A Critical Period in Cortical Interneuron Neurogenesis in Down Syndrome Revealed by Human Neural Progenitor Cells

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Key Words

Trisomy 21 • Cortex • Microarray analysis

Abstract

Down syndrome (DS) is a developmental disorder whose mental impairment is due to defective cortical development. Human neural progenitor cells (hNPCs) derived from fetal DS cortex initially produce normal numbers of neurons, but generate fewer neurons with time in culture, similar to the pattern of neurogenesis that occurs in DS in vivo. Microarray analysis of DS hNPCs at this critical time reveals gene changes indicative of defects in interneuron progenitor development. In addition, dysregulated expression of many genes involved in neural progenitor cell biology points to changes in the progenitor population and subsequent reduction in interneuron neurogenesis. Delineation of a critical period in interneuron development in DS provides a foundation for investigation of the basis of reduced neurogenesis in DS and defines a time when these progenitor cells may be amenable to therapeutic treatment.

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Introduction

Down syndrome (DS) is the most common genetic form of mental retardation. The cause of DS is known to be trisomy of chromosome 21, but it is not known how this chromosomal anomaly causes the mental impairment characteristic of DS [Lejeune, 1959]. DS may simply be the result of too much genetic material in the genome [Shapiro, 1983]. However, most lines of research have focused on the premise that specific genes located on chromosome 21 are responsible for DS symptoms. Chromosome 21 is the smallest human chromosome comprising up to 500 genes [Hattori et al., 2000; Gardiner et al., 2002]. Overexpression of chromosome 21 genes may exert direct effects that lead to manifestations of DS, or have secondary effects on other genes. Based on the variability of DS individual phenotypes, genetic, environmental, and stochastic influences are also likely to play a role in DS [Antonarakis, 2001; FitzPatrick, 2005; Reeves et al., 2001; Roper and Reeves, 2006]. It is useful, therefore, to first uncover cellular differences caused by trisomy 21 and then define the molecular mechanisms that underlie these differences.

Descriptions of neuroanatomical differences between unaffected and DS human brains have provided

clues to the specific deficiencies that occur in DS cortical development. Overall, DS brains weigh less, and the cerebral cortices have simpler convolutions than age-matched euploid brains [Becker et al., 1991; Colon, 1972; Schmidt-Sidor et al., 1990; Wisniewski, 1990]. A dramatic reduction in the number of neurons in the developing cerebral cortex of DS patients has been consistently reported dating back to the 1920s [Becker et al., 1991; Benda, 1947; Colon, 1972; Crome and Stern, 1967; Davidoff, 1928; Golden and Hyman, 1994; Ross et al., 1984; Wisniewski et al., 1984]. It is important to note that the neuron reductions are area, cell type and age specific: the missing neurons are primarily small, granular, presumably GABAergic neurons in layer II and layer IV of the cortex [Ross et al., 1984]. Cortical neuron density appears normal in early gestation, but there are fewer neurons than normal in late gestation (>23 weeks), and this paucity continues throughout early life [Golden and Hyman, 1994; Weitzdoerfer et al., 2001]. Interestingly, this neuron deficiency appears near the end of cortical neurogenesis. The major period of generation and migration of cortical neurons occurs from 10 to 25 weeks of gestation in humans [Sidman and Rakic, 1973]. Cortical neurons are generated in the proliferative ventricular zone and reach their destination in the cortical plate by both radial and nonradial migration [Rakic, 1972; O'Rourke et al., 1997; Anderson et al., 1997; Lavdas et al., 1999]. Newly born neurons are added to the cortical plate in an 'inside-out' fashion and differentiate to form the cell-specific layers of the cortex [Angevine and Sidman, 1961; Rakic, 1974]. Although most neuroblasts have been generated by the 16th gestational week, many neurons have not yet reached their final destination [Sidman and Rakic, 1973]. Furthermore, medium and small neurons, as well as glia, continue to be produced after 16 weeks of gestation [Sidman and Rakic, 1973]. Therefore, the timing of the cortical neuron deficiency revealed by morphological descriptions of developing DS brains indicates that the maturation or late phase of human cortical development is affected in DS [Sidman and Rakic, 1973]. These histological observations provide the basis for the hypothesis that there is a developmentally specified neurogenesis defect, rather than a general defect in neurogenesis.

The mechanisms that lead to fewer cortical neurons in DS cannot easily be revealed using primary human tissue because it is not amenable to systematic analysis and manipulation. In addition, studies of primary fetal brain tissue are hampered by the mixed population of cells and can only be used to evaluate one developmental time.

These limitations can be overcome to some extent by forcing expansion of progenitor cells within the tissue. Human neural progenitor cells (hNPCs) derived from fetal cortical tissue can be grown and expanded in culture [Svendsen et al., 1998; Vescovi et al., 1999; Carpenter et al., 1999]. In vitro culture of hNPCs provides an experimental paradigm in which to study the molecular mechanisms that underlie cortical development. hNPCs in culture are regionally and temporally specified [Ostenfeld et al., 2002; Wright et al., 2006; Anderson et al., 2007]. These cells follow an intrinsic developmental program with time in culture that is similar to in vivo development. hNPCs with trisomy 21 can be expanded in culture [Bahn et al., 2002; Bhattacharyya and Svendsen, 2003; Esposito et al., 2008] and have reduced neurogenesis [Bahn et al., 2002], validating trisomy 21 hNPCs as a way to study the reduced accumulation of cortical neurons in DS. The present study takes advantage of the intrinsic developmental program of hNPCs in culture to explore the striking inability of trisomy 21 hNPCs to generate normal numbers of neurons in culture. The results define a critical developmental time period when reduced neurogenesis occurs and offer a molecular basis for the reduced neurogenesis.

Materials and Methods

Tissue Collection and Cell Culture

Human fetal tissue was obtained from the Birth Defects Laboratory at the University of Washington, Seattle and the Tissue Bank for Developmental Disorders at the University of Maryland. Neurospheres were generated from two trisomy 21 cortex samples of approximately 13 weeks of gestation and three gestational age-matched euploid controls, as well as an 18-week gestation trisomy 21 cortex and an 18-week gestation-matched control. The method of collection conformed to the guidelines recommended by the National Institutes of Health for the collection of such tissues and set out by the University of Washington, the University of Maryland and the University of Wisconsin-Madison. Institutional Review Board approval was obtained for all of these studies. Cortical progenitors isolated from fetal brain were induced to proliferate as free-floating neurospheres according to previously described methods [Svendsen et al., 1998]. Briefly, freshly isolated tissue was mechanically chopped and seeded into flasks. The cells were initially expanded in DMEM/Ham's F12 media (Sigma-Aldrich, St. Louis, Mo., USA) with penicillin, streptomycin, amphotericin B (PSA, 1%; Invitrogen, Carlsbad, Calif., USA) and supplemented with B27 (2%; Invitrogen) and mitogens: EGF (20 ng/ml; Sigma-Aldrich), and FGF-2 (20 ng/ml; R & D Systems, Minneapolis, Minn., USA) with heparin (5 µg/ml; Sigma-Aldrich). Media were replenished every 3–4 days. Neurospheres were passaged every 14 days by a chopping method that does not require trypsin or mechanical dissociation, and cell-cell contact was continuously

maintained. After 4 weeks, neurospheres were grown in DMEM/Ham's F12 media with PSA and supplemented with N2 (1%; Invitrogen) and 20 ng/ml EGF. Karyotype analysis and fluorescent in situ hybridization with chromosome 21 probes confirmed retention of trisomy 21 in hNPCs from DS fetuses and absence of chromosomal abnormalities in euploid hNPCs. No hNPC lines used in this study showed trisomy 21 mosaicism.

Differentiation and Immunostaining

For short-term cultures, neurospheres were plated onto laminin/poly-L-lysine-coated glass coverslips in media without mitogens (DME/F12 + PSA + B27) for 7 days. For long-term cultures used to identify neuronal subtypes, neurospheres were plated onto laminin/poly-L-lysine flasks in media without mitogens (DME/F12 + PSA + B27) and neurotrophin-4 (20 ng/ml; Pepro-Tech, Rocky Hill, N.J., USA) for 7 days. Cells were then dissociated and grown at a density of 5,000 cells/ μ l for 9–12 weeks in neurobasal media (Invitrogen) with PSA and glutamine (Sigma-Aldrich) supplemented with fetal calf serum (10%; Invitrogen), B27 and NT4. All cultures were fixed in 4% paraformaldehyde (Sigma-Aldrich) for 20 min and washed in PBS. Fixed cells were blocked and permeabilized in 5% goat serum with 0.2% Triton X-100 (Sigma-Aldrich) and subsequently incubated with antibodies to β -III-tubulin (TuJ1; 1:5,000, Sigma-Aldrich) and vesicular GABAergic transporter or vesicular glutamate transporter 1 (Synaptic Systems, Goettingen, Germany) followed by incubation with a fluorescent secondary antibody. Hoechst 33258 (Sigma-Aldrich) was used as a nuclear stain.

Analysis of Neurosphere Growth

Individual spheres (approximately 0.30 mm in diameter) were grown in single wells of a 96-well plate ($n = 10$). Diameter measurements were taken every 3–4 days using a lens-mounted micrometer. The volume of each sphere was calculated as an index of cell number as previously described in detail [Svendsen et al., 1998].

Analysis of Cell Death and Division within Neurospheres

Neurospheres were fixed in 4% paraformaldehyde (Sigma-Aldrich) for 40 min and cryoprotected with 30% sucrose for 4 h at 4°C. Neurospheres were embedded in tissue freezing media and sectioned at 20 μ m on a cryostat. Sections were analyzed for apoptotic cells using the DeadEnd Fluorometric TUNEL assay (Promega, Madison, Wisc., USA) according to the manufacturer's directions. Sections were double labeled for Ki67 using an antibody (BD Biosciences, San Jose, Calif., USA) followed by a fluorescent secondary antibody. Hoechst 33258 (Sigma-Aldrich) was used as a nuclear stain. Positive cells were quantified using Image J and MetaMorph software. Results are expressed as mean $\pm$ SEM of 10 sections per 5 spheres of 2 lines each of trisomy 21 and control hNPCs.

RNA Extraction for Quantitative Reverse Transcriptase Polymerase Chain Reaction and Affymetrix GeneChips

To ensure biological significance, total RNA was isolated from two trisomy 21 hNPC lines (13-week gestation tissue) and three age-matched euploid control hNPC lines. RNA was extracted from hNPCs at approximately 10 weeks in culture using the RNeasy Mini Kit (Qiagen, Valencia, Calif., USA). Total RNA was additionally cleaned up and concentrated using the RNeasy Min-

Elute Cleanup Kit (Qiagen). The quality of the RNA of each sample was assessed by spectrophotometer readings (optical density 260/280 = 2.0 for each RNA). RNA from the two trisomy 21 hNPC lines and from the three age-matched control cell lines was pooled prior to cDNA synthesis.

Affymetrix GeneChip Sample Preparation

cDNA synthesis was performed for control and DS samples using the one-cycle cDNA synthesis kit (Affymetrix, Santa Clara, Calif., USA). In brief, double-stranded cDNA was synthesized using an oligo(dT)₂₄ primer at the 3' end for priming the first-strand cDNA synthesis by Superscript II reverse transcriptase and the T7 RNA polymerase promoter sequence at the 5' end. cDNA was purified using the cDNA sample cleanup module provided with the one-cycle cDNA synthesis kit and resuspended in 14 μ l elution buffer.

Biotin-labeled cRNA was synthesized using the IVT labeling kit (Affymetrix) and incubated at 37°C for 16 h. cRNA was purified with the cRNA sample cleanup module provided with the IVT labeling kit and eluted in 21 μ l. Spectrophotometric analysis was used to determine the cRNA yield as well as the quality of cRNA (optical density 260/280 = 2.0 for each cRNA). An adjusted cRNA yield was calculated to reflect any carryover of unlabeled total RNA. cRNA was fragmented using the 5 $\times$ fragmentation buffer (Affymetrix) provided, at a final concentration of 0.5 μ g/ μ l, in order to break down full-length cRNA to 35–200 base fragments. Fragmented biotin-labeled cRNA samples were then hybridized to the U133 Plus 2.0 array at 45°C for 16 h. Hybridized arrays were washed and double-stained with streptavidin-phycoerythrin using the Fluidics Station 400 (Affymetrix) as defined by the manufacturer's protocol.

Affymetrix GeneChip Analysis

Two separate RNA extractions were performed on the two pooled DS hNPC lines and the pooled euploid controls and processed separately on GeneChips. Stained U133 Plus 2.0 arrays were scanned at 3 μ m resolution using the Affymetrix GeneChip Scanner 3000 at the Gene Expression Center (University of Wisconsin, Madison, Wisc., USA). GeneChip Operating Software v1.2 was used to analyze the relative abundance of each gene derived from the average difference of intensities. log-transformed data were then analyzed further using the GeneSifter software (www.genesifter.net). Gene expression ratios were generated using control GeneChips from RNA pools from two consecutive RNA extractions as the baseline for comparison with DS GeneChips generated from two consecutive RNA extractions. Statistical analysis of GeneChip data was conducted using GeneSifter software. Student *t* tests were conducted for each data set with only genes with a *p* value <0.05 being considered in the statistical analysis.

Confirmation of mRNA Expression Regulation by Real-Time Quantitative Reverse Transcriptase Polymerase Chain Reaction

To confirm the Affymetrix GeneChip data, real-time quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) was performed using SYBR[®] Green for several genes that were found to have biological significance. In brief, RNA was extracted from two DS hNPC lines and three euploid control hNPC lines using RNeasy Mini Kit (Qiagen). Total RNA was addition-

ally cleaned up and concentrated using the RNeasy MinElute Cleanup Kit (Qiagen). RNA from two DS lines as well as from three age-matched control cell lines was pooled prior to reverse transcription. Reverse transcription was performed for control and DS samples using the Invitrogen SuperScript[®] III first-strand synthesis system (Invitrogen). qPCR was performed using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, Calif., USA). Immediate detection of the PCR product is measured by the increase in fluorescence caused by the binding of SYBR Green dye to either the control or DS double-stranded DNA. β -Actin was used as internal control in PCR amplification, and the comparative Ct method was used for relative quantification of expression. For each gene, at least two qPCR runs were completed using separate cDNAs from separate RNA pools.

Primers and sequences used:

APP forward, 5'-GGT CGA GAG GTG TGC TCT GAA-3';
APP reverse, 5'-GGT TCC TGG GTA GTC TTG AGT-3';
ATP1B4 forward, 5'-TGA TCC GGA TGA AGC GAA CCA GAA-3';
ATP1B4 reverse, 5'-TTT AGA AGG ATG CAG GGC TGT CCA-3';
 β -actin forward, 5'-GCG AGA AGA TGA CCC AGA TC-3';
 β -actin reverse, 5'-CCA GTG GTA CGG CCA GAG G-3';
CTBP1 forward, 5'-ACG ACT TCA CCG TCA AGC AGA TGA-3';
CTBP1 reverse, 5'-ATG AGG TTG GGT GCA TCC TTC AGA-3';
CXADR forward, 5'-TGC GTC TAA ACG TTG TCC CTC CTT-3';
CXADR reverse, 5'-CCA ATG AGC GCT AGA GCA AGC AAA-3';
DACH1 forward, 5'-TCT AAC TGG GCA TGG ACA ACC ACT-3';
DACH1 reverse, 5'-ACC TGT TTC TCT TGA GCT CTG GCA-3';
DLL3 forward, 5'-TCC CGG ATG CAC TCA ACA ACC TAA-3';
DLL3 reverse, 5'-TTC AGG GCG ATT CCA ATC TAC GGA-3';
DLX1 forward, 5'-GGC TGT TTG CCA ATT CAG GGT TCT-3';
DLX1 reverse, 5'-TTC GGC TCC AAA CTC TCC ATA CCA-3';
DLX2 forward, 5'-TTA CTC CGC CAA GAG CAG CTA TGA-3';
DLX2 reverse, 5'-TTG GCT TCC CGT TCA CTA TCC GAA-3';
DLX5 forward, 5'-ATC CGT CTC AGG AAT CGC CAA CTT-3';
DLX5 reverse, 5'-TTG AGA GCT TTG CCA TAG GAA GCC-3';
EMX2 forward, 5'-ATC GCT TCC AAG GGA ACG ACA CTA-3';
EMX2 reverse, 5'-CAA AGG CGT GTT CCA GCC TTA GAA-3';
GABRA2 forward, 5'-TGT GCC TGC AAG AAC TGT GTT TGG-3';
GABRA2 reverse, 5'-TGG CAG TTG CAT AAG CCA CTT TGG-3';
GABRA5 forward, 5'-AGT CCA TCG CTC ACA ACA TGA CCA-3';
GABRA5 reverse, 5'-AGC TGC CAA ATT TCA GAG GGC AAG-3';
GABRB3 forward, 5'-TGC TGT ATG GGC TCA GAA TCA CCA-3';
GABRB3 reverse, 5'-TCA ATG TCA TCC GTG GTG TAG CCA-3';
HEY1 forward, 5'-AGA GTG CGG ACG AGA ATG GAA ACT-3';
HEY1 reverse, 5'-ACC AGC CTT CTC AGC TCA GAC AAA-3';
NR2F1 forward, 5'-AAC TTA CAC ATG CCG TGC CAA CAG-3';
NR2F1 reverse, 5'-CAT GCC CAC TTT GAG GCA CTT CTT-3';
Olig1 forward, 5'-TTT CGA ACC TTC CAG TCC AGA GGA-3';
Olig1 reverse, 5'-ATC GAA CAT CCG CTC TGG TCA CTT-3';
Olig2 forward, 5'-GGT AAG TGC GCA ATG CTA AGC TGT-3';
Olig2 reverse, 5'-TAC AAA GCC CAG TTT GCA ACG CAG-3';
PRRX1 forward, 5'-CAG ATT GGT GGC TGT TAG ATT GAA-3';
PRRX1 reverse, 5'-GAT GCA CTT TTA GCA CAC ATT TGT ATT-3';
SOX3 forward, 5'-GGG ACG CCT TGT TTA GCT TTG CTT-3';
SOX3 reverse, 5'-TAA CAC AGC GAT TCC CAG CCT ACA-3';
ZIC1 forward, 5'-ACA AGT CCT ACA CGC ATC CCA GTT-3';
ZIC1 reverse, 5'-ATA AGG AGC TTG TGG TCG GGT TGT-3'.

Results

Temporal Changes in Neurogenesis from Trisomy 21 hNPCs

Histopathological observations of prenatal DS brains revealed that fewer neurons are present in specific layers of the cortex when compared to euploid brains [Davidoff, 1928; Benda, 1947; Crome and Stern, 1967; Colon, 1972; Ross et al., 1984; Wisniewski et al., 1984; Becker et al., 1991; Golden and Hyman, 1994], and this lack of cortical neurons occurs at a specific developmental time (>23 weeks) [Ross et al., 1984; Golden and Hyman, 1994]. Trisomy 21 hNPCs in culture generate fewer neurons than euploid controls and can therefore be used to define the mechanism of reduced cortical neurogenesis in trisomy 21 [Bahn et al., 2002; Bhattacharyya and Svendsen, 2003]. To test the hypothesis that trisomy 21 hNPCs have a developmentally specified neurogenesis defect, rather than a general defect in neurogenesis, we took advantage of the fact that hNPCs are temporally specified in vitro [Wright et al., 2006; Anderson et al., 2007]. hNPCs derived from trisomy 21 and euploid fetal cortices (13–18 weeks of gestation) were expanded and allowed to differentiate into neurons at successive times of expansion. Trisomy 21 hNPCs expanded for short periods of time (less than 6 weeks) and then allowed to differentiate produce a similar percentage of neurons as euploid hNPCs (fig. 1). In contrast, trisomy 21 hNPCs expanded in culture for more than 10 weeks generate far fewer neurons compared to age- and culture-matched euploid controls (fig. 1). The number of neurons generated from individual trisomy 21 hNPC lines decreases dramatically with increased time in culture while age-matched euploid hNPCs continue to generate 20–30% neurons (data not shown). Therefore, trisomy 21 hNPCs exhibit a developmentally regulated defect in neurogenesis that can be delineated in culture.

The reduced number of neurons in the DS cortex after mid-gestation is subtype specific: there are fewer small, granular, presumably GABAergic neurons in layer II and layer IV of the cortex [Ross et al., 1984]. To test whether our hNPCs differentiate into predominantly small GABAergic interneurons, euploid hNPCs were propagated for more than 10 weeks and neurons differentiated from these progenitor cells were allowed to mature for up to 10 weeks. The cultures were then fixed and immunostained to identify GABAergic and glutamatergic neurons. As shown in figure 2, these hNPCs differentiate into exclusively GABAergic neurons. These results are in agreement with recent studies reporting that hNPCs derived from fetal cortex differentiate into primarily

Fig. 1. Developmental time course of neuron production by hNPCs. Euploid and trisomy 21 cortical hNPCs expanded as neurospheres for less than 6 weeks or for more than 10 weeks were plated onto coverslips in media without mitogens for 1 week. The cells were fixed and immunostained for β -III-tubulin to label young neurons. **a** β -III-Tubulin-positive neurons were quantified in each culture. The graph shows mean $\pm$ SD of the percentage of neurons in 10–15 neurospheres in 3–4 separate neurosphere lines. * $p = 0.02$. **b, c** Immunostaining for β -tubulin 3 to label young neurons in euploid (**b**) and trisomy 21 (**c**) cultures.

Fig. 2. Expanded hNPCs generate GABAergic neurons. Euploid hNPCs were propagated for more than 10 weeks in culture plated onto coverslips in media that promoted differentiation. After 10 weeks, the cells were fixed and immunostained for β -III-tubulin

(green; color only in online version) and either vGAT (red) to identify GABAergic neurons or vGlut (red) to identify glutamatergic neurons. Arrows indicate GABAergic neurons.

GABAergic neurons [Schaarschmidt et al., 2009; Maciaczyk et al., 2009]. Trisomy 21 hNPCs were also differentiated into neurons, but few if any neurons were found at 10 weeks in culture, due to the fewer number of neurons generated and selective apoptosis of trisomy 21 neurons [Busciglio and Yankner, 1995] (data not shown). These

results suggest that trisomy 21 hNPCs have defects in GABAergic neurogenesis.

To test whether the neurogenesis defect in trisomy 21 hNPCs is due to a decrease in progenitor cells, rather than a decrease in the differentiation of neurons from progenitors, we analyzed the expansion of individual

Fig. 3. Developmental time course of hNPC growth. Control (euploid) and trisomy 21 cortical hNPCs expanded as neurospheres for less than 6 weeks or for more than 10 weeks. The diameter of individual neurospheres in single wells of a 96-well plate was measured over a period of 2 weeks. The diameter measurements were converted to volumes to allow comparison between the trisomy

21 and euploid neurospheres. Points on graphs represent mean $\pm$ SEM of the percent increase in volume of 10–15 neurospheres in 2–5 separate neurosphere lines. Pictures show representative images of euploid (c) and trisomy 21 (d) neurospheres expanded for >10 weeks at endpoint. Bar = 100 μ m.

neurospheres. Comparison of the growth of euploid and trisomy 21 neurospheres revealed that trisomy 21 neurospheres grew more slowly than their normal counterparts. Importantly, the growth of neurospheres coincided with neurogenesis: reduced growth of trisomy 21 hNPCs occurred at the time when trisomy 21 hNPCs had reduced neuron production (fig. 3). These results suggest a loss of trisomy 21 progenitor cells within neurospheres. We next tested whether loss of progenitor cells within trisomy 21 neurospheres was due to increased cell death or decreased cell division. Cell death was assessed using a fluorometric TUNEL assay and dividing cells were identified with antibodies to Ki67 within sections of tri-

somy 21 and euploid neurospheres that had been in culture for >10 weeks. We were unable to detect significant differences in either cell death or division within trisomy 21 neurospheres compared to controls. Euploid neurospheres had $4.7 \pm 0.5\%$ TUNEL-positive cells compared to $2.87 \pm 0.4\%$ TUNEL-positive cells in trisomy 21 neurospheres. Euploid neurospheres had $6.7 \pm 2.4\%$ proliferating cells compared to $8.2 \pm 3.4\%$ Ki67-positive cells in trisomy 21 neurospheres.

Gene Expression Differences in Trisomy 21 hNPCs

Our results show that trisomy 21 hNPCs have impaired neurogenesis that occurs at a defined time in cul-

Table 1. Gene ontology of gene changes in trisomy 21 hNPCs

Gene names	Number of genes	Biological process
<i>ABL1, ACTN2, ACVR1, ADRA1A, AGT, AHR, APOE, ARF6, ATP7A, ATXN1, BCAR1, BCL2L11, BCL2L14, BECN1, BRCA2, BRE, BTG1, BTG2, C16orf5, CASP1, CASP6, CASP7, CDKN2A, CDKN2C, CFLAR, CLN8, CTNNA1, DBC1, DIP, DLG5, EAF2, EEF1A2, EGLN3, EIF5A, ELMO1, ERC1, FAS, GADD45A, GAL, GJA1, GSK3B, GSPT1, HDAC1, HIP1, IGFBP3, IL12A, IL24, ISG20L1, LITAF, MCL1, MLL, NLRP1, NOTCH2, PAX3, PDCD4, PDCD6, PEG3, PHLDA1, PLAGL1, PPP3CC, RYBP, SERPINB9, SFRP1, SIVA1, SOCS2, SOD2, STK17A, STK17B, STK4, TCF7L2, TGFB3, TIMP3, TNFAIP3, TNFRSF10A, TNFRSF11B, TRAF7, TRIM69, ZAK, ZBTB16</i>	81	cell death apoptosis
<i>ABL1, ACSL6, ACVR1, ACVR2A, ADRA1A, AGT, AHR, AIM2, ANG, APC, APOE, ARHGAP20, AURKB, BCAR1, BCAT1, BIN1, BLM, BLZF1, BMP2, BRCA2, BTG1, BTG2, CABLES1, CALCRL, CCNE1, CCNE2, CDC14B, CDC25A, CDCA7, CDK3, CDK6, CDK9, CDKN2A, CDKN2C, CENPE, CENPF, CHRNA7, CLIP1, CP110, CSRP2, CTBP1, CTNNA1, CTNNBIP1, DAB2IP, DBC1, DBF4, DIAPH2, DIRAS3, DLG5, DSCC1, EDN1, EGF, EIF5A, ERBB2, ERBB3, FGF9, FGFR1OP, FLT4, GADD45A, GAL, GAS7, GJA1, GLI3, GMNN, GNL3, GSPT1, HBP1, HDAC6, HELLS, HHEX, HLA-DQB1, HRASLS3, HUS1, IL12A, JAG2, KANK1, KLF4, KLHDC3, LYN, MAPK4, MCM2, MKI67, MPHOSPH9, MTSS1, MXD1, NEK1, NOTCH2, NOX4, NPY, NR1P, NUMBL, PARD6B, PDCD4, PDGFC, PDGFD, PDGFRA, PDS5A, PDS5B, PES1, PLAGL1, PMS1, PPP1CB, PRR5, PSMF1, PTCH1, RAD51, RBBP8, RIF1, S100A11, S100A6, SASS6, Septin 11, Septin 8, SFTPD, SMC2, SMC4, STAG2, STARD13, STIL, TBX2, TCF7L2, TERF2, TFDP2, TGFB3, TGFBI, TXN, TXNRD1, UHMK1, USP16, XRN1, ZAK</i>	131	cell division cell cycle cell proliferation
<i>ACSL6, AFF2, ALK, APOE, ATP2B2, ATP7A, B3GNT2, BMP2, BPTF, BRCA2, BTG2, C17orf47, CDK5RAP2, CHL1, CLN8, CTNNA1, CYFIP1, DLX2, DLX5, EGF, EGR2, EMX2, EN2, EPHB3, ERBB2, FARP2, FEZ2, GJA1, GLI3, HHEX, JAG2, KLF4, MAPT, MCL1, NOTCH2, NR2F1, NR1P, NRXN3, NTNG1, NTNG2, NUMBL, OLIG2, PARD6B, ROBO1, RTN1, S100A6, SEMA4F, SLIT1, SLITRK1, SMAD2, SOX3, TCF7L2, ZIC1</i>	54	cell fate generation of neurons brain development

ture. One way to assess the molecular differences in trisomy 21 neural progenitor cells that lead to decreased neurogenesis is by transcriptional analysis. Euploid and trisomy 21 hNPCs were analyzed by microarray analysis prior to the critical time at which trisomy 21 hNPCs show the decreased neurogenesis phenotype. Microarray analysis is most useful when comparing two populations of cells that are uniform, and therefore, we used gestational age-matched (13 weeks) and culture-matched hNPCs. Furthermore, we pooled the RNA from the different hNPC lines to reduce line-to-line differences in our small number of samples.

Overall Changes in Gene Expression

Transcriptional expression in hNPCs was assessed using Affymetrix U133 Plus 2.0 arrays that allow analysis of approximately 47,000 transcripts. Comparison of transcriptional expression between euploid and trisomy 21 hNPCs focused on gene changes between the two groups that had a *p* value of <0.05. Gene changes ≥ 1.2 -fold were included in order to embrace changes in chromosome 21

genes that should be increased 1.5-fold based on the gene dosage hypothesis. Overall, expression of 2,430 transcripts was changed greater than 1.2-fold in trisomy 21 hNPCs compared to euploid hNPCs (online suppl. table 1, www.karger.com/doi/10.1159/000236899). Only approximately 3% of the expression changes were in genes located on chromosome 21, indicating that trisomy 21 alters transcriptional regulation throughout the genome. Expression of equal numbers of probes was increased and decreased, indicating that trisomy 21 causes both up- and downregulation of gene expression. Expression of less than 20% (453) of the transcripts was changed more than 2-fold in trisomy 21 hNPCs. The expression of 165 probes was changed more than 3-fold in trisomy 21 hNPCs, which is much greater than the number of genes whose expression is altered 3-fold in hNPCs that carry a single gene mutation in the *FMR1* gene [Bhattacharyya et al., 2008].

Defective neurogenesis in trisomy 21 hNPCs suggests that there may be defects in the progenitor cells that cause reduced neurogenesis. We therefore focused our analysis

Fig. 4. qPCR confirmation of selected genes involved in interneuron progenitor cell development. **a** Fold change in expression of three chromosome 21 genes whose expression is upregulated >1.5-fold. **b** Fold change in expression of three DLX genes involved in interneuron development. **c** Fold change in expression

of transcription factor genes involved in neural progenitor development. **d** Fold change in expression of three GABA receptor subunit genes. Bars are mean fold change $\pm$ SEM of 2–4 separate PCR runs on distinct cDNAs. Numbers in parentheses refer to fold changes as assessed by microarray analysis.

on genes that may alter progenitor cell behavior. Gene ontology analysis of the biological function of the 2,430 transcripts altered in trisomy 21 hNPCs revealed that many changed genes are involved in cell death, cell division and neural progenitor development (table 1). We were unable to detect significant changes in cell death or division at the cellular level within trisomy 21 neurospheres, but these microarray results suggest that more subtle defects in these processes may be occurring in the cells.

Changes in Expression of Chromosome 21 Genes

Changes in the expression of chromosome 21 genes represent a small proportion (3%) of the total expression differences in trisomy 21 hNPCs. Virtually all of the chromosome 21 transcripts whose expression is altered greater than 1.2-fold are increased with an average in-

crease of 1.77 ± 0.61 and varying from 1.21- to 3.43-fold (online suppl. table 1; table 2). The expression of only one DS critical region gene (DSCR2) was increased. However, the expression of many chromosome 21 genes was not changed in trisomy 21 hNPCs, including *SOD1*, *APP*, *DSCAM*, and *S100B*. *DYRK1* was not expressed in either trisomy 21 or euploid hNPCs, as assessed by both microarray analysis and qPCR (data not shown). Expression of three chromosome 21 genes was increased greater than 3-fold: *OLIG1*, *OLIG2* and *CXADR* (online suppl. table 1; table 2). The overexpression of these three genes and the lack of misexpression of *APP* in trisomy 21 hNPCs was confirmed by qPCR (fig. 4).

Genes Involved in Neural Development

Many genes whose expression is changed in trisomy 21 hNPCs are transcription factor genes or genes associ-

Table 2. Changes in chromosome 21 gene expression

Gene ID	Gene identifier	Ratio	Direction	p value	Gene ID	Gene identifier	Ratio	Direction	p value
<i>C21orf62</i>	NM_019596	5.32	up	0.02	<i>GART</i>	NM_000819	1.49	up	0.00
<i>OLIG2</i>	AI870776	5.02	up	0.01	<i>DIP2A</i>	BC033718	1.48	up	0.01
<i>OLIG1</i>	AL355743	4.14	up	0.05	<i>SETD4</i>	NM_017438	1.47	up	0.04
<i>CXADR</i>	AY072911	3.44	up	0.02	<i>NDUFV3</i>	AI129346	1.47	up	0.04
<i>OLIG2</i>	AA757419	3.19	up	0.01	<i>UBE2G2</i>	BC001738	1.47	up	0.00
<i>CXADR</i>	BG260087	3.16	up	0.01	<i>UBE2G2</i>	AL355686	1.46	up	0.05
<i>CXADR</i>	NM_001338	3.14	up	0.04	<i>ATP5J</i>	NM_001685	1.45	up	0.00
<i>C21orf57</i>	AW945538	2.59	up	0.00	<i>GART</i>	BE966876	1.43	up	0.03
<i>C21orf57</i>	AI146751	2.39	up	0.00	<i>COL6A1</i>	AA292373	1.42	up	0.03
<i>ADARB1</i>	NM_015833	2.32	up	0.00	<i>C21orf88</i>	NM_153754	1.41	up	0.05
<i>NCAM2</i>	NM_004540	2.24	up	0.04	<i>SETD4</i>	AB004853	1.41	up	0.04
<i>C21orf66</i>	NM_016631	2.10	up	0.01	<i>U2AF1</i>	NM_006758	1.39	up	0.04
<i>IL10RB</i>	BC001903	1.99	up	0.04	<i>IL10RB</i>	BF526978	1.38	up	0.01
<i>JAM2</i>	NM_021219	1.88	up	0.01	<i>IFNAR1</i>	NM_000629	1.36	up	0.01
<i>C21orf66</i>	NM_013329	1.88	up	0.04	<i>TIAM1</i>	U90902	1.34	up	0.03
<i>TTC3</i>	AU131711	1.85	up	0.02	<i>DSRC2</i>	NM_003720	1.34	up	0.04
<i>C21orf57</i>	BF026326	1.77	up	0.05	<i>POFUT2</i>	BC000626	1.33	up	0.04
<i>C21orf86</i>	AV705934	1.76	up	0.03	<i>BRWD1</i>	AA807344	1.32	up	0.02
<i>PDE9A</i>	NM_002606	1.71	up	0.02	<i>BACH1</i>	NM_001186	1.32	up	0.03
<i>ATP5J</i>	BF195118	1.69	up	0.01	<i>BACH1</i>	AF317902	1.30	up	0.03
<i>TTC3</i>	D83077	1.67	up	0.00	<i>C21orf51</i>	AY033902	1.30	up	0.05
<i>TTC3</i>	NM_003316	1.65	up	0.00	<i>PKNOX1</i>	AL570914	1.29	up	0.03
–	AL355741	1.60	up	0.02	<i>H2BFS</i>	NM_017445	1.29	up	0.00
<i>C21orf33</i>	NM_004649	1.59	up	0.03	<i>PRMT2</i>	NM_001535	1.29	up	0.03
<i>GART</i>	D32051	1.56	up	0.00	<i>USP16</i>	AI435036	1.26	up	0.02
<i>SYNJ1</i>	NM_003895	1.56	up	0.02	<i>SUMO3</i>	BG338532	1.25	up	0.01
<i>TCP10L</i>	AI336920	1.56	up	0.01	–	BC034606	1.25	up	0.05
<i>RUNX1</i>	D43968	1.55	up	0.02	<i>SUMO3</i>	NM_006936	1.24	up	0.02
<i>DIP2A</i>	AW027812	1.55	up	0.00	<i>C21orf63</i>	AI744591	1.24	up	0.04
<i>C21orf56</i>	AL136871	1.54	up	0.03	<i>D21S2056E</i>	NM_003683	1.23	up	0.03
<i>NDUFV3</i>	NM_021075	1.53	up	0.05	<i>GART</i>	AF008655	1.23	up	0.03
<i>C21orf33</i>	D86062	1.52	up	0.05	<i>DIP2A</i>	AI590053	1.22	up	0.00
<i>IFNAR1</i>	AA811138	1.52	up	0.04	<i>SNF1LK</i>	AV763520	1.22	up	0.04
<i>PRMT2</i>	AI928367	1.51	up	0.04	<i>USP16</i>	NM_006447	1.21	up	0.01
<i>MRPS6</i>	AL555227	1.50	up	0.02	–	AA452540	3.43	down	0.04
<i>PRMT2</i>	AL050065	1.50	up	0.02	<i>ITSN1</i>	AF003737	1.23	down	0.05
<i>GART</i>	AF008655	1.49	up	0.01					

ated with signal transduction pathways known to be crucial in nervous system development, including the Jak-STAT, Notch, Wnt and cytokine signaling pathways (table 1; online suppl. table 1). We focused our study on the expression of genes involved in progenitor cell development and confirmed the expression changes of 20 genes of interest by qPCR (fig. 4). As we would expect when interneuron development is faulty, the expression of interneuron-specific genes, *DLX1*, *DLX2* and *DLX5*, is down-regulated in trisomy 21 hNPCs. The expression of many cell fate and progenitor cell genes is concomitantly increased, and the expression of at least two cell fate genes,

DACH1 and *SOX3*, is increased. Moreover, the expression of many members of the Notch signaling pathway is also increased: *DLL3*, *DLL1*, *DLK1*, *JAG2*, *MAML2*, *Numb*, *HEY1* and *RBPJ*. *EMX2* and *NR2F/COUP-TF1*, which are crucial in neural progenitor specification, are overexpressed in trisomy 21 hNPCs. The expression of *CTBP1*, a C-terminal binding protein 1 that acts as a transcriptional corepressor downstream of many signaling pathways, is also increased in trisomy 21 hNPCs. In addition, the expression of three GABA_A receptor subtypes are altered. GABA_A receptor α_2 (*GABRA2*) is up-regulated, while GABA_A α_5 (*GABRA5*) and GABA_A re-

ceptor β_3 (*GABRB3*) are downregulated. Taken together, these expression changes suggest that the trisomy 21 progenitors have intrinsic changes that lead to decreased GABAergic interneuron neurogenesis.

Discussion

Modeling Trisomy 21 Human Cortical Neurogenesis in vitro

The bulk of cortical development occurs prenatally in humans with the generation of most cortical neurons occurring from 10 to 25 weeks of gestation [Sidman and Rakic, 1973]. Trisomy 21 causes a developmentally regulated reduction in cortical neurons: the number of cortical neurons appears normal in early gestation, but there are fewer neurons present in the DS cortex after 23 weeks of gestation [Golden and Hyman, 1994; Weitzdoerfer et al., 2001]. Using hNPCs in culture to model fetal cortical neurogenesis, we find that hNPCs derived from 13-week trisomy 21 fetal cortex and expanded for a short time exhibit neurogenesis that is indistinguishable from euploid controls. These young neural progenitors correspond to early cortical development, a time at which trisomy 21 cortices are largely normal. The same trisomy 21 hNPCs expanded in culture for longer times, and therefore later cortical progenitors, generate fewer neurons compared to controls.

The timing of the cortical neuron deficiency in DS fetal brain (>23 weeks) coincides with the later phase of human cortical development when medium and small neurons, as well as glia, continue to be produced from neural progenitor cells [Sidman and Rakic, 1973]. Deficits during this time period would be expected to affect the generation of small neurons and glia. Indeed, small, granular, presumably GABAergic neurons in cortical layers II and IV appear to be missing in DS brains [Ross et al., 1984]. Our in vitro results suggest that trisomy 21 hNPCs fail to generate the proper number of GABAergic interneurons, due to defects in progenitor cells rather than in survival of generated neurons. Furthermore, since neurogenesis from hNPCs in vitro proceeds through the division of committed neuronal progenitor cells rather than directly from a neural progenitor cell [Ostenfeld and Svendsen, 2004; Nelson and Svendsen, 2006], our results suggest that committed interneuron progenitor cells are affected in trisomy 21, leading to subsequent loss of interneurons. Taken together, these results mimic the observed time-dependent loss of neurons in vivo and support hNPCs as a system to investigate cortical neurogenesis in DS.

Microarray Analysis Reveals Changes in the Neural Progenitor Cell Pool in Trisomy 21 hNPCs

Microarray analysis of trisomy 21 and unaffected hNPCs was carried out to define gene expression changes that cause the loss of neurogenesis in trisomy 21 hNPCs. Results from this comparison may also identify cell-intrinsic changes in these cells that lead to subsequent problems as the cells mature into neurons and glia. Finally, this information can be used to discover new pathways of study on the pathophysiology of trisomy 21 and potential therapeutic intervention. Our microarray results are consistent with previous reports analyzing human trisomy 21 tissue or cells in that the overexpression of different chromosome 21 genes is cell type and developmental time specific [Gross et al., 2002; Mao et al., 2003; Lubec and Sohn, 2003; Giannone et al., 2004; Mao et al., 2005; Chung et al., 2005; Li et al., 2006; Ait Yahya-Graison et al., 2007; Rozovski et al., 2007]. Overexpression of chromosome 21 genes in our study is nonlinear (>1.5-fold), again in agreement with previous reports [Lockstone et al., 2007]. Our results differ from previous results obtained using differential display comparing trisomy 21 and euploid hNPCs that revealed that genes known to be regulated by the REST/NRSF transcription factor were selectively repressed in the DS hNPCs [Bahn et al., 2002]. The basis for this discrepancy is likely due to the different gestational ages of the euploid and DS fetal tissue. In this study, we analyzed trisomy 21 hNPCs that were derived from the same gestational age fetal cortical tissue as control hNPCs. Our results also vary from a recent microarray analysis of trisomy 21 hNPCs implicating changes in *S100B* and aquaporin 4 in cell injury [Esposito et al., 2008]. We do not detect expression changes in these genes and our results show changes in neural progenitor cell genes that were not detected in the study by Esposito et al. [2008]. The differing results are probably due to the different gestational ages of the fetal tissue from which hNPCs are generated (13 weeks vs. 19 weeks). Esposito et al. [2008] also report that their hNPCs exhibit characteristics of glial progenitors, as would be expected late in gestation [Sidman and Rakic, 1973; Esposito et al., 2008]. By focusing on a specific cell type (interneuron progenitors) at a developmental time when they show a phenotype (reduced interneuron neurogenesis), we have been able to narrow down the gene expression changes that are likely responsible for this particular manifestation of DS.

Transcriptional analysis of trisomy 21 hNPCs defines potential molecular causes of the reduced neurogenesis of GABAergic neurons. The most striking gene expres-

sion change relating to interneuron development in trisomy 21 hNPCs is the nearly absent expression of the *DLX* homeobox transcription factors, *DLX1*, *DLX2* and *DLX5*. The *DLX* genes are required for generation of forebrain interneurons [Anderson et al., 1997; Letinic et al., 2002] and dysregulation of these genes has been observed in DS fetal and adult brain [Lockstone et al., 2007]. It is not clear, however, what causes the lack of *DLX* gene expression in trisomy 21 hNPCs. Changes in other genes, primarily transcription factors, suggest at least two possibilities for the lack of interneuron-specific gene expression and subsequent loss of interneurons: (1) inhibition of the interneuron progenitor pool or interneuron neurogenesis, and/or (2) promotion of undifferentiated progenitors or committed progenitors of another lineage.

Overexpression of *COUP-TF1/NR2F1* in trisomy 21 hNPCs may prevent the generation of interneuron progenitors. Recently, transient expression of *COUP-TF1/NR2F1* has been reported to be required for the temporal specification of cortical progenitor cells by regulating the balance of early- and late-born neurons [Faedo et al., 2008; Naka et al., 2008]. Overexpression of *COUP-TF1/NR2F1* causes increased neurogenesis of early-born neurons and depletion of progenitors for late-born neurons [Faedo et al., 2008; Naka et al., 2008]. It is therefore possible that increased expression of *COUP-TF1/NR2F1* contributes to the depletion of late-born neuron-specific progenitor cells in trisomy 21 hNPCs. Overexpression of *COUP-TF1/NR2F1* may occur only transiently in trisomy 21 and cause depletion of progenitors for late-born neurons without the initial increase in neurogenesis of early-born neurons. Alternatively, *COUP-TF1/NR2F1* may be overexpressed earlier in DS cortical development, but its manifestations have not been detected or have been compensated for. In addition, trisomy 21 hNPCs overexpress many components of the Notch signaling pathway. Up-regulation or activation of Notch components inhibits neurogenesis [Yoon and Gaiano, 2005; Androutsellis-Theotokis et al., 2006; Guentchev and McKay, 2006; Louvi and Artavanis-Tsakonas, 2006]. Therefore, initiation of pathways that antagonize neurogenesis in trisomy 21 hNPCs may prevent these progenitor cells from differentiating into committed *DLX*-expressing interneuron progenitors.

There are many gene expression changes in trisomy 21 hNPCs that may function to increase the proportion of undifferentiated progenitors or committed progenitors of another lineage. The cell fate genes, *DACH1* and *SOX3*, both contribute to the maintenance of undifferentiated progenitor cells [Li et al., 2002; Machon et al., 2002; Wang

et al., 2006; Saarimaki-Vire et al., 2007], and so their increased expression in trisomy 21 hNPCs may reflect a shift away from neurogenesis. Interneuron progenitors in trisomy hNPCs may also be lost at the expense of another committed progenitor. Expression of the chromosome 21 *OLIG* genes is increased >3-fold in trisomy 21 hNPCs. *OLIG1* and *OLIG2* are basic helix-loop-helix transcription factors described initially in the ventral spinal cord in a subpopulation of neural progenitors that give rise first to motor neurons and then oligodendrocytes [Lu et al., 2000; Zhou et al., 2000; Takebayashi et al., 2000]. While *OLIG* gene function is necessary for the development of oligodendrocytes [Lu et al., 2002; Zhou and Anderson, 2002], additional functions of the *OLIG* genes have been revealed in both neural development and in disease [for a review, see Ligon et al., 2006]. The appearance of oligodendrocyte progenitor cells and subsequent myelin formation occurs earlier in the human cortex than in rodents, and so it is possible that more oligodendrocytes are specified at this time in DS cortex [Jakovcevski and Zecevic, 2005]. In addition to being required for interneuron differentiation, *DLX* genes have been shown to repress oligodendrocyte precursor cell formation by acting on a common progenitor [Petryniak et al., 2007]. Our results show that trisomy 21 hNPCs have decreased *DLX* expression and increased expression of at least one oligodendrocyte-specific gene (*OMG*, oligodendrocyte myelin glycoprotein), supporting the idea that there may be a shift from interneurons to oligodendrocytes in the trisomy 21 progenitor pool. Unfortunately, our culture system does not support the differentiation of oligodendrocytes from either control or trisomy 21 hNPCs, so we cannot make any conclusions on oligodendrocyte development. Trisomy 21 hNPCs from later gestational ages show a more glial progenitor phenotype [Esposito et al., 2008], implying that more glial cells may be generated from these progenitors.

Misexpression of several other genes in trisomy 21 hNPCs may also contribute to the lack of interneurons, but their roles in cortical development are less well known. For example, Coxsackie and adenovirus receptor (*CXADR* or *CAR*) is a chromosome 21 gene whose expression is greatly increased in trisomy 21 hNPCs. *CXADR* is a transmembrane cell adhesion molecule of the immunoglobulin superfamily implicated in the process of adenoviral infection in various systems. In the nervous system, *CXADR* is expressed in some neurons within layers II–VI of the adult human cortex [Persson et al., 2006], but has a poorly defined role in CNS development. *CXADR* is expressed in developing CNS [Honda et

al., 2000; Tomko et al., 2000] and in neurospheres, and has been suggested to be an important player in the stem cell niche [Hauwel et al., 2005]. *CXADR* can bind *LNK* (Ligand of Numb) which (with Numb) influences Notch signaling to regulate stem cell fate [Sollerbrant et al., 2003]. Furthermore, *CXADR* is highly expressed in human neuroblastoma and medulloblastoma, which are cancers of progenitor cells [Persson et al., 2007]. Taken together, these studies suggest that *CXADR* may have an important contribution to the development of neural progenitor cells. Expression of *CTBP1*, a C-terminal binding protein 1, is also increased in trisomy 21 hNPCs. C-terminal binding protein family members function predominantly as transcriptional corepressors in association with sequence-specific DNA-binding transcriptional repressors. Genetic studies indicate that the *CTBP1* genes play important roles in development [Hildebrand and Soriano, 2002; Chinnadurai, 2007]. However, the large number of transcription factors that are predicted to bind *CTBP1* make its role in cortical neurogenesis difficult to ascertain. Signaling through GABA_A receptors has been implicated in proliferating ventricular zone cells during early cortical neurogenesis [Loturco et al., 1995], and so it is conceivable that the misexpression of GABA_A receptors in trisomy 21 hNPCs alters the normal extracellular cues that these cortical progenitors receive and therefore affects their decision to divide or differentiate. The role of these genes in DS cortical development as well as normal human cortical development warrants further investigation.

Due to the large number of transcriptional changes that occur in trisomy 21 hNPCs, it is likely that a combination of these mechanisms is responsible for the decreased differentiation of interneurons in the trisomy 21 cortex. Furthermore, epigenetic mechanisms also shape the development of the cortex and add further complexity. Our results therefore represent a starting point for the dissection of the molecular mechanisms that lead to interneuron loss in DS cortex and provide a basis to explore potential interventions to correct the loss.

Conclusion

Our results indicate that trisomy 21 hNPCs are impaired in their ability to give rise to GABAergic interneurons. Gene expression changes in these trisomy 21 hNPCs reveal possible dysregulation of progenitor cell processes. Concurrent downregulation of interneuron genes with upregulation of genes involved in specification and main-

tenance of progenitors suggests that there is a shift away from interneuron progenitor specification and neurogenesis in trisomy 21 hNPCs. The results raise further questions about the development of the DS cortex that have ramifications on normal cortical development in general. It is not clear from our results, for example, whether the gene changes are causative and lead to decreased precursor pool or whether the gene changes are the result of upstream/earlier changes and select the precursor pool. It is also not known why there is not a global neurogenesis defect in DS, as many of the dysregulated genes function in other neuronal lineages. It is possible that these changes are triggered at this particular time, but the expression of these genes is normal at other times. Further work on hNPCs and mouse models where there are also defects in timing of cortical neurogenesis [Haydar et al., 2000; Chakrabarti et al., 2007] will hopefully shed light on these unanswered questions and lead to a fuller understanding of cortical development in DS.

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