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Notch activation induces apoptosis in neural progenitor cells through a p53-dependent pathway

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Abstract

Notch signaling is involved in a variety of cell-fate decisions during development. Here we investigate the role of Notch signaling in apoptotic cell death of neural progenitors through the generation and analysis of cell type-specific conditional transgenic and knockout mice. We show that conditional expression of a constitutively active form of Notch1 in early neural progenitor cells, but not postmitotic neurons, selectively induces extensive apoptosis, resulting in a markedly reduced progenitor population. Conversely, attenuation of Notch signaling in *Notch1* conditional knockout or *Presenilin-1*−/− mice results in reduced apoptosis of early neural progenitor cells. Furthermore, Notch activation in neural progenitor cells leads to elevated levels of nuclear p53 and transcriptional upregulation of the target genes *Bax* and *Noxa*, and the promotion of apoptotic cell death by Notch activation is completely suppressed by p53 deficiency. Together, these complementary gain-of-function and loss-of-function studies reveal a previously unappreciated role of Notch signaling in the regulation of apoptotic cell death during early mammalian neural development.

Keywords: Neural development; Genetic; Mouse; Conditional; Transgenic; Knockout; *Drosophila*; Presenilin; Notch; Apoptosis; p53

Introduction

Notch signaling is an evolutionarily conserved signaling pathway that mediates cell–cell interactions required for a variety of cell-fate decisions during development (Artavanis-Tsakonas et al., 1999; Justice and Jan, 2002). Notch receptors are activated upon ligand binding from neighboring cells and then proteolytically processed by an ADAM/TACE family metalloprotease (Brou et al., 2000; Mumm et al., 2000) followed by a presenilin-dependent protease to release the Notch intracellular domain (NICD) (De Strooper et al., 1999; Schroeter et al., 1998; Song et al., 1999; Struhl and Greenwald, 1999; Ye et al., 1999). NICD translocates into the nucleus and activates transcription of its downstream targets primarily by forming a complex with CSL (CBF1/RBP-Jκ, Suppressor of hairless, Lag-1) (Greenwald, 1998; Kimble and Simpson, 1997; Mumm and Kopan, 2000; Weinmaster, 1998). Within the cell, Notch activity can also be regulated through ubiquitin-mediated proteolysis involving the E3 ligases SEL-10 (Gupta-Rossi et al., 2001; Oberg et al., 2001; Wu et al., 2001), Itch (McGill and McGlade, 2003; Qiu et al., 2000), or Suppressor of deltex (Cornell et al., 1999).

During mammalian neural development, activation of the Notch signaling pathway is known to be involved in the maintenance of neural progenitor identity and the suppression of neuronal differentiation (Schuurmans and Guillemot, 2002). Targeted disruption of *Notch1* or *RBP-Jκ* in mice results in upregulation of pro-neuronal transcription factors and depletion of neural progenitor cells (Conlon et al., 1995; de la Pompa et al., 1997; Hitoshi et al., 2002;

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Oka et al., 1995; Swiatek et al., 1994). Attenuation of Notch signaling in *Presenilin-1* (*PS1*) null mice leads to premature neuronal differentiation and subsequently reduced neural progenitor and neuronal populations (Handler et al., 2000; Hitoshi et al., 2002; Shen et al., 1997). The role of Notch signaling in the maintenance of neural progenitor cells is further supported by gain-of-function studies showing that Notch activation prevents neuronal differentiation (Chambers et al., 2001; Dorsky et al., 1995; Henrique et al., 1997; Hitoshi et al., 2002; Nye et al., 1994; Scheer et al., 2001).

More recently, Notch signaling has been shown to influence gliogenesis (Gaiano and Fishell, 2002; Lundkvist and Lendahl, 2001; Morrison, 2001; Wang and Barres, 2000). In the peripheral nervous system, Notch signaling acts instructively in neural crest stem cells to promote an irreversible switch to gliogenesis (Morrison et al., 2000). In the central nervous system (CNS), activated Notch1 and 3 in multipotent progenitor cells derived from adult hippocampus also favors astroglial differentiation (Tanigaki et al., 2001). Notch activation similarly promotes the generation of Müller glia in the retina (Furukawa et al., 2000; Hojo et al., 2000; Scheer et al., 2001). In the mouse telencephalon, one study has shown that Notch activation promotes radial glial identity (Gaiano et al., 2000). Subsequent findings indicate that radial glia can cause both neurons and glia (Hartfuss et al., 2001; Malatesta et al., 2000; Miyata et al., 2001; Noctor et al., 2001), making it less clear whether Notch signaling promotes terminal glial differentiation in the embryonic forebrain (Schuurmans and Guillemot, 2002). Another study found that the consequence of Notch activation in neural progenitor cells is dependent upon their temporal and spatial context: it drives progenitor cells into a nondifferentiating, nonmigratory, quiescent state at an earlier developmental stage, while it promotes proliferation of glia at later stages (Chambers et al., 2001).

In addition to neurogenesis and gliogenesis, Notch signaling regulates cell proliferation in a cell type-dependent manner. Its activation can increase proliferation in mammalian astrocytes (Chambers et al., 2001; Furukawa et al., 2000; Tanigaki et al., 2001) and *Drosophila* wing disc cells (Baonza et al., 2000), and can cause T cell lymphoma (Ellisen et al., 1991) and mammary tumors (Jhappan et al., 1992). Conversely, Notch activation promotes cell cycle arrest in the vertebrate retina (Dorsky et al., 1995; Scheer et al., 2001), telencephalon (Chambers et al., 2001), and keratinocytes (Rangarajan et al., 2001), which is in agreement with findings showing that loss of Notch1 and PS1 in the mouse skin results in development of tumors (Nicolas et al., 2003; Xia et al., 2001).

The effect of Notch activation on apoptotic cell death is also highly cell type-specific. It promotes cell death in the *Drosophila* retina (Cagan and Ready, 1989; Miller and Cagan, 1998; Yu et al., 2002), wing imaginal disc (Milan et al., 2002), and sensory organ (Orgogozo et al., 2002). In the zebrafish retina, Notch activation plays a similar proapoptotic role (Scheer et al., 2001). In mammalian lymphocyte development, Notch signaling serves as a binary switch influencing apoptotic cell death: it protects T cells from apoptosis (Deftos et al., 1998; Jehn et al., 1999; Miele and Osborne, 1999) while promoting apoptosis in B cells (Morimura et al., 2000). These observations raise the possibility that during mammalian neural development, Notch signaling may play a role in the regulation of apoptotic cell death, which is an important mechanism that controls the size and shape of the nervous system (Kuan et al., 2000). However, there has been no report demonstrating an involvement of Notch signaling in apoptosis during mammalian neural development.

All of the mammalian gain-of-function studies described above employed retroviral infection or transfection approaches to express NICD in a small percentage of neural progenitor cells, which is advantageous for tracing distinct neuronal or glial fates of infected progenitors, but it is technically difficult to trace the progeny of the infected cells that have undergone cell death. To overcome this limitation and to determine whether Notch activation is involved in the regulation of apoptotic cell death in neural progenitor cells or postmitotic neurons, we took advantage of a cell type-specific conditional transgenic approach which allows expression of NICD in most, if not all, neural progenitor cells (NPC-NICD) or postmitotic neurons (PN-NICD) in mice. Here we show that expression of the intracellular domain of Notch1 in neural progenitor cells, but not postmitotic neurons, selectively induces extensive apoptotic cell death in a subset of progenitor cells, resulting in marked reduction in both neural progenitor and neuronal population. Notch activation promotes apoptotic cell death via a p53-dependent pathway as demonstrated by upregulation of the p53 pathway in NPC-NICD mice and the complete suppression of apoptotic cell death by p53 deficiency. To complement our gain-of-function genetic approach, we furthered our study to loss-of-function mutant mice. Attenuation of Notch activity in *Notch1* conditional knockout (cKO) or *PS1*-null mice results in a marked reduction in cell death of early neural progenitor cells. Together, these results show that Notch signaling plays a physiological role in the regulation of programmed cell death of neural progenitors during early mammalian neural development.

Material and methods

Generation of Notch1 conditional transgenic and knockout mice

The human NICD (amino acid residues 1762–2304), including the RAM23 domain, CDC10/Ankyrin repeats, and two nuclear localization signals, was generated by PCR using a vector containing full-length human Notch1 cDNA (gift of Dr. S. Artavanis-Tsakonas). The cDNA sequence

was confirmed by sequencing both strands of the cDNA. The cDNA, together with an SV40 late polyadenylation signal, was cloned into the restriction sites following the floxed "stop" cassette of the chicken β-actin-loxP-stop-loxP (pCALSL) vector (gift of Dr. R. Kelleher). The transgene was injected into the pronucleus of C57BL/6J embryos to obtain *CALSL-NICD* transgenic mice. Southern analysis was performed on six independent lines of transgenic mice to determine the number of the transgene insertion sites, approximate copy numbers of the transgene, and the orientation of the transgenes relative to one another. We eliminated four of the six lines that may carry transgenes in two or more insertion sites because Cre recombinase can potentially mediate site-specific recombination between any two *loxP* sites present in these insertion sites, resulting in large chromosomal deletions. The remaining two lines carrying transgenes in a head-to-tail orientation in a single insertion site were selected for further characterization. Both lines were further confirmed by PCR to carry intact transgenes in a head to tail orientation and were termed *CALSL-NICD* (*H*) (high; 10–20 copies) and *CALSL-NICD* (L) (low; 1–2 copies) lines. Each of the two lines was then bred to *Nestin-Cre* (*Nes-Cre*) mice (Tronche et al., 1999), which were generated in C57BL/6J and SJL hybrid and backcrossed to C57BL/6J mice for more than 10 generations, to obtain *CALSL-NICD;Nes-Cre* (NPC-NICD) mice. Because the expression level of NICD in the NPC-NICD (L) line was below detection by Western and immunohistochemical analyses, the NPC-NICD (H) line, which expressed detectable but very low levels of NICD, was selected to assess the effects of Notch activation in neural progenitor cells. To simplify the nomenclature, "(H)" was omitted henceforth, while transgenic mice carrying the (L) locus were always specified. Similarly, *CALSL-NICD* mice were crossed with *Synapsin I-Cre* (*Syn-Cre*) mice (Zhu et al., 2001) to generate *CALSL-NICD;Syn-Cre* (PN-NICD) mice.

For the generation of *CALSL-NICD;Nes-Cre;p53−/−* mice, *CALSL-NICD* and *Nes-Cre* mice were crossed to *p53−/−* mice (Jackson Laboratory) (Jacks et al., 1994) to obtain *CALSL-NICD*; *p53+/−* and *Nes-Cre*; *p53+/−* mice, respectively. Further crossing to *p53−/−* mice to obtain *CALSL-NICD;p53−/−* or *Nes-Cre;p53−/−* mice was made difficult due to the incomplete penetrance of lethality and exencephaly associated with *p53−/−* mice (Armstrong et al., 1995). Therefore, *CALSL-NICD;p53+/−* mice were crossed with *Nes-Cre;p53+/−* mice to generate *CALSL-NICD;Nes-Cre;p53−/−* mice. Genotyping revealed that even at E10.5–11.5, the ratio of *CALSL-NICD;Nes-Cre;p53−/−* (approximately 1/20) was lower than the expected Mendelian ratio of 1/16. The usable *CALSL-NICD; Nes-Cre; p53−/−* embryos were further reduced to approximately 1/30 due to exencephaly in a subset of *p53−/−* embryos (Armstrong et al., 1995; Sah et al., 1995).

A modified *Notch1* allele was generated similarly as described in a previous report (Radtke et al., 1999). Briefly, the targeting vector containing a floxed *PGK-neo* selection cassette and a *loxP* site in the upstream and downstream of the first coding exon was transfected into RW-4 ES cells (Simpson et al., 1997). The floxed *Notch1* allele (*fN1*) was then generated by transient transfection of a cDNA encoding Cre recombinase. Removal of the floxed *PGK-neo* cassette in ES cells was confirmed by PCR and Southern analyses. ES cells carrying the *fN1* allele were injected into mouse blastocysts to generate chimeric mice, which were then used to generate homozygous *fN1* mice. The *fN1* mouse was then crossed with *Nes-Cre* transgenic mice to generate *Notch1* conditional knockout (*N1* cKO) mice.

Preparation of embryos and brain sections

Embryos at day 9.5 (E9.5, 23 pairs of somites) and E10.0 (29 pairs of somites) were fixed in 4% paraformaldehyde overnight at 4°C and then processed for whole-mount in situ hybridization. For brain sections, embryos were fixed in 4% paraformaldehyde at 4°C for 40 min (E10.0), 1.5 h (E11.5), or 2 h (E12.5 and 13.5), cryoprotected, embedded in OCT, and then sectioned at 7 μm. For embryos between 9.0 and 10.0, only those with identical number of somites were used. In addition, for *p53−/−* mice, only those embryos with properly closed neural tube were used for analysis.

Immunostaining and in situ hybridization

For immunostainings, sections were blocked with a solution containing 1% BSA, 3% goat serum, and 0.1% Triton X-100 for 3 h at room temperature, and incubated with desired primary antibodies overnight at 4°C. The following primary antisera or antibodies were used: anti-activated-caspase-3 rabbit antiserum (1:300, Cell Signaling Technology), anti-β-tubulin III antibody (1:100, Sigma), anti-p53 rabbit antiserum (1:100, Santa Cruz Biotechnology), and anti-mouse NICD antiserum (1:800, gift of Dr. A. Israel). Sections were then washed in PBS and incubated with appropriate Alexa Fluor 488- or Alexa Fluor 594-conjugated secondary antibodies (1:300, Molecular Probes) for 2 h at room temperature. Images were viewed on a Zeiss Axioskop and collected with a SPOT 2 Cooled Color Digital camera (Diagnostic Instruments) or a Zeiss confocal laser scanning microscope. Whole-mount in situ hybridization and in situ hybridization of brain sections were performed as described in Handler et al. (2000) using probes specific for full-length *Cre* or the stop cassette.

TUNEL assay and quantification of apoptotic cells

TUNEL assay was performed on comparable brain sections at E10.0 and E11.5 as described in Handler et al. (2000). At E11.5, the number of apoptotic cells was quantified by counting three serial sections per brain in the entire

telencephalon under ×20 magnification. For E10.0, the number of apoptotic cells in the forebrain–midbrain junction was quantified by counting three serial sections per brain using a micrometer grid eyepiece to select an area of 320 × 320 μm^2 that encompasses all TUNEL+ cells in this region. Statistical significance was determined by Student's *t* test.

Real-time quantitative RT-PCR

Brains at E11.5 were collected for the preparation of total RNA using Tri Reagent (Sigma). The cDNA was synthesized using Superscript II first-strand cDNA synthesis system (Gibco BRL). Oligonucleotide primers were designed using Primer Express software 1.0 (Applied Biosystems). The specificity of primers was determined by gel electrophoresis of PCR products to ensure a single band with predicted size. Quantitative PCR reaction was performed using SYBR Green on an ABI PRISM 7700 Sequence Detection System (Applied Biosystems). Relative quantification of gene expression was performed using Comparative CT method according to manufacturer's recommended protocol (User Bulletin #2, ABI PRISM 7700 Sequence Detection System, Perkin-Elmer). Levels of target mRNA are normalized according to 18S rRNA. The change of the expression of a target gene in the NPC-NICD brain relative to the littermate control is calculated as: fold change $= 2^{-(\Delta CT,Tg-\Delta CT,control)}$.

Results

Generation of conditional NICD transgenic mice

Previous studies in *Drosophila* and zebrafish have shown that Notch is involved in apoptotic cell death of certain cell populations in the developing retina (Cagan and Ready, 1989; Miller and Cagan, 1998; Scheer et al., 2001) and

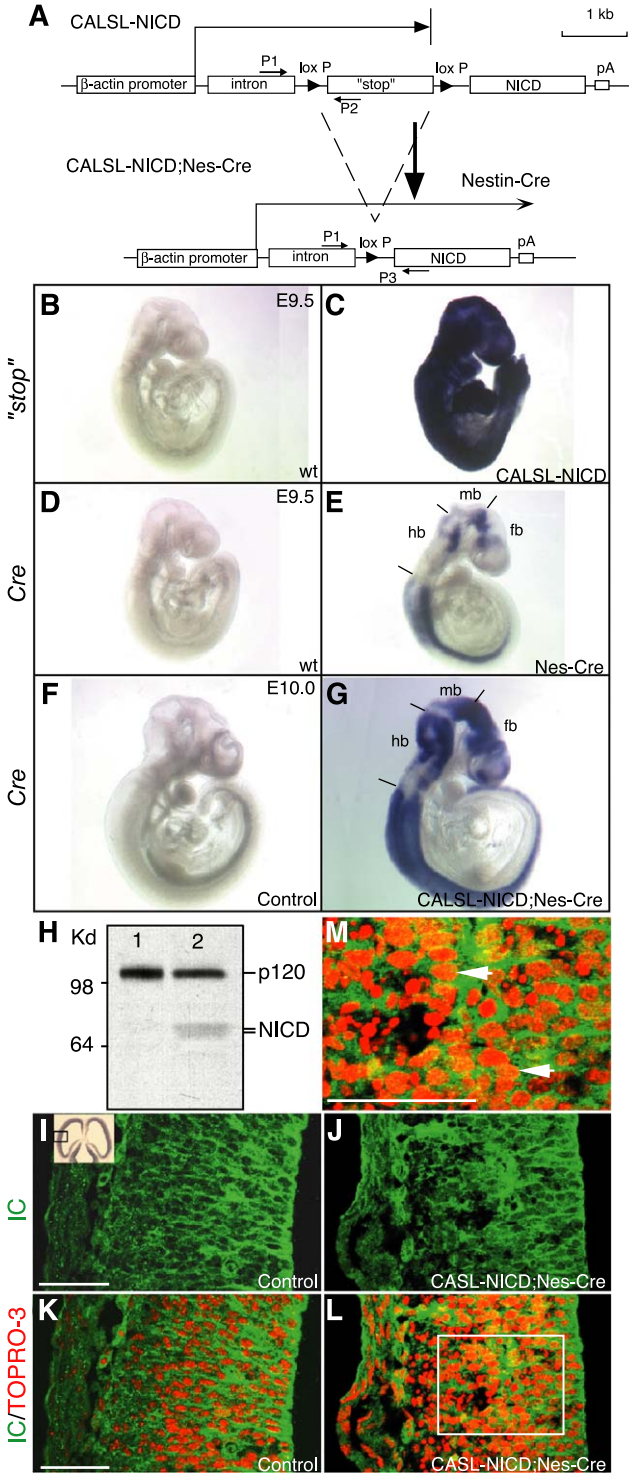

Fig. 1. Generation of NPC-NICD mice. (A) The *CALSL-NICD* transgene contains the chicken *β-actin* promoter, a hybrid 5′intron, a transcriptional and translational stop cassette flanked by two loxP sites, cDNA encoding human NICD (amino acid residues 1762–2304), and the SV40 polyadenylation signal. In *CALSL-NICD* transgenic mice, transcription and translation of the *NICD* cDNA are blocked by multiple polyadenylation sites and stop codon present in the stop cassette. In the presence of Cre recombinase, the stop cassette is excised, thus allowing the transcription and translation of NICD (see arrow). P1, P2, and P3 are PCR primers used to confirm the excision of the stop cassette. (B and C) Whole-mount in situ hybridization analysis of *CALSL-NICD* and wild-type embryos at E9.5. The stop cassette transcript is ubiquitously expressed in *CALSL-NICD* transgenic mice. (D and E) Whole-mount in situ hybridization analysis of *Nes-Cre* and wild-type embryos at E9.5. The *Cre* transcript is expressed in the forebrain (fb, telencephalon and diencephalon), midbrain (mb, mesencephalon), and hindbrain (hb, metencephalon and myelencephalon) of *Nes-Cre* transgenic mice. (F and G) Whole-mount in situ hybridization analysis of NPC-NICD double transgenic mice (G) and *CALSL-NICD* single transgenic control (F) at E10.0. *Cre* is expressed broadly in the forebrain, midbrain, and hindbrain of NPC-NICD mice. (H) Western analysis of E13.5 brain extracts revealed a doublet band corresponding to the appropriate size of NICD in NPC-NICD (lane 2) but not control mice (lane 1). (I–L) Immunohistochemical analysis of the control and NPC-NICD telencephalon at E11.5 using the IC antiserum (green) double labeled with a nuclear dye, TOPRO-3 (red). In contrast to the below detection level of endogenous NICD in the nucleus of most progenitor cells in control mice (I and K), most live progenitor cells in the NPC-NICD telencephalon express detectable levels of NICD in their nuclei (J and L). (M) Higher power view of the boxed area in L. Arrows indicate nuclei that are in yellow, resulting from overlapping NICD immunoreactivity (green) and nuclear DNA labeling (red). Scale bar: 100 μm.

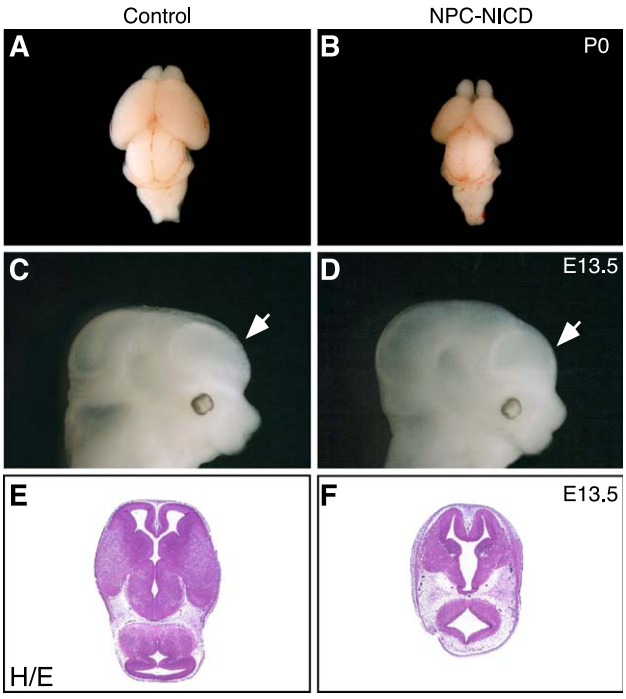

Fig. 2. Markedly reduced brain size in NPC-NICD mice. (A–D) Gross morphology of NPC-NICD and control (wild-type or single transgenic littermates) brains at P0 and E13.5. NPC-NICD mice exhibit a smaller brain, especially the cerebral cortex or telencephalon. Arrows indicate the telencephalon. (E and F) Hematoxylin and eosin staining of NPC-NICD and control brain sections at E13.5. All brain subregions are markedly reduced in NPC-NICD mice.

sensory organ (Orgogozo et al., 2002). Whether Notch signaling regulates cell death in the developing mammalian brain, however, was unknown. Gain-of-function studies employing retroviral approaches to express NICD in neural progenitor cells showed that expression of NICD at E9.5 promotes radial glial lineage (Gaiano et al., 2000), while expression of NICD at E14.5 results in cessation of proliferation of progenitor cells and dramatic reduction of neuronal generation, and subsequently increased glial proliferation leading to overproduction of glia (Chambers et al., 2001). The advantage of the retroviral approach is the readily imposable temporal control of NICD expression, while the disadvantages are that only a small percentage of neural progenitor cells can be infected by retroviruses and that the infected cells are traced by expression of marker or reporter genes, making it technically difficult to determine which fraction of the infected cells have undergone cell death. To circumvent this technical limitation, we chose to use a transgenic approach expressing the intracellular domain of Notch1 in most, if not all, neural progenitor cells to determine the consequences of Notch activation in neural progenitor cells. Because expression of the intracellular domain of Notch3 under the direct control of the *Nestin* promoter resulted in early embryonic lethality, which precluded establishment of transgenic lines for further analysis (Lardelli et al., 1996), we took advantage of the Cre/loxP technology for recombination-activated transgene expression (Lakso et al., 1992) to generate conditional transgenic mice that express the intracellular domain of Notch1 either in neural progenitor cells or in postmitotic neurons.

We first generated *CALSL-NICD* transgenic mice in which *NICD* expression is under the control of the chicken *β-actin* promoter and a floxed transcriptional and translational "stop" cassette (Fig. 1A). Two independent lines of transgenic mice, which were confirmed by Southern and PCR analyses to carry intact transgenes in a head-to-tail fashion in a single insertion site, were designated *CALSL-NICD* (H) (high; 10–20 copies) and *CALSL-NICD* (L) (low; 1–2 copies) lines. Both lines were crossed with *Nes-Cre* mice (Tronche et al., 1999) to obtain *CALSL-NICD;Nes-Cre* (H) and *CALSL-NICD;Nes-Cre* (L) mice. To simplify the nomenclature, *CALSL-NICD* (H) and *CALSL-NICD;Nes-Cre* (H) mice are termed *CALSL-NICD* and NPC-NICD

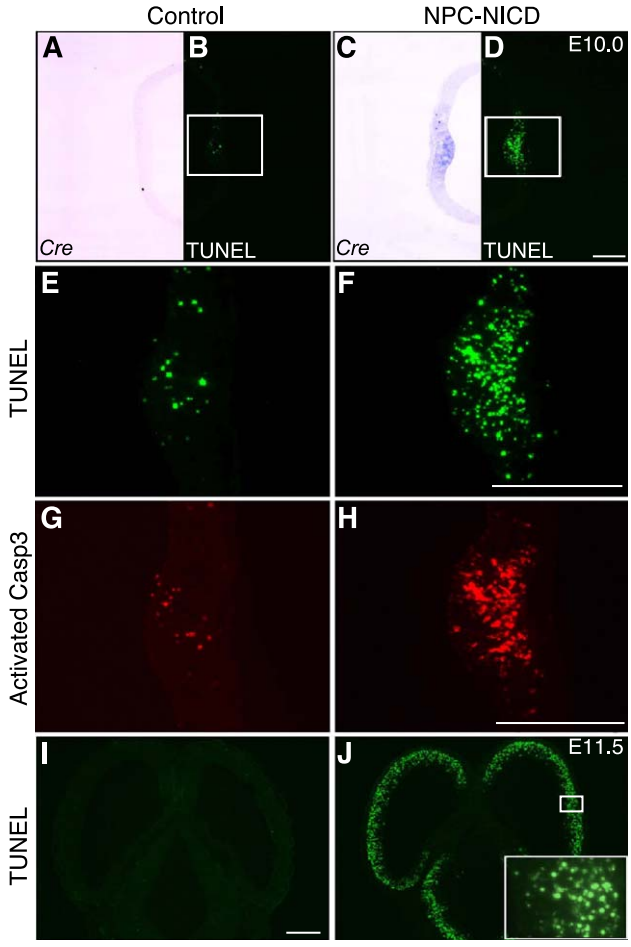

Fig. 3. Extensive apoptosis induced by NICD in early neural progenitor cells. (A–D) In situ hybridization and TUNEL staining of control and NPC-NICD brain sections at E10.0. *Cre* is expressed in the forebrain–midbrain junction of NPC-NICD mice (C), where extensive apoptosis is observed (D). (E and F) Higher power views of the boxed areas in B and D, respectively. (G and H) Increased apoptosis is also revealed by increased activated Caspase-3 immunoreactivity. (I and J) TUNEL staining of the control (I) and NPC-NICD (J) telencephalon at E11.5, when apoptosis is most severe. Scale bar: 200 μm.

mice, respectively, while *CALSL-NICD* (L) and NPC-NICD (L) mice are always specified.

Whole-mount in situ hybridization analysis of *CALSL-NICD* embryos at E9.0 and 9.5 (18 and 23 pairs of somites) using the stop cassette sequence as probe confirmed ubiquitous expression of the stop transcript driven by the chicken *β-actin* promoter (Figs. 1B and C, data not shown). The spatial and temporal pattern of Cre expression was determined by in situ hybridization analysis of *Nes-Cre* and *CALSL-NICD;Nes-Cre* mice. The *Cre* transcript was already detectable at E9.0 (18 pairs of somites) in the spinal cord and the forebrain–midbrain junction (data not shown). At E9.5 (23 pairs of somites), *Cre* expression extended to the diencephalon, mesencephalon, and metencephalon (Figs. 1D and E). By E10.0 (29 pairs of somites), *Cre* expression was evident throughout the CNS, though its expression appeared to be more restricted in the telencephalon and was highest in the forebrain–midbrain junction (area connecting the diencephalon and mesencephalon) (Figs. 1F and G). This expression pattern is consistent with a previous report in which a *lacZ* transgene was expressed under the control of the same *Nestin* promoter and regulatory sequences (Zimmerman et al., 1994). We further confirmed the excision of the "stop" cassette in the *CALSL-NICD;Nes-Cre* brain at E9.5 (23 pairs of somites) by PCR using three primers specific for the 5′intron, "stop" cassette, and NICD coding sequence (Fig. 1A, data not shown). In addition, it was demonstrated previously that *Cre*-mediated site-specific recombination occurs in neural progenitor cells localized in the ventricular zone during early embryonic development using the same *Nes-Cre* mouse crossed with a *ROSA26* reporter mouse (Graus-Porta et al., 2001).

RT-PCR analysis revealed higher levels of the *NICD* transcript in NPC-NICD mice than in NPC-NICD (L) mice at E11 (data not shown). Western analysis using an antiserum (IC) specific for the intracellular domain of Notch1 detected very low levels of NICD in NPC-NICD but not in control or NPC-NICD (L) brains, whereas levels of furin-cleaved products (p120) were similar (Fig. 1H and data not shown). We then performed immunohistochemical analysis using the IC antiserum which could detect all Notch products containing the intracellular domain (e.g., full-length, p120, NICD). The expression of the NICD protein, indicated by increased IC immunoreactivity in the nucleus of neural progenitor cells of the NPC-NICD telencephalon relative to the control, becomes clear at E11.5. In the control telencephalon, the predominant pattern of the IC immunoreactivity was perinuclear (Figs. 1I and K). The IC immunoreactivity in the nucleus, which corresponds to the endogenous NICD, was barely detectable (Fig. 1K). In the NPC-NICD telencephalon, the perinuclear immunoreactivity was similar to the control, but the IC immunoreactivity in the nucleus was slightly more intense compared to the control (Figs. 1J, L, and M), consistent with the Western result showing very low levels of NICD expressed in these mice. The specificity of the IC antiserum for Notch1 was confirmed by the greatly diminished IC immunoreactivity in neural progenitor cells deficient in Notch1, as shown in Fig. 5.

Extensive apoptosis in neural progenitor cells of NPC-NICD mice

NPC-NICD mice between E9.0 and 13.5 were obtained at the expected Mendelian ratio. Although some NPC-NICD mice died in utero, most of them survived to the perinatal stage. The brain, particularly the cerebral cortex, of NPC-NICD mice at postnatal day 0 was much smaller (Figs. 2A and B). At E12.0–13.5, it was already evident that the telencephalon of NPC-NICD mice was smaller than the control (Figs. 2C and D). Histological examination of NPC-

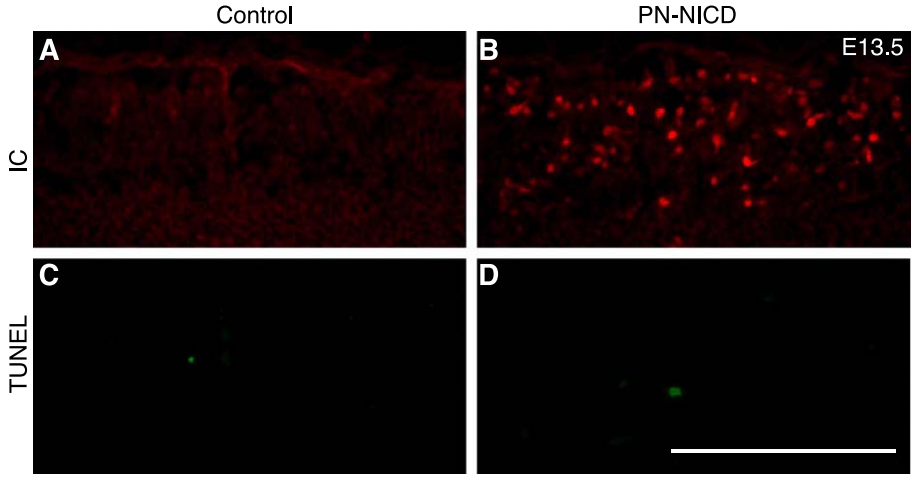

Fig. 4. Expression of NICD in postmitotic neurons does not promote apoptosis. (A and B) Immunostaining of control and PN-NICD (*CALSL-NICD;Syn-Cre*) mice at E13.5 using the IC antiserum. NICD is highly expressed in postmitotic neurons in the cortical plate of the PN-NICD telencephalon. (C and D) TUNEL staining reveals no increase in apoptosis in the PN-NICD brain. Scale bar: 200 μm.

NICD brain sections stained with hematoxylin and eosin at E12.5–13.5 revealed that all brain subregions were significantly reduced in size (Figs. 2E and F). Fewer postmitotic neurons labeled by MAP2 immunoreactivity were detected in the NPC-NICD brain at this age (data not shown). In addition, the ventricular zone harboring neural progenitor cells was thinner in the NPC-NICD brain (Figs. 2E and F, data not shown).

The presence of pyknotic nuclei shown by TOPRO-3 staining (Fig. 1L) and the reduction of neural progenitor population in NPC-NICD mice prompted us to determine whether there is an increase in apoptotic cell death. At E10.0, the TUNEL assay showed an increase in the number of apoptotic cells in the forebrain–midbrain junction of NPC-NICD mice, where *Cre* transcripts were expressed, while much lower numbers of apoptotic cells were found in the comparable region of control brains (Figs. 3A–F). The increase in apoptosis was further confirmed by an increase in the number of cells immunoreactive for activated Caspase-3 (Figs. 3G and H). The number of TUNEL-positive cells in this region of the NPC-NICD and control neuroepithelium was 95 ± 32 and 26 ± 6, respectively ($n = 3$). By E11.5, the extent of apoptosis in the telencephalon was more widespread, and TUNEL+ cells represented approximately 30% of all cells (Fig. 3J). Because the induction of apoptosis in NPC-NICD mice was robust, we reasoned that even though NICD was below detection by Western and immunohistochemical analyses in the NPC-NICD (L) line, the residual amount of NICD expressed in neural progenitor cells, which could be detected by RT-PCR, should similarly induce apoptotic cell death. Indeed, we found a 60% increase in the number of TUNEL+ cells in the telencephalon of NPC-NICD (L) mice at E11.5 (57 ± 6, $n = 3$) compared to the control (36 ± 6, $n = 3$; $P < 0.01$). These results show that even very low levels of NICD promote apoptotic cell death in neural progenitor cells. However, expression of NICD can only induce apoptotic cell death in a subset of progenitor population.

NICD does not promote apoptosis in postmitotic neurons

To determine whether the induction of apoptosis by NICD is restricted to dividing progenitor cells, we generated a second cell type-specific conditional transgenic mouse *CALSL-NICD;Syn-Cre* (PN-NICD) by breeding *CALSL-NICD* mice with *Syn-Cre* mice, in which Cre is expressed in postmitotic neurons under the control of the *Synapsin I* promoter (Zhu et al., 2001). NICD was expressed highly in postmitotic neurons of PN-NICD mice at E13.5 as visualized by the IC immunoreactivity (Figs. 4A and B). In contrast to many apoptotic cells observed in NPC-NICD mice, expression of NICD in postmitotic neurons did not lead to an increase in apoptosis (Figs. 4C and D). Similarly, expression of NICD in mature pyramidal neurons under the control of the *αCaMKII* promoter also failed to induce apoptosis (XY and JS, unpublished results). Therefore, expression of NICD alone, even at much higher levels, is insufficient to induce apoptosis. Neural progenitor cells, where Notch1 is expressed at higher levels relative to postmitotic neurons, appear to be more sensitive to alterations in NICD levels. These results demonstrate that expression of NICD selectively induces apoptotic cell death in dividing progenitor cells but not in postmitotic (immature or mature) neurons.

Attenuation of Notch signaling reduces apoptosis in early neural progenitor cells

Gain-of-function approaches used in our study and many others (Chambers et al., 2001; Furukawa et al., 2000;

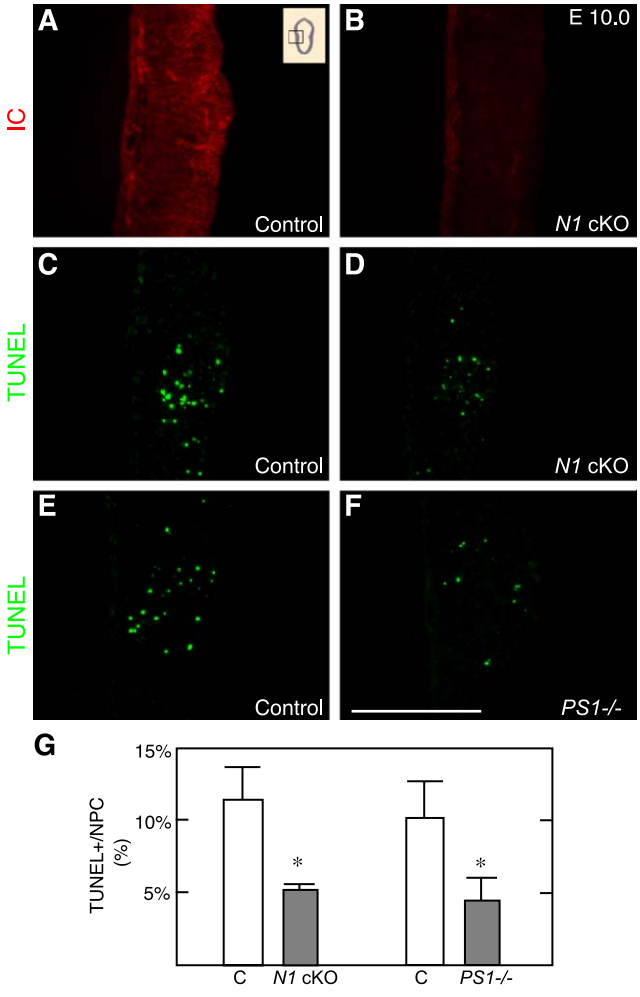

Fig. 5. Reduction of apoptotic cell death in *N1* cKO and *PS1*−/− mice at E10.0. (A and B) Immunohistochemical analysis of Notch1 expressions using IC antiserum. Notch1 protein is markedly reduced in *N1* cKO mice in the forebrain–midbrain junction at E10.0 (B). (C and D) TUNEL analysis on comparable brain sections from *N1* cKO and littermate control embryos. Apoptotic cells are enriched in the forebrain–midbrain junction (C and D). TUNEL-positive cells are greatly reduced in the *N1* cKO brain (D). (E and F) TUNEL-positive cells are also greatly reduced in the *PS1*−/− brain. (G) Quantification of the percentage of TUNEL+ cells to neural progenitor cells (NPC) in the forebrain–midbrain junction of comparable brain sections of *N1* cKO, *PS1*−/−, and control mice. Scale bar: 200 μm.

Gaiano et al., 2000; Hojo et al., 2000; Morrison et al., 2000; Scheer et al., 2001; Tanigaki et al., 2001) by forced expression of NICD have their intrinsic limitations and may reveal nonphysiological activities of Notch. To complement our gain-of-function studies and to assess the role of Notch signaling in cell death, we employed the Cre/loxP recombination system to generate a *Notch1* conditional knockout (*N1* cKO) mouse. We first generated a floxed *N1* (*fN1)* mouse in which two loxP sites were introduced to flank the first coding exon of *Notch1*. The *fN1* mouse was then bred to the *Nes-Cre* mouse to generate *N1* cKO mice. Immunohistochemical analysis at E10.0 and E11.5 revealed greatly reduced IC immunoreactivity in neural progenitor cells of *N1* cKO mice relative to control (Figs. 5A and B, data not shown).

Brains of *N1* cKO and littermate controls (*fN1*/+) were examined for cell death at E10.0. TUNEL analysis of comparable brain sections of *N1* cKO and control embryos revealed significantly reduced numbers of apoptotic cells in the forebrain–midbrain junction of *N1* cKO mice (19 ± 2) compared to control (39 ± 8, $n = 4$) (Figs. 5C and D). Because reduced Notch signaling is known to cause premature neuronal differentiation and reduction of the progenitor population (de la Pompa et al., 1997; Handler et al., 2000; Hitoshi et al., 2002; Shen et al., 1997), we sought to determine whether the reduction in the absolute number of TUNEL+ cells could be secondary to a reduction in the number of neural progenitor cells in *N1* cKO mice. We therefore determined the number of neural progenitor cells by subtracting TuJ1+ neurons from total cell numbers and then calculated the ratio of TUNEL+ apoptotic cells to TuJ1-negative neural progenitor cells. We found that the percentage of apoptotic cells relative to all progenitor cells is reduced by approximately 50% in the forebrain–midbrain junction of *N1* cKO mice (5.1 ± 0.4%, $n = 4$) relative to control (11.5 ± 2.2%, $n = 4$; $P < 0.01$) (Fig. 5G).

We previously showed that the intramembrane cleavage of Notch1 is reduced in *PS1*−/− cells (Song et al., 1999), and that Notch activity is attenuated in *PS1*−/− brains, as evidenced by downregulation of Notch downstream effector *Hes5* and upregulation of Notch ligand *Dll1* (Handler et al., 2000). TUNEL analysis of comparable brain sections of *PS1*−/− and control embryos at E10.0 revealed an approximately 50% reduction in apoptotic cells in the forebrain–midbrain junction of *PS1*−/−mice (13 ± 5, $n = 5$) relative to control (30 ± 8, $n = 6$; $P < 0.005$) (Figs. 5E and F). The percentage of apoptotic cells to progenitor cells in this region is also reduced by approximately 50% in *PS1*−/− mice (4.4 ± 1.5%, $n = 5$) relative to control (10.2 ± 2.5%, $n = 6$; $P < 0.005$) (Fig. 5G). These results are consistent with the observation showing reduced apoptosis in *N1* cKO mice, providing further evidence supporting for Notch1 serving as a key target of PS1 in the regulation of early neural development.

Although it is possible that reductions in apoptotic cell death in these loss-of-function mutant mice could result in a higher number of neural progenitor cells, which over time could affect the ratio of apoptotic cells to total progenitor cells, this is unlikely the case in the present study for the following reasons. First, we examined cell death at E10.0, immediately after the *Cre* transgene is expressed in this area (the midbrain–forebrain junction) at E9.5. Therefore, this effect should be at the minimum at this early developmental stage. Second, the absolute number of progenitor cells is quite comparable between mutant and control mice at this age, and the relatively low percentages of cell death observed (approximately 5% for mutants and approximately 10% for controls) would not be

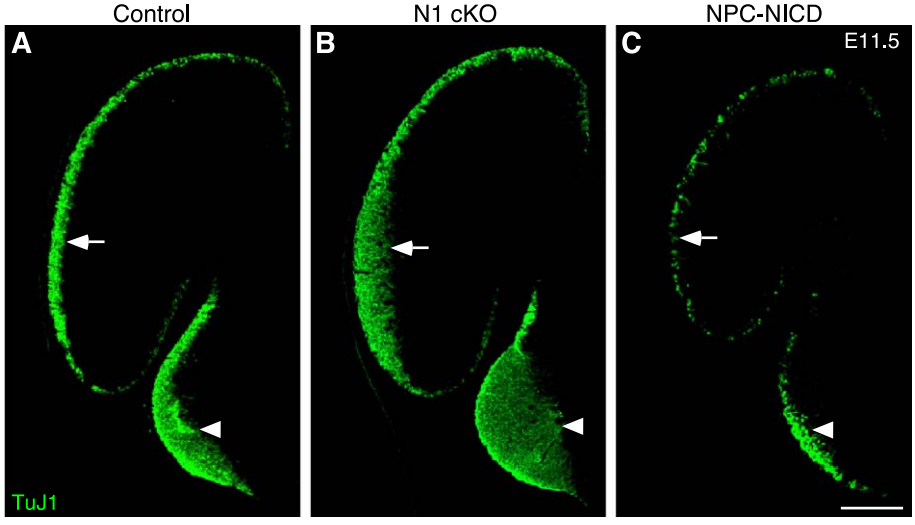

Fig. 6. Neuronal differentiation in *N1* cKO and NPC-NICD mice. (A–C) Immunohistochemical analysis on comparable brain sections from control (A), *N1* cKO (B), and NPC-NICD (C) mice using TuJ1 antibodies. (B) Increased neuronal differentiation is observed in the *N1* cKO telencephalon (arrow) and diencephalon (arrow head), while in C, reduced number of neurons is observed in the NPC-NICD telencephalon (arrow) and diencephalon (arrow head) compared to control (A). Scale bar: 200 μm.

expected to result in any substantial reduction in the overall progenitor population. Third, the magnitude of the difference in percent cell death between genotypes (approximately 50%) is quite large in comparison to the relatively low rate of cell death observed (5–10%), indicating that any minor inaccuracies in estimation of actual cell death rates would not alter our conclusions.

By E11.5, the naturally occurring apoptosis in all areas became much less frequent, and few TUNEL+ cells were detected in the control telencephalon (Fig. 3I). No significant difference in the number of TUNEL+ cells was observed in the telencephalon of control and *N1* cKO mice, consistent with our previous studies indicating that there was no significant difference in the number of apoptotic cells in the telencephalon of *PS1*−/− and littermate controls at E11.5 and 12.5 (Handler et al., 2000). More postmitotic neurons labeled by TuJ1 were found in the telencephalon and diencephalon of *N1* cKO mice (Figs. 6A and B), which is in agreement with our previous study showing that reduction of Notch activity in *PS1*−/− mice results in premature neuronal differentiation at this age (Handler et al., 2000). On the other hand, fewer TuJ1-positive differentiated neurons were present in NPC-NICD telencephalon and diencephalon (Fig. 6C), resulting from reduction of the progenitor population and possibly inhibition of neuronal differentiation by NICD.

NICD activates the p53 tumor suppression pathway

We next looked for the molecular mechanism by which constitutive activation of Notch signaling could lead to apoptotic cell death. Constitutive expression of Notch signaling, caused by a naturally occurring truncation mutation of human Notch1 homologue, has been shown to cause T cell lymphoma (Ellisen et al., 1991). Expression of oncogenes often induces apoptosis through the activation of the p53 tumor suppression pathway (Levine, 1997). We therefore examined NPC-NICD brains to determine whether levels of p53 were elevated. In contrast to very low levels of p53 in the control brain, highly elevated levels of nuclear p53 immunoreactivity were observed in the progenitor cells of NPC-NICD mice at E10.5 and E11.5 (Figs. 7A–D). Double-labeling analysis using antibodies specific for p53 and β-tubulin III showed that most, if not all, of p53+ cells did not express β-tubulin III, confirming that p53 upregulation occurred in neural progenitor cells (Fig. 7F).

p53 responds to oncogenic stimuli by inducing apoptosis through the activation of target effector genes, which include *Bax* (Miyashita and Reed, 1995) and *Noxa* (Oda et al., 2000). These gene products, which contain the BH3-domain, can bind to Bcl-2 and antagonize its function, resulting in cytochrome *c* release from mitochondria and activation of the apoptosis cascade. To confirm functional upregulation of the p53 pathway, we examined whether elevated levels of nuclear p53 in NPC-NICD mice would lead to increased expression of *Bax* and *Noxa*, which are the better known downstream targets of the p53-mediated apoptosis pathway (Vousden, 2000). Quantitative real-time RT-PCR analysis of NPC-NICD and control brains at E11.5 revealed significant increases in *Bax* (180 ± 33%, $P < 0.05$) and *Noxa* (550 ± 112%, $P < 0.005$) expression in the NPC-NICD brain (Fig. 7E). Thus, elevated levels of nuclear p53 in these mice also lead to transcriptional activation of p53-responsive apoptotic effector genes, providing a molecular mechanism for the induction of apoptosis by NICD.

p53 deficiency suppresses apoptosis induced by NICD

To determine whether NICD induces apoptosis through a p53-dependent pathway, we crossed *CALSL-NICD;Nes-Cre* mice to the *p53*−/− background (Jacks et al., 1994) and examined whether inactivation of p53 can block the extensive apoptotic cell death in NPC-NICD brains. *CALSL-*

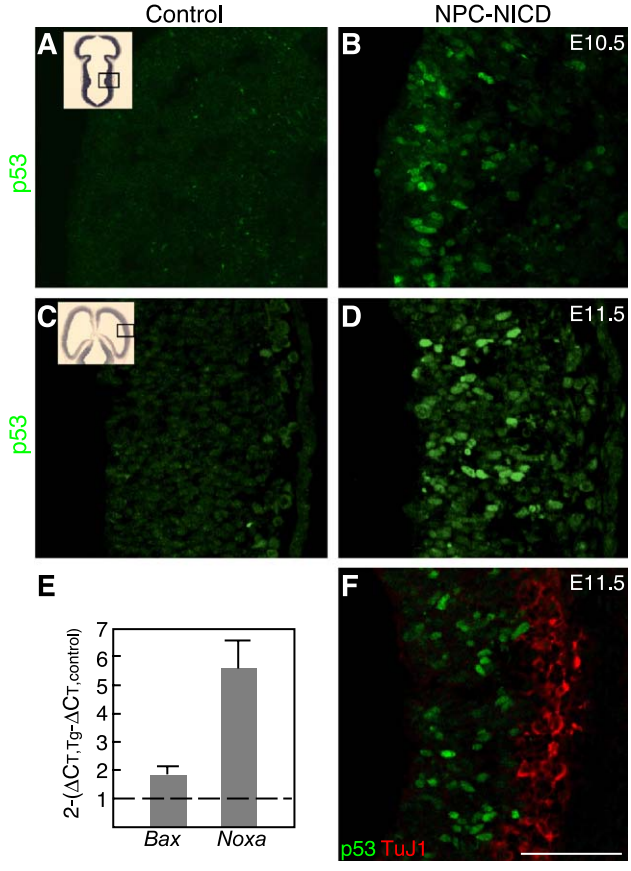

Fig. 7. Activation of the p53 pathway in NPC-NICD mice. (A–D) Immunohistochemical analysis of control and NPC-NICD brains at E10.5 and E11.5. Levels of p53 are highly elevated in the nucleus of neural progenitor cells in NPC-NICD brains in contrast to very low levels of p53 in the control. (E) Quantitative real-time RT-PCR analysis of *Bax* and *Noxa* expression at E11.5. The *y*-axis represents the fold change of mRNA levels in NPC-NICD brains relative to the control. Expression of *Bax* and *Noxa* is increased (approximately 2- and 6-fold, respectively) in NPC-NICD brains ($n = 3–5$). (F) Confocal microscopy analysis of p53+ cells (green) and newly generated postmitotic neurons labeled by TuJ1 (red) in NPC-NICD mice. Most, if not all, p53+ cells are immunonegative for TuJ1. Scale bar: 100 μm.

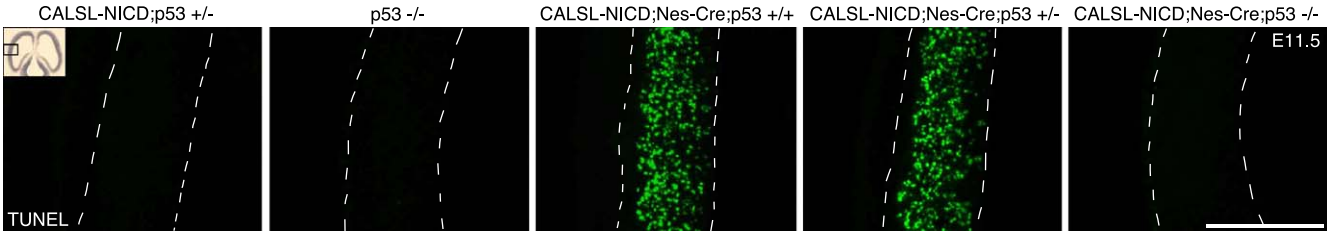

Fig. 8. p53 deficiency suppresses apoptotic cell death in NPC-NICD mice. TUNEL analysis at E11.5 reveals extensive apoptotic cell death in the telencephalon of *CALSL-NICD;Nes-Cre* and *CALSL-NICD;Nes-Cre;p53*+/− mice. In contrast, few apoptotic cells are detected in the telencephalon of *CALSL-NICD;Nes-Cre;p53*−/−, *p53*−/−, and *CALSL-NICD;p53*+/− mice. Scale bar: 200 μm.

NICD;Nes-Cre;p53−/− mice were obtained from the cross between *CALSL-NICD;p53*+/− and *Nes-Cre;p53*+/− mice at a frequency lower than the expected Mendelian ratio (1/16) due to the incomplete penetrance of early embryonic lethality associated with *p53*−/− deficiency (Armstrong et al., 1995). TUNEL analysis of *CALSL-NICD;Nes-Cre;p53*−/− mice at E10.5–11.5 revealed that the dramatic increase in apoptotic cells observed in *CALSL-NICD;Nes-Cre* brains was completely blocked by inactivation of p53 in these mice (Fig. 8). Removal of one *p53* allele in *CALSL-NICD;Nes-Cre;p53*+/− brains, however, appeared to have no effect on the suppression of the extensive apoptosis. These results show that NICD induces apoptosis in neural progenitor cells via the p53-dependent pathway.

Discussion

Notch1 regulates apoptotic cell death in mammalian early neural progenitor cells

Notch has been reported to regulate apoptosis in interommatidial precursor cells of the developing retina (Cagan and Ready, 1989; Miller and Cagan, 1998) and sensory organ primary precursor cells (Orgogozo et al., 2002) during *Drosophila* development. Similarly, Notch activation causes retinal cells to enter apoptosis in the zebrafish retina (Scheer et al., 2001). However, it was unknown whether Notch signaling is involved in the regulation of apoptotic cell death during mammalian neural development, in addition to its well-established roles in neurogenesis (Schuurmans and Guillemot, 2002). We therefore used unbiased genetic approaches to address this important question because modulation of apoptosis contributes to the final determination of the brain size and its shape (Kuan et al., 2000).

In the present study, we employed complementary conditional transgenic and knockout approaches to determine the effects of up- or downregulation of Notch signaling on apoptotic cell death in neural progenitor cells. We show that expression of even very low levels of NICD in early neural progenitors induces extensive apoptosis and results in a markedly reduced progenitor population and ultimately a smaller brain (Figs. 2 and 3). The promotion of cell death by NICD is restricted to a narrow temporal window: its expression in immature or mature neurons even at much higher levels in *CALSL-NICD;Syn-Cre*, *αCaMKII-NICD*, or *CALSL-NICD;αCaMKII-Cre* mice fails to promote cell death (Fig. 4, XY and JS, unpublished results). These results are consistent with previous findings showing that NICD induces extensive apoptosis in the zebrafish retina, particularly when expressed at an early stage (Scheer et al., 2001), demonstrating a conserved pro-apoptotic role of Notch activation in neural progenitor cells in vertebrates.

However, the gain-of-function approach widely used in Notch function studies has its intrinsic limitations and may reveal nonphysiological functions. We therefore generated and analyzed *Notch1* conditional KO mice in which Notch1 inactivation is restricted to neural progenitor cells. We found that attenuation of Notch activity in *N1* cKO and *PS1*−/− mice results in a marked (approximately 50%) reduction in the apoptotic death of early neural progenitor cells at E10 (Fig. 5). These results corroborate our findings from NPC-NICD mice, supporting a physiological role of Notch signaling in the regulation of apoptotic cell death during early mammalian neural development.

As seen in NPC-NICD mice, loss of Notch1 function also influences cell death in neural progenitors in a narrow temporal window (narrower than NICD): while compared to the control, the number of apoptotic cells in *N1* cKO or *PS1*−/− mice is reduced at E10, but unchanged at E11.5. Combined, our results indicate that Notch1 activity selectively affects apoptotic cell death in early neural progenitors. One explanation for this observation is that under normal physiological conditions, early neural progenitor cells at E10 are more sensitive than progenitor cells at E11.5 to cell death-promoting signals. Precedents for such context-dependent protection from apoptotic cell death promoted by Notch signaling have been reported in the *Drosophila* interommatidial lattice (Cagan and Ready, 1989; Miller and Cagan, 1998).

NICD induces apoptosis in neural progenitor cells via a p53-dependent pathway

The increased frequency of apoptotic cell death in NPC-NICD mice allowed us to probe the underlying molecular pathways. Previous studies in *Drosophila* (Milan et al., 2002; Miller and Cagan, 1998; Orgogozo et al., 2002) and

mammalian developing B cells (Morimura et al., 2000) did not explore the involvement of p53 pathway in cell death induced by Notch. Here, we show that a p53-dependent pathway is activated in neural progenitor cells in association with the extensive apoptotic cell death induced by Notch activation. This is evidenced by the markedly increased immunoreactivity of p53 in the nucleus of neural progenitor cells in NPC-NICD mice (Fig. 7). Further, we observed significant transcriptional upregulation of *Bax* and *Noxa*, two major genes mediating p53-dependent apoptotic cell death (Fig. 7), although the direct link between p53 and Bax–Noxa is not shown in the present study. Genetic rescue experiment unequivocally demonstrates that p53 deficiency is sufficient to suppress the increased cell death phenotype caused by Notch activation (Fig. 8).

These results agree with the current view that p53 family members participate in embryonic development in mice (Yang et al., 2002), in addition to their role in tumor suppression in adult tissues. Mice deficient for *p63*, a homologue of *p53*, lack limbs and a wide range of epithelial structures including skin, prostate, breast, and urothelia (Mills et al., 1999; Yang et al., 1999). Inactivation of another *p53* homologue, *p73*, results in congenital hydrocephalus and hippocampal dysgenesis, presumably due to disappearance of Cajal-Retzius neurons (Yang et al., 2000). *p53* and its downstream target *Bax* have been shown to be highly expressed and colocalize in the ventricular and subventricular zone at embryonic and early postnatal stages in mice (van Lookeren Campagne and Gill, 1998). *p53*−/− mice have an incomplete penetrant phenotype: although some survive into adulthood, approximately 40% of the embryos display severe defects in neural tube closure (Armstrong et al., 1995; Sah et al., 1995), suggesting that other family members, such as p63 and p73, may compensate for the lack of p53 in the proper development of the nervous system.

It is not yet clear how Notch triggers p53 activation and the downstream apoptotic cascades in neural progenitor cells. Several possibilities exist. First, a constitutively active form of Notch1 can act as an oncogene in neural progenitor cells, as reported in nonneural cell types (Ellisen et al., 1991; Jhappan et al., 1992). We did observe an increase in proliferation at E11.5 in *NPC-NICD; p53*−/− (55.0 ± 4.5%) compared to *CALSL-NICD; p53*−/− mice (44.3 ± 2.0%, $P < 0.05$), as measured by the ratio of BrdU-labeled cells to all progenitor cells. The proliferation rate of the dying p53+ progenitor cells (BrdU+/p53+) is also higher than that of live progenitor cells (BrdU+/Ki67+). These observations are consistent with our hypothesis that Notch activation increases proliferation and results in apoptotic cell death in a subset of progenitor cells. Second, Notch may act as a specification signal for cell death. However, the programmed cell death induced by Notch activation is only limited to a subpopulation of neural progenitor cells (approximately 30% in the NPC-NICD telencephalon at E11.5). Further, Notch activation in other cell types, such as postmitotic neurons (this study), cultured adult neural progenitor cells (Tanigaki et al., 2001), or neural crest stem cells (Morrison et al., 2000), does not lead to increased cell death. Therefore, expression of NICD per se appears insufficient to induce apoptosis. Third, in NPC-NICD mice, we observed ectopically expressed *NeuroD* transcripts distributed in the forebrain–midbrain junction at E10.0 and in the ventricular zone of the telencephalon at E11.5 (XY and JS, unpublished results). However, we did not observe any increase in terminally differentiated neurons labeled by TuJ1 or MAP2 (Fig. 6, data not shown). It is therefore possible that higher levels of NICD in NPC-NICD mice may prevent these *NeuroD*-expressing cells from completion of differentiation and trigger the apoptosis cascade. Finally, Notch activation may also act in opposition of trophic or survival factors that are essential at early developmental stages (E10) but become redundant at later stages (E11.5). It has been shown that the pro-apoptotic role of Notch blocks EGFR-dependent survival signaling in the *Drosophila* retina (Miller and Cagan, 1998; Yu et al., 2002). In conclusion, although the exact mechanism is not yet completely clear, Notch activation induces apoptosis in a subset of neural progenitor cells by activation of the p53 pathway.

Notch1 regulates mammalian neural development in a context-dependent manner

Our observation that Notch activation promotes cell death in neural progenitor cells appears to be at odds with one previous study showing that expression of NICD promotes radial gliogenesis (Gaiano et al., 2000). In both studies, NICD is expressed in telencephalic neural progenitor cells beginning at E9, although the methodologies by which NICD was introduced differ. Our genetic approach allows expression of NICD in most, if not all, progenitor cells, while their retroviral infection approach limits NICD expression to a small percentage of the progenitor cells. In addition, their use of a reporter gene to identify infected cells essentially restricts subsequent analysis to surviving cells, selecting a subset cell population that does not respond to Notch activation with cell death. Therefore, it is likely that the cell death induced by NICD was unintentionally overlooked in the infection experiment, but was better revealed by our genetic experiment. Furthermore, our present genetic study is in agreement with prior genetic studies in *Drosophila* and zebrafish, all showing that Notch signaling regulates programmed cell death during development (Cagan and Ready, 1989; Miller and Cagan, 1998; Orgogozo et al., 2002; Scheer et al., 2001).

Although our transgenic approach is advantageous in revealing apoptotic cell death, the resulting depletion of neural progenitor cells makes it difficult to determine conclusively the effects of NICD expression on neuronal differentiation. Expression of NICD in NPC-NICD mice results in a reduction in postmitotic neurons (Fig. 6C),

which could be explained by depletion of progenitor cells caused by increased apoptosis or suppression of neuronal differentiation. Due to the very early expression of NICD at the onset of neurogenesis, NPC-NICD mice are not good models for the study of the effect of Notch activation on gliogenesis. Although introduction of NICD into other types of progenitors by retroviral vectors has been shown to promote gliogenesis (Furukawa et al., 2000; Morrison et al., 2000; Tanigaki et al., 2001), the expression of NICD in our study and these previous reports differs in cell type (early neural progenitors of the developing brain versus other progenitor types such as neural crest cells of the PNS), timing (E9.5 versus later stages), or extent (all progenitors versus a small percentage of progenitors). Because Notch can activate the *GFAP* promoter and promote astroglial fates only during the gliogenesis phase (Ge et al., 2002; Tanigaki et al., 2001), it is not surprising that we did not observe an increase in radial glia or astrocytes in NPC-NICD mice during the neurogenesis phase (XY and JS, unpublished results). At later developmental stages, the extensive loss of the progenitor cells makes it too difficult to determine the impact of NICD on gliogenesis. Theoretically, the role of Notch1 in neurogenesis and gliogenesis can be studied in NPC-NICD mice in the *p53*−/− background in which cell death is suppressed. However, *p53*−/− embryos display a variety of neurodevelopmental defects with incomplete penetrance (Armstrong et al., 1995; Sah et al., 1995), rendering it difficult to discern the phenotypes caused by Notch activation alone.

In summary, Notch signaling regulates neurogenesis, gliogenesis, and apoptotic cell death in a highly context-dependent manner. Notch activation consistently suppresses neurogenesis and promotes alternative cell fates by maintenance of the progenitor state, promotion of glial cell types, or induction of apoptotic cell death in a manner dependent upon the spatial and temporal context. Thus, our results provide an important addition to the previously described function of Notch signaling in the suppression of terminal neuronal differentiation during the neurogenesis phase and promotion of terminal glial differentiation during the gliogenesis phase, demonstrating a crucial role for Notch in the regulation of apoptosis in early neural progenitors through a p53-dependent mechanism.

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