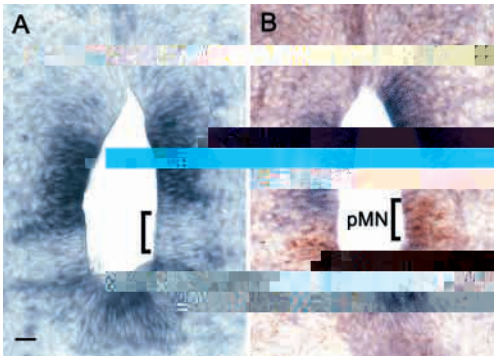

Fig. 2. Expression of *Fgfr3* and *Olig2*. Transverse sections through stage 35 (E9) chicken spinal cords were subjected to in situ hybridisation for *Fgfr3* (A) or double in situ for *Fgfr3* and *Olig2* (B). At this age, *Fgfr3* expression is confined to the VZ and a few scattered cells outside the VZ. The two spatially separated domains of *Fgfr3* expression are clearly visible (A). *Olig2* is expressed predominantly within the ventral 'gap' of *Fgfr3* expression (B). This suggests that pMN (brackets), which generates *Pdgfra*$^+$ oligodendrocyte progenitors (OLPs), does not also generate *Fgfr3*$^+$ putative astrocyte progenitors. Scale bar: 50 μm.

after stage 34 (E8), both lateral and dorsal to the *Fgfr3*$^{+}$

DISCUSSION

On the basis of their spatial distribution and time of appearance, Peters et al. (Peters et al., 1993) suggested that *Fgfr3*

surface. However, radial glia are distributed all around the spinal cord lumen, unlike *Fgfr3*, so one would have to postulate that only a subset of radial glia express *Fgfr3*.

In double-knockout mice that lack the two basic helix-loop-helix (bHLH) transcriptions factors Olig1 and Olig2, the pMN domain of the VZ undergoes a homeotic transformation into p2, its immediate dorsal neighbour (Rowitch et al., 2002). As a result, pMN no longer generates motoneurones followed by OLPs, but instead produces V2 interneurones followed by astrocytes (Zhou and Anderson, 2002; Takebayashi et al., 2002). By implication, this is the usual fate of p2 precursors in wild-type mice. This is consistent with our observation that *Fgfr3*$^{+}$ astrocytes apparently originate within an extended part of the ventral VZ, including p2 but excluding pMN.ig2,l VZ, including

REFERENCES

Altman, J. and Bayer, S. A. (1984). The development of the rat spinal cord. *Adv. Anat. Embryol. Cell Biol.* **85**, 1-166.

Baas, D., Dalencon, D., Fressinaud, C., Vitkovic, L. and Sarlieve, L. L. (1998). Oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells express glutamine synthetase: developmental and cell type-specific regulation. *Mol. Psychiatry* **3**, 356-361.

Bansal, R., Kumar, M., Murray, K., Morrison, R. S. and Pfeiffer, S. E. (1996). Regulation of FGF receptors in the oligodendrocyte lineage. *Mol. Cell. Neurosci*. **7**, 263-275.

Benjelloun-Touimi, S., Jacque, C. M., Derer, P., de Vitry, F., Maunoury, R. and Dupouey, P. (1985). Evidence that mouse astrocytes may be derived from the radial glia. An immuno-histochemical study of the cerebellum in the normal and reeler mouse. *J. Neuroimmunol*. **9**, 87-97.

Bignami, A. and Dahl, D. (1974). Astrocyte-specific protein and radial glia in the cerebral cortex of newborn rat. *Nature* **252**, 55-56.

Bottenstein, J. E. and Sato, G. H. (1979). Growth of a rat neuroblastoma cell line in serum-free supplemented medium. *Proc. Natl. Acad. Sci. USA* **76**, 514-517.

Briscoe, J. and Ericson, J. (1999). The specification of neuronal identity by graded Sonic Hedgehog signalling. *Semin. Cell Dev. Biol.* **10**, 353-362.

Briscoe, J., Pierani, A., Jessell, T. M. and Ericson, J. (2000). A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube. *Cell* **101**, 435-445.

Choi, B. H., Kim, R. C. and Lapham, L. W. (1983). Do radial glia give rise to both astroglial and oligodendroglial cells? *Dev. Brain Res.* **8**, 119-130.

10*rk(,)Tj -30.0430.*

Alzheimer's disease indicates a profound impairment of metabolic interactions with astrocytes. *Neurochem. Int.* **36**, 471-482.

Rogister, B., Ben-Hur, T. and Dubois-Dalcq, M. (1999). From neural stem cells to myelinating oligodendrocytes. *Mol. Cell. Neurosci.* **14**, 287-300.

Rowitch, D. H., Lu, R. Q., Kessaris, N. and Richardson, W. D. (2002). An 'oligarchy' rules neural development. *Trends Neurosci.* **25**, 417-422.

Sommer, I. and Schachner, M. (1981). Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: an immunocytological study in the central nervous system. *Dev. Biol.* **83**, 311-327.

Spassky, N., Olivier, C., Perez-Villegas, E., Goujet-Zalc, C., Martinez, S., Thomas, J.-L. and Zalc, B. (2000). Single or multiple oligodendroglial lineages: a controversy. *Glia* **29**, 143-148.

Stallcup, W. B. and Beasley, L. (1987). Bipotential glial progenitor cells of the optic nerve express the NG2 proteoglycan. *J. Neurosci.* **7**, 2737-2744.

Stanimirovic, D. B., Ball, R., Small, D. L. and Muruganandam, A. (1999). Developmental regulation of glutamate transporters and glutamine synthetase activity in astrocyte cultures differentiated in vitro. *Int. J. Dev. Neurosci.* **17**, 173-184.

Sun, T., Pringle, N. P., Hardy, A. P., Richardson, W. D. and Smith, H. K. (1998). Pax6 inluences the time and site of origin of glial precursors in the ventral neural tube. *Mol. Cell. Neurosci.* **12**, 228-239.

Takebayashi, H., Nabeshima, Y., Yoshida, S., Chisaka, O., Ikenaka, K. and Nabeshima, Y. (2002). The basic helix-loop-helix factor olig2 is essential for the development of motoneuron and oligodendrocyte lineages. *Curr. Biol.* **12**, 1157-1163.

Theiler, K. (1972). *The House Mouse. Development and Normal Stages from Fertilization to 4 Weeks of Age*. Berlin, Heidelberg, New York: Springer-Verlag.

Unsicker, K., Grothe, G., Ludecke, G., Otto, D. and Westermann, R. (1993). Fibroblast growth factors: their roles in the central and peripheral nervous system. In: *Neurotrophic Factors* (ed. S. E. Loughlin and J. H. Fallon), pp. 313-338. San Diego, CA: Academic Press.

Voigt, T. (1989). Development of glial cells in the cerebral wall of ferrets: direct tracing of their transformation from radial glia into astrocytes. *J. Comp. Neurol.* **289**, 74-88.

Woodward, W. R., Nishi, R., Meshul, C. K., Williams, T. E., Coulombe, M. and Eckenstein, F. P. (1992). Nuclear and cytoplasmic localization of basic fibroblast growth factor in astrocytes and CA2 hippocampal neurons. *J. Neurosci*. **12**, 142-152.

Zhou, Q., Wang, S. and Anderson, D. J. (2000). Identification of a novel family of oligodendrocyte lineage-specific basic helix-loop-helix transcription factors. *Neuron* **25**, 331-343.

Zhou, Q. and Anderson, D. J. (2002). The bHLH transcription factors Olig2 and Olig1 couple neuronal and glial subtype specification. *Cell* **109**, 61-73.