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THE DIFFERENTIATION POTENTIAL OF PRECURSOR CELLS FROM THE MOUSE LATERAL GANGLIONIC EMINENCE IS RESTRICTED BY *IN VITRO* EXPANSION

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Abstract—We have investigated whether the differentiation potential of attached cultures derived from the mouse lateral ganglionic eminence (LGE) is influenced by *in vitro* expansion. Primary neuronal cultures derived from the LGE give rise to neurons expressing the striatal projection neuron markers Islet1 (ISL1) and dopamine and cAMP-regulated phosphoprotein of 32 kilodaltons (DARPP-32) as well as the olfactory bulb interneuron marker Er81. Our previous results showed that after expansion *in vitro*, LGE precursor cells can be induced to differentiate into neurons which exhibit molecular characteristics of the LGE, such as the homeobox transcription factors DLX and MEIS2. We show here that while attached LGE cultures maintain Er81 expression through five passages, they lose the ability to generate ISL1- or dopamine and cAMP-regulated phosphoprotein of 32 kilodaltons-expressing neurons already after the first passage. This indicates that the expansion of LGE precursor cells restricts their differentiation potential *in vitro*. Interestingly, the undifferentiated LGE cultures retain the expression of both the *Isl1* and *Er81* genes, suggesting that precursor cells for both striatal projection neurons and olfactory bulb interneurons are present in these cultures. Thus the restriction in differentiation potential of the expanded LGE cultures likely reflects deficiencies in the differentiation conditions used.

Key words: striatal projection neuron, olfactory bulb interneuron, neurogenesis.

Transplantation studies have shown that the lateral ganglionic eminence (LGE), located in the floor of the embryonic telencephalon, gives rise to both striatal projection neurons and olfactory bulb interneurons (Deacon et al., 1994; Olsson et al., 1995, 1997, 1998; Wichterle et al., 1999, 2001). These two neuronal subtypes exhibit many differences such as their capacity for long distance axonal outgrowth as well as their migratory properties. The striatal projection neurons migrate radially into the developing striatum, while the olfactory bulb interneurons migrate rostrally to populate the olfactory bulb (Wichterle et al., 2001). Also, the striatal projection neurons arise almost exclusively during embryogenesis, while the olfactory bulb interneurons are predominantly generated at postnatal periods (Bayer and Altman, 1995). Both neuronal subtypes utilize GABA as a neurotransmitter (Kita and Kitai, 1988; Shipley et al., 1995) but only the striatal projection neurons express the phosphoprotein dopamine and cAMP-regulated phosphoprotein of 32 kilodaltons (DARPP-32) (Ouimet et al., 1984; Anderson and Reiner, 1991).

Olfactory bulb interneurons and striatal projection neurons have recently been suggested to derive from distinct progenitor pools in the LGE (Stenman et al., 2003). The olfactory bulb interneurons were proposed to derive from the progenitor domain marked by the expression of the ETS transcription factor Er81 in the dorsal third of the LGE, while the striatal projection neurons derive from a domain marked by the LIM homeobox protein Islet1 (ISL1) located in the ventral two-thirds of the LGE. Both of these LGE domains express the *Dlx* homeobox genes (Stenman et al., 2003).

We have recently made use of two different *in vitro* systems to study LGE neurogenesis, neurosphere cultures (Reynolds et al., 1992) and attached cultures (Skogh et al., 2001). Neurospheres were grown under serum-free conditions in the presence of both epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) (Parmar et al., 2002). After expansion *in vitro* (i.e. six passages) followed by differention, neurons were produced that expressed the homeobox proteins MEIS2, PBX and DLX, each of which are characteristic of LGE-derived neurons (Parmar et al., 2002). Attached cultures derived from the LGE, on the other hand, have been expanded (between five and 25 passages) in serum and EGF (Skogh et al., 2001). The undifferentiated attached cultures displayed many molecular characteristics of glial cells. In fact, at least a portion of the neurons generated in these cultures were derived from *glial fibrillary acidic protein* (GFAP, an astrocyte marker; Bignami et al., 1972)-expressing precursors (Skogh et al., 2001). The neurons generated in these LGE cultures also displayed molecular characteristics typical of their *in vivo* counterparts, such as DLX and MEIS2 expression (Skogh et al., 2001). Interestingly, neither the neurosphere cultures nor the attached glial cultures gave rise to neurons expressing DARPP-32 (Skogh et al., 2001; Parmar et al., 2002), indicating that that these expanded cultures cannot generate fully differentiated striatal projection neurons under the differentiation conditions used.

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Abbreviations: bFGF, basic fibroblast growth factor; DARPP-32, dopamine and cAMP-regulated phosphoprotein of 32 kilodaltons; EGF, epidermal growth factor; FBS, fetal bovine serum; GFAP, glial fibrillary acidic protein; ISL1, Islet1; LGE, lateral ganglionic eminence; PN, primary neuronal; RT-PCR, reverse transcription-polymerase chain reaction; SHH, sonic hedgehog.

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In the present report, we studied the neurons generated in attached LGE cultures at different points during the expansion (after the first and fifth passage), and compared them with those in primary LGE cultures. Our results show that already after one passage the LGE cells lose the ability to generate DARPP-32-positive neurons. This restriction in differentiation potential correlates with a loss in the expression of certain developmental control genes such as *Isl1*. The undifferentiated LGE cultures express both *Isl1* and *Er81*, suggesting that precursor cells for both striatal projection neurons and olfactory bulb interneurons are present in these cultures. However, the differentiation conditions used here are not sufficient to promote striatal neuron differentiation.

EXPERIMENTAL PROCEDURES

Dissection and growth conditions

All animal-related procedures were conducted in accordance with local ethical guidelines and approved animal care protocols. LGEs were dissected from E13.5 wild-type NMRI mouse embryos, and cells were isolated and dissociated as previously described (Toresson et al., 1999). All cell cultures were initiated and cultured as described in Skogh et al. (2001) according to the flowchart in Fig. 1. Two types of cultures were established, primary neuron cultures and expanded cultures. The expanded cultures were differentiated either after one passage (P1) or after five passages (P5). The growth medium for each culture condition is described in the legend to Fig. 1. For differentiation, all cultures were kept in differentiation medium for 7 days before fixation as previously described (Skogh et al., 2001).

Immunocytochemistry

For immunocytochemistry, cells grown on chamber slides were fixed in ice cold 4% PFA for 15 mins, followed by three rinses in PBS. All immunostainings were performed following our previously published protocol (Skogh et al., 2001; Parmar et al., 2002). Primary antibodies used were: mouse anti-β tubulin III (used at 1:333; Sigma, St. Louis, MO), rabbit anti-Er81 (provided by T. Jessell and S. Morton, used at 1:3500), rabbit anti-ISL1 (provided by H. Edlund, used at 1:400), rabbit anti-MEIS2 (provided by A. Buchberg, used at 1:5000), rabbit anti-panPBX (used at 1:400; Santa Cruz Biotechnology, Santa Cruz, CA), mouse anti-DARPP-32 (provided by P. Greengard, used at 1:20 000). Two or 3 independent cultures were immunostained for each marker and between 2 and 8 wells were analyzed for each culture condition.

RT-PCR

For reverse transcription-polymerase chain reaction (RT-PCR) RNA was isolated from dissected postnatal day 3 (P3) mouse forebrain, dissected E13.5 LGE tissue pieces, three different primary neuronal (PN) cultures, three cultures initiated as expansion cultures, but not passaged (P0), three cultures passaged once (P1), and three cultures passaged five times (P5), each lysed either directly after growth in expansion medium (proliferating P5) or after 7 days in differentiation medium (differentiated P5). Each culture was generated from a separate dissection, except the P5 cultures where proliferating P5 culture 1 is the same as differentiated P5 culture 1, etc. Total RNA was prepared from tissue pieces or cell cultures as previously described (Parmar et al., 2002) using the following programs for *G3PDH*, *Pax6, Meis2*, *Mash1*, *Dlx1*, *Dlx5* and *Islet-1*, denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, and extension at 72 °C for 1 min. For *Gsh2*, denaturation for 1 min, annealing at 60 °C for 1 min, and extension for 1.5 min. For *Ebf1*, denaturation for 30 sec, annealing at 55 °C for 30 sec, and extension for 45 s. For *Darpp-32*, denaturation for 1 min, annealing at 60 °C for 1 min, and extension for 1 min. And for *Er81*, denaturation for 30 sec, annealing at 54 °C for 30 sec, and extension for 45 sec.

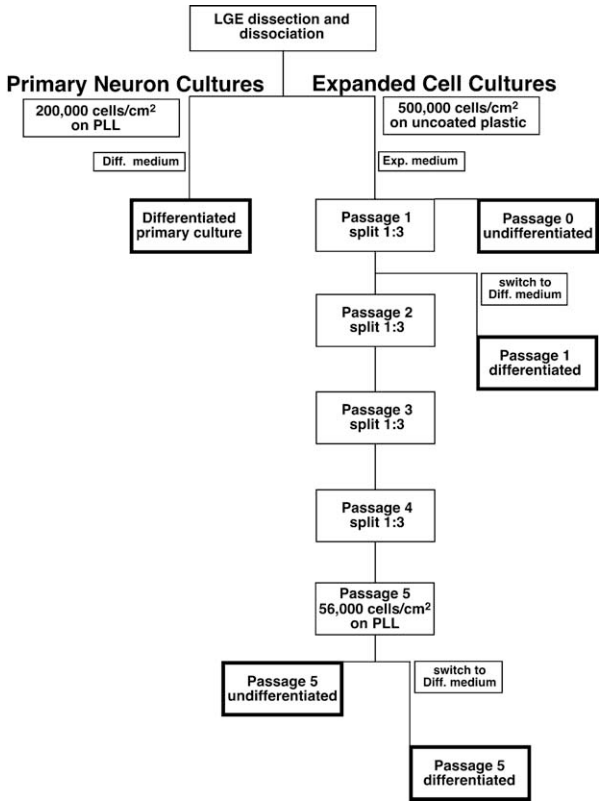

Fig. 1. Flowchart for the generation and differentiation of the LGE cultures used in the present study. Bold boxes indicate the points where cultures were fixed and studied further. All differentiated cultures were maintained in Differentiation medium for 7 days before fixation. Differentiation medium: Dulbecco's modified Eagle's medium (DMEM)/F12, defined hormone and salt mixture (Reynolds et al., 1992), 2 mM glutamine, penicillin/streptomycin and 1% FBS. Expansion medium: DMEM/F12, defined hormone and salt mixture (Reynolds et al., 1992), 2 mM glutamine, penicillin/streptomycin, 10% FBS and 20 ng/ml EGF. PLL, poly-L-lysine.

PCR products were amplified using 25–35 cycles. Primers used were: **G3PDH**: sense 5′-ACC ACA GTC CAT GCC ATC, antisense 5′-TCC ACC ACC CTG TTG CTG TA, **Meis2**: sense 5′-AAG ACT CCG AGA GTT AT, antisense 5′-GTT TGA AAC TAA AGG ACA, **Isl1**: sense 5′-GCA GCA TAG GCT TCA GCA AG, antisense 5′-GTA GCA GGT CCG CAA GGT G, **Ebf1**: sense 5′-CGA CTC CAT GAT AAA ACA AGC C , antisense 5′-CAG AGA CTG CCA GGA CAT GGC, **Darpp-32:** sense 5′-CTG TGC CTA TAG GCC CCC ATC , antisense 5′-GGG ATG CTG AGG TTC CTC TCC AGG, **Er81:** sense 5′-CAG AGA TCT GGC TCA TGA TTC AG, antisense 5′-CAC ATG CAG CCT TCT GTT CTG C. **Mash1:** sense: 5′AGC AGC TGC TGG ACG AGC A, antisense: 5′CCT GCT TCC AAA GTC CAT TC **Gsh2:** sense: 5′CAG CTT TCC GGA CAG TGC TC, antisense: 5′GGT AGC AGA AGG AGC CTC TG, **Dlx1:** sense: 5′ATG ACC ATG ACC ACC ATG CC, antisense: 5′TCA CAT CAG TTG AGG CTG CT, **Dlx5:** sense: 5′ATG ACA GGA GTG TTT GAC AG, antisense: 5′CTA ATA AAG CGT CCC GGA GG. **Pax6:** sense: 5′AGT CAC AGC GGA GTG AAT CAG, antisense: 5′AGC CAG GTT GCG AAG AAC TCT. An RT-negative control was included for each sample, to exclude

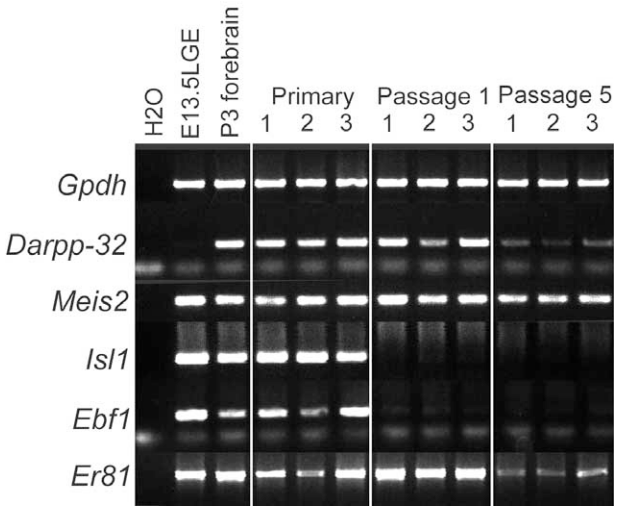

Fig. 2. RT-PCR results for *Darpp-32, Meis2, Isl1, Ebf1,* and *Er81* on RNA derived from primary E13.5 LGE neuron cultures, and differentiated P1 and P5 cultures, derived from E13.5 LGE and expanded in EGF and 10% serum. Control RNA was derived from dissected E13.5 LGE and P3 forebrain tissue. *Gpdh* was used as a control.

genomic DNA contamination. These controls never resulted in amplification.

RESULTS

Differentiation of primary LGE cultures

Our previous study demonstrated that LGE cultures can be expanded extensively *in vitro* and subsequently induced to undergo neurogenesis (Skogh et al., 2001). While the *in vitro* generated neurons exhibit many molecular characteristics of the LGE, they do not express the striatal projection neuron marker DARPP-32. For comparison with the expanded cultures we characterized the differentiated neurons in primary neuronal (PN) cultures derived from the LGE. These cultures were grown for 7 days in Differentiation medium (see Experimental Procedures) before fixation and immunostaining or RT-PCR analysis. Using β tubulin III and morphology to identify the neurons, we determined that 55.3% of all the cells in these cultures were neuronal. Many of the cells in the primary cultures also exhibited glial characteristics such as GFAP expression; however, this was not quantified.

As would be expected, the differentiated primary LGE cultures expressed *Darpp-32* mRNA (Fig. 2). Using immunocytochemistry, DARPP-32 protein was detected in 23.8% of all the neurons. Thus a significant number of primary LGE cells can be differentiated into neurons exhibiting a striatal projection neuron phenotype, using these culture conditions.

The E13.5 LGE expresses a number of developmental control genes, such as the atypical homeobox gene *Meis2* (Fig. 2; Toresson et al., 2000a). This gene is also expressed in the differentiated primary LGE cultures (Fig. 2). As shown previously, MEIS2 is expressed by neurons in the LGE (Fig. 3a inset) as well as by neurons in the primary LGE cultures (Fig. 3b; 61.6% of neurons) (Skogh et al., 2001). PBX proteins, which represent another group of atypical homeodomain proteins normally expressed in neurons of the LGE (Fig. 3e, inset; Toresson et al., 2000a), were also found in primary LGE neurons (Fig. 3f; 30.8% of neurons).

The LIM homeobox gene *Isl1* and the HLH gene *Ebf1* are both known to be expressed by differentiating striatal projection neurons (Garel et al., 1999; Toresson et al., 2000b; Toresson and Campbell, 2001; Wang and Liu, 2001). Accordingly, these genes are expressed in the E13.5 LGE (Fig. 2). Both *Isl1* and *Ebf1* are detected in the primary LGE cultures as well (Fig. 2). Moreover, as in the E13.5 LGE (Fig. 3i, inset), many of the neurons in the primary cultures express ISL1 protein (Fig. 3j; 32.2%).

In addition to the striatal projection neurons, the LGE also gives rise to interneurons, which migrate rostrally to the olfactory bulb (Wichterle et al., 1999, 2001). The ETS transcription factor Er81 marks the portion of the LGE from which the olfactory bulb interneurons have been proposed to originate (Fig. 4a; Stenman et al., 2003). This domain occupies the dorsal portion of the LGE (Yun et al., 2001) and is distinct from the ISL1 domain (Stenman et al., 2003). *Er81* is expressed in the differentiated LGE cultures (Fig. 2) along with its protein product (data not shown). Taken together, these findings indicate that the primary LGE cultures represent a good system for comparisons with the expanded cultures since the neurons they generate are very similar to their *in vivo* counterparts.

Differentiation of expanded LGE cultures

The LGE cultures used in this study were prepared and expanded as previously described (Toresson et al., 1999; Skogh et al., 2001); however, in the present study we used a differentiation medium containing 1% fetal bovine serum (FBS), instead of being serum-free as before (Skogh et al., 2001). The reason for this was that the PN cultures from the E13.5 LGE survive better in 1% FBS than in serum-free medium and we wanted to be able to compare these cultures. Expanded cultures differentiated equally well in 1% FBS as in serum-free medium (data not shown).

Although the LGE is known to be the source for striatal projection neurons (Deacon et al., 1994; Olsson et al., 1995, 1997, 1998; Wichterle et al., 2001), expanded LGE cultures do not generate neurons expressing the striatal projection neuron marker DARPP-32 (Skogh et al., 2001). To determine when the LGE cells lose the ability to generate DARPP-32 neurons, we differentiated the cultures after one passage (P1) as well as after five passages (P5). In expanded cultures that were differentiated at P1, 30.4% of all the cells were β tubulin III-positive neurons; however, in P5 cultures, the numbers of neurons varied widely from 0 to 14.8% between cultures prepared at different times. We used only neurogenic cultures at P5 in the present study, which produced an average of 10% neurons after differentiation.

LGE cultures differentiated at P1 and P5 both expressed weak but detectable levels of *Darpp-32* mRNA (Fig. 2), however, these cultures never contained neurons expressing the DARPP-32 protein (data not shown). As

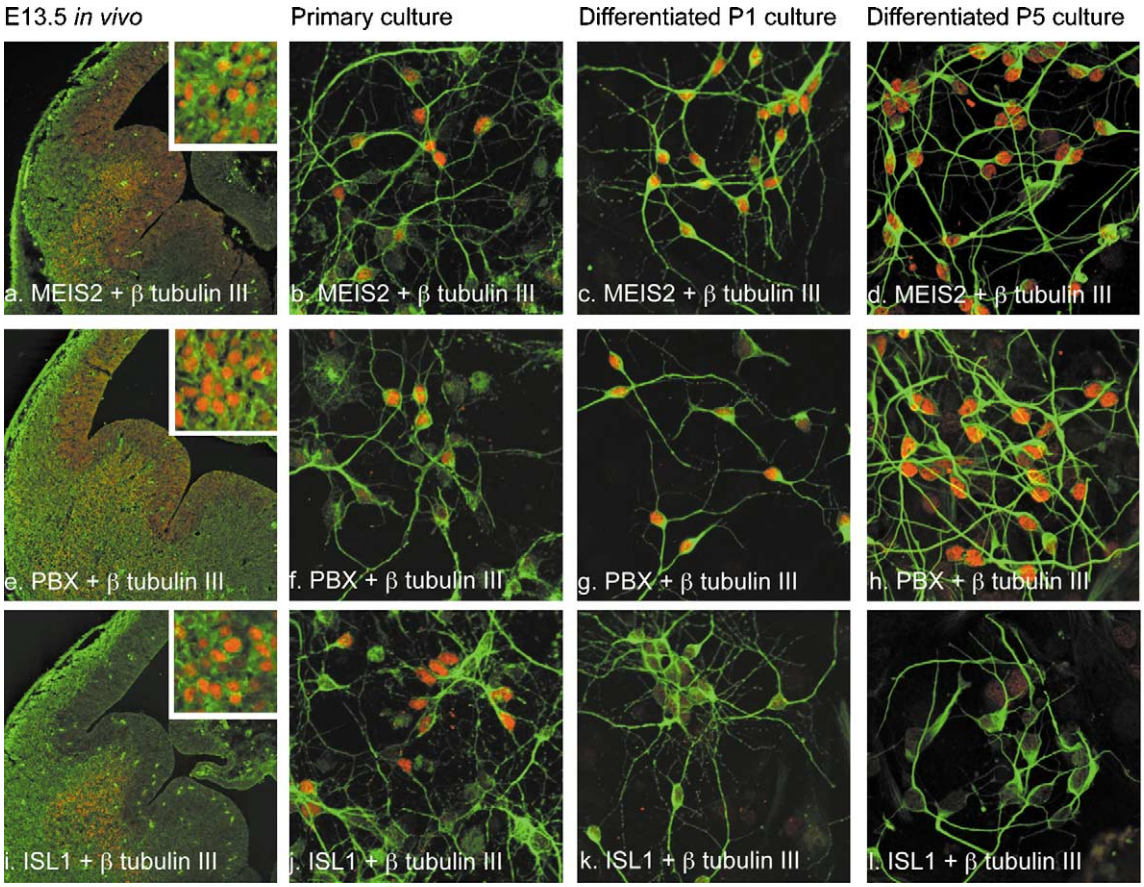

Fig. 3. Expression of developmental control proteins *in vivo* and *in vitro*. MEIS2 (red in a–d) and PBX (red in e–h) are highly expressed in β tubulin III-positive neurons (green) of the LGE SVZ (insets in a and e), and in primary (b, f), P1 (c, g), and P5 (d, h) cultures. ISL1 (i–l) is expressed in neurons of the E13.5 LGE (inset in i), and in PN cultures (j), but not in expanded cultures passaged once (k) or five times (l).

previously shown (Skogh et al., 2001), the *Meis2* gene (Fig. 2) and its protein product (Fig. 3c, d) are expressed in neurons of the expanded LGE cultures differentiated at both P1 and P5. The numbers of MEIS2 positive neurons in the P1 and P5 cultures was 95.1% and 44.3%, respectively. PBX proteins were also found in many of the neurons from expanded cultures (Fig. 3g, h; P1=82.2% and P5=93.1%).

Interestingly, the expression of the striatal progenitor/neuron markers, *Isl1* and *Ebf1,* was very sensitive to the expansion of the LGE cells *in vitro*. Already after one passage, neither gene was detectable in the differentiated cultures (Fig. 2). This was further confirmed by immunostaining for ISL1, which demonstrated that no neurons in the differentiated P1 or P5 cultures express ISL1 (Fig. 3k, l). Together with the lack of DARPP-32 expressing neurons in the expanded and differentiated cultures, these findings indicate that the ability of LGE cultures to generate neurons exhibiting striatal projection neuron phenotypes is lost already at the beginning of the expansion (i.e. at the

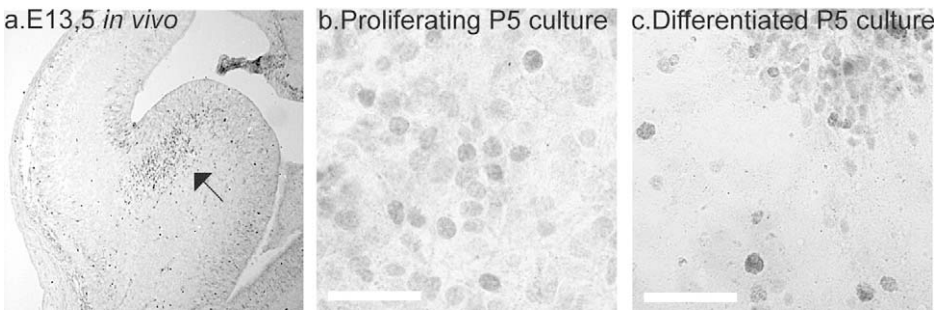

Fig. 4. Expression of Er81 *in vivo* and in cell cultures expanded in EGF and serum. (a) Cells expressing Er81 are confined to the dorsal third of the E13.5 LGE (indicated by the arrow). Er81-positive cells are seen both in undifferentiated proliferating (b) and differentiated (c) cultures passaged five times. Scale bar=50 μm.

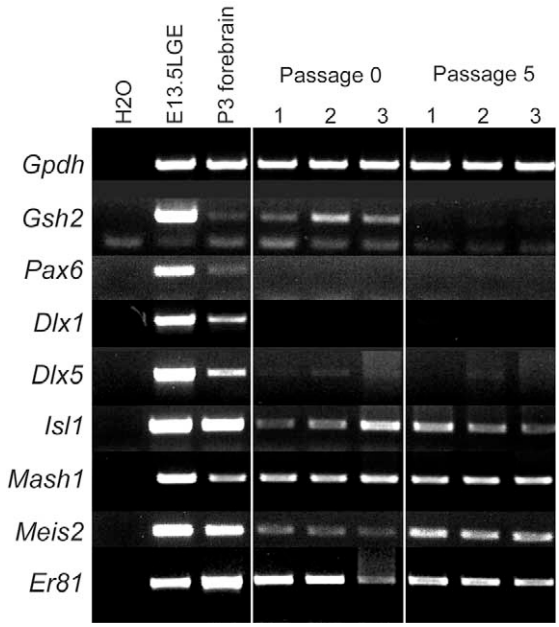

Fig. 5. RT-PCR results for *Gsh2, Pax6, Dlx1, Dlx5, Isl1, Mash1, Meis2,* and *Er81* on RNA derived from proliferating cultures, before the first passage (P0) and after five passages (P5), derived from E13.5 LGE and grown in EGF and serum. Control RNA was derived from dissected E13.5 LGE and P3 forebrain tissue. *Gpdh* was used as a control.

first passage of the cultures). This was not the case for cells expressing the olfactory bulb interneuron marker Er81. Indeed, *Er81* gene expression was found in both P1 and P5 differentiated cultures (Fig. 2), as was its protein product (Fig. 4c).

Effect of expansion on gene expression in LGE cultures

The fact that expanded cultures loose the ability to generate neurons with striatal projection neuron phenotypes suggests that the expansion process alters the molecular composition of the precursors, thereby limiting their differentiation potential. To look at this possibility we examined the proliferating cultures either at the point just before their first passage (P0) or at P5. We assayed for a number developmental control genes, which are known to be expressed in the E13.5 LGE ventricular zone and/or subventricular zone (Fig. 5; *Gsh2* (Hsei-Li et al., 1995), *Pax6* (Sussel et al., 1999), *Dlx1* and *Dlx5* (Eisenstat et al., 1999), *Isl1* (Toresson et al., 2000b; Wang and Liu, 2001), *Mash1* (Guillemot and Joyner, 1993; Horton et al., 1999), *Meis2* (Toresson et al., 2000a) and *Er81* (Yun et al., 2001)).

Although *Gsh2* is highly expressed in the E13.5 LGE, it is progressively lost in the P0 and P5 proliferating cultures (Fig. 5). *Pax6*, *Dlx1* and *Dlx5* are barely detectable already in the undifferentiated P0 cultures, unlike the case in the LGE *in vivo* (Fig. 5). The expression of *Mash1*, *Meis2* and *Er81* (along with its protein product) are all maintained in the expanding (i.e. undifferentiated) cultures (Figs. 4b and 5) Surprisingly, despite the absence of *Isl1* in differentiated cultures, *Isl1* is expressed in the proliferating cultures both at P0 and P5 (Fig. 5). This suggests that the gene is actively down-regulated by the differentiation conditions used. Thus, it seems that expansion of the LGE cells significantly alters their molecular profile. While certain genes are maintained in the proliferating LGE cells, others are reduced or lost. These changes in gene expression are likely to contribute to the restricted differentiation potential of the expanded LGE cells.

DISCUSSION

The present findings demonstrate that the ability of attached LGE cultures to generate neurons expressing striatal projection neuron phenotypes, as marked at different stages of maturation by ISL1, *Ebf1* or DARPP-32, is lost already at the beginning of the expansion period (i.e. P1). However, many cells in the expanded and differentiated LGE cultures express the olfactory bulb interneuron marker Er81. *In vitro* generated neurons in expanded LGE cultures have previously been shown to express GABA and DLX proteins (Skogh et al., 2001), both of which are found in mature or developing olfactory bulb interneurons (Shipley et al., 1995; Bulfone et al., 1998) as well as by mature or developing striatal projection neurons (Kita and Kitai, 1988; Eisenstat et al., 1999). Taken together, these facts suggest that the neurons formed in the expanded LGE cultures are most similar to olfactory bulb interneurons. In support of this notion, the human equivalents to these cells (Skogh et al., 2001) have been transplanted into the subventricular zone of neonatal rats and the cells appear to behave similarly to endogenous olfactory bulb progenitors (Parmar et al., 2003).

This is interesting, considering the glial nature of the proliferating LGE cultures. As previously shown, at least a portion of the precursor cells which give rise to neurons in the expanded LGE cultures express *GFAP* (Skogh et al., 2001). A previous study has shown that the neuronal precursors/stem cells in the postnatal subventricular zone also express *GFAP* (Doetsch et al., 1999). These *GFAP*-positive subventricular zone cells were shown to give rise to olfactory bulb interneurons. Thus, it may be that the neurogenic *GFAP*-positive cells in the proliferating LGE cultures (Skogh et al., 2001) represent olfactory bulb interneuron progenitors. It remains unclear, however, what fraction of the neurogenic precursor cells in the expanded LGE cultures are *GFAP*-positive.

The lack of DARPP-32-positive neurons in the differentiated LGE cultures could suggest that a selection has occurred as a result of the expansion *in vitro*, and that striatal projection neuron progenitors are lost. This seems unlikely, however, since the *Isl1* gene is detected in the undifferentiated cultures. Moreover, *Darpp-32* gene expression is detected (albeit rather weakly) in the differentiated cultures. It is unclear why the *Darpp-32* gene expression does not lead to stable DARPP-32 protein expression in the neurons. It should be mentioned, however, that the granule cell layer and periglomerular layer of the olfactory bulb do express low, but detectable levels of *Darpp-32* mRNA (Schalling et al., 1990), in the absence of

significant DARPP-32 protein (Hemmings and Greengard, 1986). The lack of DARPP-32 protein in the *in vitro* generated neurons may also be due to the loss of the developmental regulators *Isl1* and *Ebf1*, both of which are expressed at an earlier stage in the differentiation of striatal projection neurons than *Darpp-32* (Gustafson et al., 1992; Garel et al., 1999; Toresson et al., 2000; Toresson and Campbell, 2001; Wang and Liu, 2001). Although it is currently unclear whether *Isl1* is required for striatal neurogenesis, *Ebf1* has been shown to regulate this process (Garel et al., 1999). Thus, these cultures represent an excellent system to determine the role of *Isl1* in striatal projection neuron differentiation. Reconstitution of *Isl1* expression during the differentiation process, perhaps via retroviral infection, may direct striatal projection neuron differentiation, *in vitro*.

Despite that neither the *Isl1* gene nor its protein expression was detected in differentiating neurons, the proliferating cultures did contain detectable levels of *Isl1* mRNA. This result indicates that the differentiation conditions used here are lacking the factor(s) normally found in the LGE that is required to up-regulate or at least maintain *Isl1* expression in differentiating LGE neurons. It may be that this factor is present in serum since the proliferating cultures (which express *Isl1*) contain 10% serum while the differentiating cultures contain only 1% serum. At present, however, factors that directly regulate *Isl1* expression in the LGE and developing striatum are unknown. The signaling molecule Sonic Hedgehog (SHH) is known to be a regulator of *Isl1* expression in ventral regions of the CNS (Roelink et al., 1994; Ericson et al., 1995). However, SHH is not expressed in the LGE (Shimamura et al., 1995; Kohtz et al., 1998). Furthermore, at least some *Shh* mutants display ISL1 expression in the mutant telencephalon (Toresson, 2001).

The restriction in the differentiation potential of LGE cells after expansion *in vitro* prompted us to analyze the molecular composition of the cells in the proliferating (i.e. undifferentiated) cultures, before the first passage (P0) and at P5. By comparing the cells in dissected starting material with the P0 cultures it is clear that the composition of cells have already changed. Notably, *Gsh2* and *Pax6* are severely reduced and/or missing in the undifferentiated cultures. That genes expressed by post-mitotic cells are lost is expected, but *Gsh2* and *Pax6* are expressed in proliferating progenitors within the E13.5 LGE ventricular zone. It is interesting to compare these results to a similar analysis of undifferentiated neurosphere cultures derived from the LGE (Parmar et al., 2002). In the neurosphere cultures, *Gsh2* and *Pax6* are detected. On the other hand, undifferentiated attached cultures, but not neurosphere cultures, express *Isl1*, which is normally found in the LGE subventricular zone. Undifferentiated neurosphere cultures as well as attached cultures express *Meis2, Mash1*, and *Er81*, while neither culture expresses high levels of *Dlx1* or *Dlx5* (Parmar et al., 2002; data shown here). Each of these genes are expressed in both the ventricular and subventricular zone, albeit at different levels (Guillemot and Joyner, 1993; Eisenstat et al., 1999; Horton et al., 1999; Toresson et al., 1999; Yun et al., 2001). Thus, the molecular identity of precursor cells in the attached undifferentiated cultures studied here is most similar to that of subventricular zone cells. In contrast, the precursors that are expanded in the neurosphere cultures may be more similar to LGE ventricular zone cells.

The differences in the culture conditions between the neurosphere cultures and the attached glial cultures probably contribute significantly to the observed differences in gene expression between the precursor cells in these two culture paradigms. Firstly, the neurospheres are grown in both EGF and bFGF, while the culture medium for the attached cultures contain only EGF. Interestingly, the FGF receptor expression is mostly confined to the ventricular zone at this stage of development (see e.g. Hébert et al., 2003), while the EGF receptor is expressed at higher levels in subventricular zone (Eagleson et al., 1996). Secondly, the attached cultures are grown in the presence of serum, while neurospheres are grown serum-free. Finally, the cells that contribute to the attached cultures do so by first adhering to the plastic culture plate, while the neurospheres form as floating aggregates. Despite these differences, the neurons that develop when either of these LGE-derived cultures are differentiated (using 1% serum and no mitogens) are virtually identical with respect to the molecular markers they express (e.g. DLX, MEIS2, PBX and GABA) and lack (e.g. ISL1 and DARPP-32) (Skogh et al., 2001; Parmar et al., 2002), Moreover, the two culture systems exhibit similar neurogenic potential (i.e. neurons comprise approx. 10% of all cells in the differentiated culture).

In summary, our results show that the *in vitro* expansion of LGE cells as attached cultures results in a restriction of the differentiation potential of these cells. Specifically, the ability to generate neurons with striatal projection neuron phenotypes is lost, likely due to both alterations in the molecular identity of the expanded LGE cells as well as deficiencies in the differentiation conditions used.

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