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Genetic interaction between DNA polymerase β and DNA-PKcs in embryogenesis and neurogenesis

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Abstract

DNA polymerase β (Polβ) has been implicated in base excision repair. Polβ knockout mice exhibit apoptosis in postmitotic neuronal cells and die at birth. Also, mice deficient in nonhomologous end-joining (NHEJ), a major pathway for DNA double-strand break repair, cause massive neuronal apoptosis. Severe combined immunodeficiency (SCID) mice have a mutation in the gene encoding DNA-dependent protein kinase catalytic subunit (DNA-PKcs), the component of NHEJ, and exhibit defective lymphogenesis. To study the interaction between Polβ and DNA-PKcs, we generated mice doubly deficient in Polβ and DNA-PKcs. Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ embryos displayed greater developmental delay, more extensive neuronal apoptosis, and earlier lethality than Pol$\beta^{-/-}$ and DNA-PKcs$^{scid/scid}$ embryos. Furthermore, to study the involvement of p53 in the phenotype, we generated Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$p53$^{-/-}$ triple-mutant mice. The mutants did not exhibit apoptosis but were lethal with defective neurulation at midgestation. These results suggest a genetic interaction between Polβ and DNA-PKcs in embryogenesis and neurogenesis.

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Keywords: apoptosis; DNA polymerase β; knockout mouse; neural development; SCID

Abbreviations: BER, base excision repair; CNS, central nervous system; DNA-PK, DNA-dependent protein kinase; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; DSBs, double-strand breaks; E, embryonic day; NHEJ, nonhomologous end-joining; PNS, peripheral nervous system; Polβ, DNA polymerase β; PPL, primordial plexiform layer; SCID (DNA-PKcs$^{scid/scid}$), severe combined immunodeficiency; SSBs, single-strand breaks; VZ, ventricular zone

Introduction

The genome is continuously damaged by a variety of endogenous and exogenous agents. Repair of such damage is a crucial mechanism for maintaining genomic integrity. A failure in faithful repair causes mutations with an increased risk of cancer. Multicellular animals have an additional mechanism for eliminating damaged cells called apoptosis. DNA polymerase β (Polβ) is a key factor in base excision repair (BER),[1,2] which is the major pathway for the repair of small lesions such as apurinic/apyrimidinic (AP) sites and oxidized or alkylated bases. In fact, Polβ-deficient cells are clearly more sensitive than wild-type cells to DNA alkylating agents[3] and hydrogen peroxide.[4] Polβ-null fibroblasts survive under culture conditions,[3] suggesting that Polβ is not essential for all cell types. On the other hand, Polβ knockout mice die immediately after birth.[5–7] We reported that the mutant mice exhibit extensive apoptosis in newly generated postmitotic neuronal cells in the central nervous system (CNS) and peripheral nervous system (PNS). In neurogenesis of the cerebral cortex,[8] neuronal progenitor cells initiate DNA replication and cell division in the ventricular zone (VZ). After mitosis, immature neuronal cells migrate through the intermediate zone (IZ) and the primordial plexiform layer (PPL), and become mature neurons in the cortical plate. In Polβ-null mice, abnormally increased numbers of neuronal apoptotic cells are detected in E12.5–E14.5 PPL (where neurogenesis in the cortex peaks) but disappear almost completely at E18.5, following completion of neurogenesis. Polβ expression is known to be high in brain, thymus and testis.[9,10] However, in Polβ-deficient embryos,[7] development of tissues other than the nervous systems appears normal. These observations indicate that Polβ plays an important role in neural development. So far, some knockout mice defective in BER factors have been generated; mice deficient in FEN1,[11] APE,[12] XRCC1[13] and DNA ligase I (LigI)[14] are all embryonic lethal at E3.5–E16.5. These findings clearly indicate that BER plays critical roles in development.

As in Polβ-deficient mice, neuronal apoptosis has been observed in mice deficient in factors for nonhomologous end-joining (NHEJ), the major pathway that repairs DNA double-strand breaks (DSBs) in mammalian cells and is essential for V(D)J recombination.[15] NHEJ relies on DNA-dependent protein kinase consisting of three subunit proteins Ku70, Ku80 and DNA-PKcs, together with Artemis, XRCC4 and DNA ligase IV (LigIV).[16] Mice null for XRCC4[17] or LigIV[18] undergo massive neuronal apoptosis, resulting in embryonic lethality around E14.5.[17,19] Ku70[20] or Ku80[21] null mice exhibit similar but less increased apoptotic cells between the VZ and PPL in E12.5–E14.5 cerebral cortex but are viable. Severe combined immunodeficiency (SCID) is known to result from a nonsense mutation that truncates the C-terminal region of DNA-PKcs protein homologous to phosphatidylinositol 3 kinase (PI3K).[22,23] The kinase activity of the DNA-PKcs protein in SCID mice is lost, but can still form a complex with

Ku protein and bind to DSBs. DNA-PKcs$^{scid/scid}$ mice fail to develop mature T and B lymphocytes owing to impaired V(D)J recombination, but are viable and normal in body size.[24,25] The mutant mice also exhibit slightly elevated neuronal apoptosis between the VZ and PPL at E14.5.[26,27] Taken together, it is evident that apoptosis occurs in early postmitotic, immature neurons and that NHEJ plays a crucial role in neurogenesis.

Recently, a variety of interactions between factors involved in different repair pathways and cell cycle checkpoints have been reported.[28–31] For example, mice defective in ataxia telangiectasia mutated (ATM) that controls cell cycle checkpoints in response to DSBs[32] are viable, but ATM$^{-/-}$DNA-PKcs$^{scid/scid}$ mice are lethal around E11.5,[33] suggesting functional interactions between the two proteins. We observed a similar interaction between Polβ and ATM by generating their double-mutant mice (Sugo *et al*., unpublished data). Although the similarity of Polβ-deficient mice and mice deficient in NHEJ proteins in neurogenesis is clear,[7,19,34,35] there is no evidence for potential interaction between Polβ and DNA-PKcs. Hence, to explore this, we generated mice defective in both Polβ and DNA-PKcs. The resulting double-mutant mice exhibited greater developmental delay, more extensive neuronal apoptosis and earlier lethality than mice with either single defect. We also studied the involvement of p53 in the phenotype by generating triple-mutant mice deficient in Polβ, DNA-PKcs, and p53. Neuronal apoptosis was found to be rescued by p53 deficiency, indicating dependency on the p53 pathway, but the lethality was not rescued. We suggest a genetic interaction between Polβ and DNA-PKcs in embryonic development and neurogenesis.

Results

Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ mice are embryonic lethal earlier than Pol$\beta^{-/-}$DNA-PKcs$^{+/+}$ mice

To assess the effect of Polβ and DNA-PKcs deficiency on embryogenesis, we first bred Pol$\beta^{+/-}$ mice with SCID (DNA-PKcs$^{scid/scid}$) mice. The resulting Pol$\beta^{+/-}$DNA-PKcs$^{+/scid}$ mice were intercrossed to obtain Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ mice. Among the offspring, Pol$\beta^{+/+}$DNA-PKcs$^{+/scid}$, Pol$\beta^{+/-}$DNA-PKcs$^{+/+}$ and Pol$\beta^{+/-}$DNA-PKcs$^{+/scid}$ mice developed normally into adulthood. Pol$\beta^{+/-}$DNA-PKcs$^{scid/scid}$ mice developed normally, similar to Pol$\beta^{+/+}$DNA-PKcs$^{scid/scid}$ mice. Pol$\beta^{-/-}$DNA-PKcs$^{scid/+}$ mice, like Pol$\beta^{-/-}$DNA-PKcs$^{+/+}$ mice, died immediately after birth as described previously,[7] and these mice were born at ratios close to Mendelian law (Table 1). In contrast, Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ double-mutant mice were represented at E11.5 but not at E12.5 (Table 1). The double-mutant embryos exhibited a profound developmental delay that was clearly evident by E9.5 (compare Figure 1a with g), and looked like E8.5

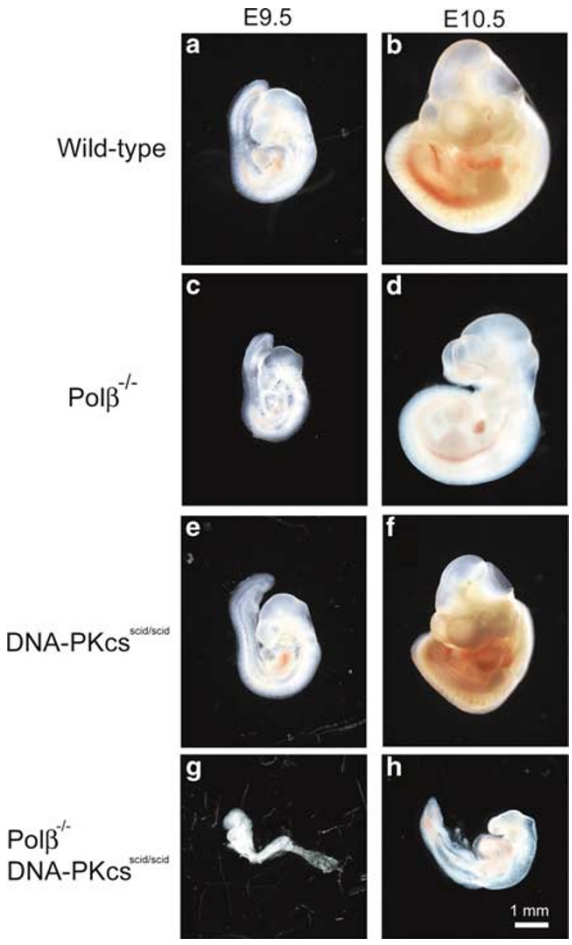

Figure 1 Lateral view of E9.5 and E10.5 embryos with different genotypes. Note that Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ mutant mice (**g**, **h**) display severe developmental delay

Table 1 Embryonic lethality of Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ mice

		Genotype								
		Pol$\beta^{+/+}$			Pol$\beta^{+/-}$			Pol$\beta^{-/-}$		
Stage	Total no. of litters (no. of embryos)	+/+	+/scid	scid/scid	+/+	+/scid	scid/scid	+/+	+/scid	scid/scid
E9.5	15 (89)	5	14	5	12	26	11	5	7	4
E10.5	22 (172)	12	23	12	19	45	21	11	16	13
E11.5	6 (48)	5	0	9	6	7	7	6	3	5
E12.5	8 (44)	2	4	10	2	10	9	1	6	0
P0	16 (116)	17	18	9	16	28	19	2	7	0
3 weeks	20 (135)	19	23	14	18	38	23	0	0	0

At stages E9.5, E10.5, P0 and 3 weeks is shown the number of offspring from the intercrosses of Pol$\beta^{+/-}$DNA-PKcs$^{+/scid}$ mice. At stages E11.5 and E12.5, the number of offspring from the intercrosses of Pol$\beta^{+/-}$DNA-PKcs$^{scid/scid}$ mice is added to the above number at the same stage.

wild-type embryos (data not shown). Similarly, E10.5 double-mutant embryos looked like wild-type controls at E9.0, although any specific malformations were not detected. At E11.5, double-mutant mice were resorbed *in utero* (data not shown). The developmental delay was not observed in either Pol$\beta^{-/-}$ (Figure 1c, d) or DNA-PKcs$^{scid/scid}$ (Figure 1e, f) mice, although both the single-mutant embryos were slightly smaller relative to the wild-type, as reported.[7,36] These results suggest that Polβ and DNA-PKcs play an overlapping role that is important for embryogenesis.

Neuronal apoptotic cells increase in E10.5 Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ mice

We postulated that the lethality of double-mutant mice might be attributed to defective neuronal development. Important events in the development of mouse embryos at E9.5 are neurulation and migration of neural crest cells.[37] At this stage, wild-type, Pol$\beta^{-/-}$DNA-PKcs$^{+/+}$ and Pol$\beta^{+/+}$DNA-PKcs$^{scid/scid}$ mice have completed neurulation (Figure 2a, c, e). However, in double-mutant mice it was delayed until E10.5 (compare Figure 2a, b with g, h). In previous studies, E12.5–E16.5 Pol$\beta^{-/-}$DNA-PKcs$^{+/+}$ mice exhibited extensive neuronal apoptosis, whereas E14.5 Pol$\beta^{+/+}$DNA-PKcs$^{scid/scid}$ mice did mild apoptosis.[7,26] To examine whether similar apoptosis occurred in mice bred in this study, we performed immunohistochemical analyses on sections of the nervous system. We used anti-cleaved caspase-3 antibody to detect apoptosis and antineuron-specific type-III β-tubulin antibody to detect neuronal differentiation. In wild-type, Pol$\beta^{-/-}$ DNA-PKcs$^{+/+}$, and Pol$\beta^{+/+}$DNA-PKcs$^{scid/scid}$ mice at E9.5–E10.5, apoptotic cells stained positive with anti-cleaved caspase-3 antibody were detected (Figure 3a–l, green); these cells were also positive for β-tubulin staining and were detected in neural crest cells developing into trigeminal ganglions (Figure 3a–l, red). Similarly, in Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ mice at E9.5, a few neural crest cells were detected at the junction between the roof plate of neural tube and surface ectoderm (Figure 3m, inset); these cells migrated to neural crest tissues. However, at this stage, neuronal apoptotic cells were scarcely observed (Figure 3m, n). Strikingly, in E10.5 double-mutant mice, neuronal apoptotic cells markedly increased compared with either Pol$\beta^{-/-}$ DNA-PKcs$^{+/+}$ or Pol$\beta^{+/+}$DNA-PKcs$^{scid/scid}$ mice (Figure 3h, l, p). We counted cells stained with both cleaved caspase-3 and type-III β-tubulin antibodies in E10.5 trigeminal ganglions and found that the number of neuronal apoptotic cells in the double mutant mice was 2.5-fold greater than the sum of those observed in both single-mutant mice. In addition, these apoptotic cells obviously increased during E9.5–E10.5. In Polβ-deficient,[7] SCID[19,26] or NHEJ-deficient mice,[17,19,38,39] neuronal apoptosis has been observed in the CNS of E12.5–E16.5 mice. In this study, however, we found that in Pol$\beta^{-/-}$ DNA-PKcs$^{+/+}$ and Pol$\beta^{+/+}$DNA-PKcs$^{scid/scid}$ mice, a fraction of neuronal cells in the PNS undergoes apoptosis at earlier stages. We also found that more extensive apoptosis of neuronal cells occurs in the PNS of Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ mice, indicating a synergistic effect of Polβ and DNA-PKcs mutations on neuronal apoptosis.

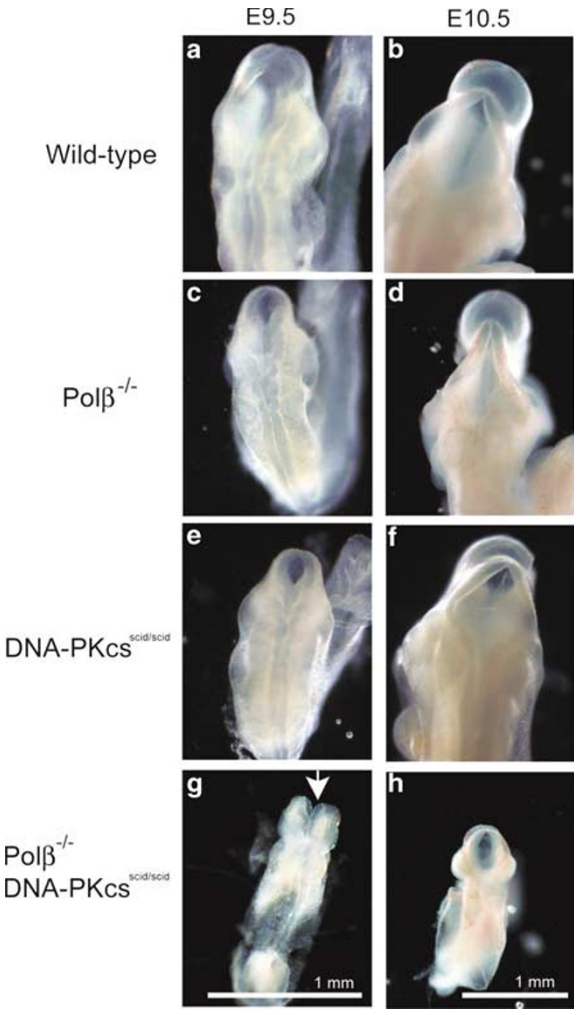

Figure 2 Dorsal view of E9.5 and E10.5 embryos with different genotypes during neurulation. Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ mice (**g**, **h**) display retardation of neurulation. The arrow in **g** represents disclosure of neural tube in E9.5 Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ mice

p53 deficiency rescues neural apoptosis but not lethality in Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ mice

We observed that apoptosis associated with Polβ deficiency is mediated by the p53-dependent apoptosis pathway in the nervous system.[40] In SCID mice, there is no report on whether p53 deficiency rescues apoptosis in the nervous system. It is controversial whether DNA-PKcs is an upstream mediator of the p53 response to DNA damage.[41] To clarify whether neuronal apoptosis in Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ mice occurred via the p53-dependent pathway, we examined stabilization (and/or activation) of p53 protein in the region of trigeminal ganglion by immunohistochemical analysis. In wild-type, Pol$\beta^{-/-}$DNA-PKcs$^{+/+}$ or Pol$\beta^{+/+}$DNA-PKcs$^{scid/scid}$ mice at E9.5 and E10.5, a small number of p53-stained cells were observed (Figure 4a–c, e–g). In E9.5 Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ mice, we detected a larger number of cells (with higher p53 levels) than those in the wild type and both single-mutant mice (Figure 4d). However, as compared with the E9.5 double-mutant mice, p53-stained cells were

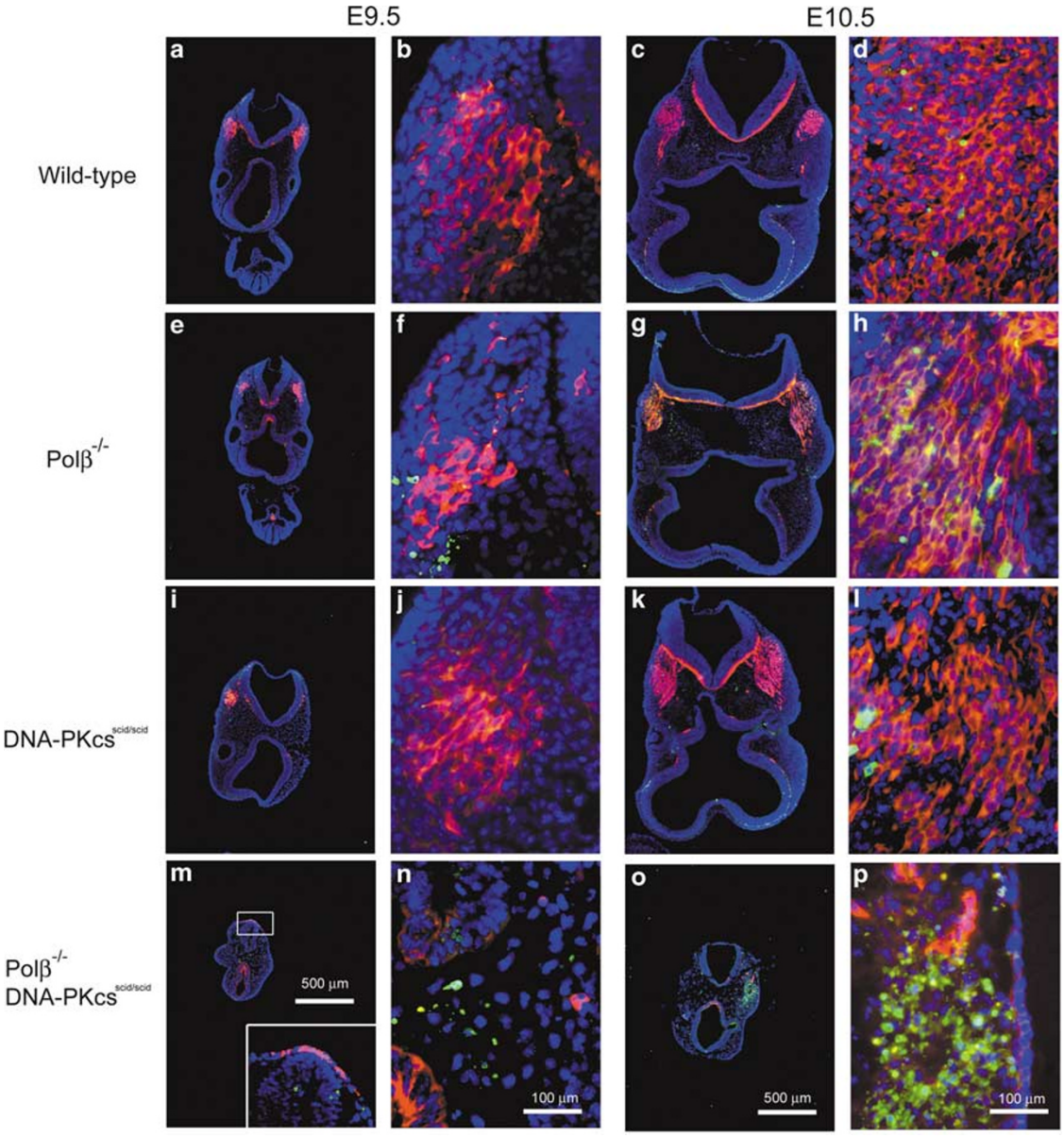

Figure 3 Immunohistochemical analysis of transverse sections in the region of trigeminal ganglions with different genotypes, using mouse anti neuron-specific type-III β-tubulin (red) antibody and rabbit anti-cleaved caspase-3 (green) antibody. Note that extensive neuronal apoptosis occurs in E10.5 Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ mice (**o**–**p**). Magnifications are: **a**, **c**, **e**, **g**, **i**, **k**, **m**, **o**, $\times 4$; **b**, **d**, **f**, **h**, **j**, **l**, **n**, **p**, $\times 40$

obviously decreased in E10.5 mice (Figure 4h *versus* d). It appears that the higher levels of p53 seen in the E9.5 mice were followed by increased apoptosis in E10.5 neural crest cells (Figure 3o, p). These results suggest that the neuronal apoptosis in Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ mice depends on the p53 pathway that is activated in response to DNA damage.

Recently, we and others have shown that p53 deficiency rescues neuronal apoptosis in Pol$\beta^{-/-}$, LigIV$^{-/-}$ and XRCC4$^{-/-}$ mice.[38,40,42] Furthermore, p53 deficiency is able to rescue lethality of the LigIV$^{-/-}$ and XRCC4$^{-/-}$ mice, concomitant with the disappearance of apoptosis. These results indicate that p53 is a key factor for neuronal apoptosis responsive to DNA DSBs. In contrast, p53 deficiency cannot rescue lethality of Polβ-deficient mice,[40] suggesting that Polβ is a critical factor for neurogenesis. To examine whether p53 deficiency rescued apoptosis and lethality observed in Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ double-mutant mice, we generated Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$p53$^{-/-}$ triple-mutant mice. At E9.5, triple-mutant mice showed normal morphology (Figure 5a, b; compare with wild-type views in Figures 1a and 2a). However, at E10.5 the mutant mice displayed markedly abnormal morphology characterized by defects in neural tube closure, like exencephaly (Figure 5d, e; compare with wild-type views in Figures 1b and 2b). Importantly, the mutant mice (Figure 5c, f) exhibited markedly reduced neuronal apoptosis

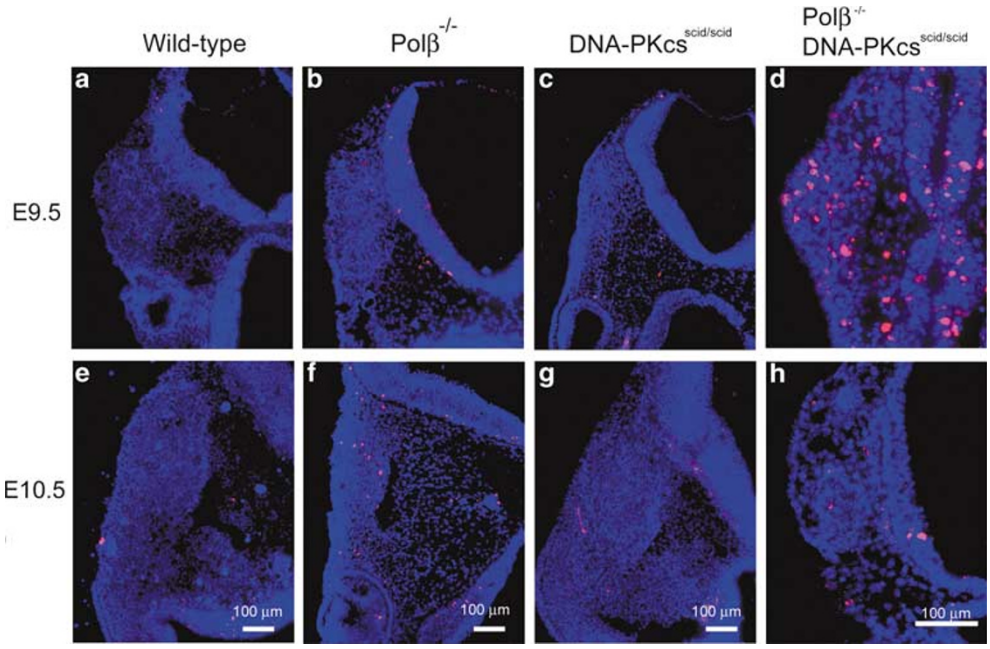

Figure 4 Immunohistochemical analysis of transverse sections in the region of trigeminal ganglions with different genotypes, using rabbit anti-p53 antibody (red). Magnifications are: **a**–**c**, **e**–**g**, × 10; **d**, **h**, × 20

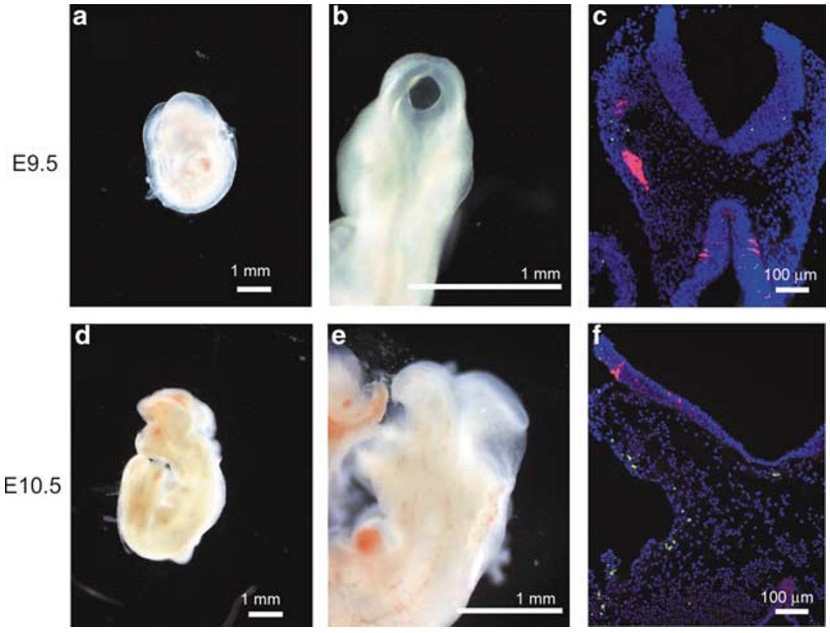

Figure 5 Lateral (**a**, **d**) and dorsal (**b**, **e**) view of Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$p53$^{-/-}$ mice at E9.5 and E10.5. (**c**, **f**) Immunohistochemical analysis of transverse sections in the region of trigeminal ganglion of the triple-mutant mice, using anti neuron-specific type-III β-tubulin antibody (red) and anti-cleaved caspase-3 antibody (green). Magnifications are: **c**, **f**, × 10

compared with Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ mice (Figure 3m–p), as judged by staining with anti cleaved caspase-3 antibody. The triple-mutant embryos stopped development around E10.5 and could not be seen at E12.5. These results indicate that both Polβ and DNA-PKcs are indispensable for the process of neural tube formation via the p53-dependent pathway.

Discussion

In this study, we have generated mice doubly deficient in Polβ and DNA-PKcs. Double-mutant mice exhibited more extensive neuronal apoptosis than did wild-type, Pol$\beta^{-/-}$, or SCID mice (Figure 3). Double-mutant mice were lethal at an earlier

embryonic stage (E11.5) than Pol$\beta^{-/-}$ mice (which die at birth). These results suggest a genetic interaction between Polβ and DNA-PKcs during neurogenesis and embryonic development. This might indicate a cooperation of the BER and NHEJ pathways in mammalian cells, although further studies will be required to prove the view.

Polβ is a key enzyme in the BER pathway.[1,2] A modified base produced by various agents is first removed by a damage-specific DNA *N*-glycosylase to leave an AP site (this site also arises from spontaneous loss of a base). The 5′ side of the AP site is cleaved by APE generating a 5′-deoxyribose phosphate (5′-dRP) residue. Polβ excises the 5′-dRP residue by its 5′-dRP lyase activity and fills one nucleotide in the gap, followed by ligation with LigI or the DNA ligase III/XRCC1 complex (short-patch BER). Alternatively, the 5′-dRP is excised by FEN1; the resulting gap of a few nucleotides is filled in by Polδ/ε (along with or without Polβ) followed by ligation with LigI (long-patch BER). The Polβ-dependent, short-patch BER is dominant and proceeds so rapidly that accumulation of unrepaired BER intermediates (mostly DNA single-strand breaks (SSBs)) is prevented. However, in Polβ-deficient cells, loss of Polβ would lead to the accumulation of these intermediates.[43,44] As suggested previously,[7] this may be the cause of the defective phenotypes including neuronal apoptosis and lethality observed in Polβ-deficient mice. Mice deficient in NHEJ factors XRCC4[17] and LigIV[18] exhibit similar but more severe apoptosis phenotypes and even earlier embryonic lethality (E14.5). In contrast, mice defective in either of the DNA-PK components (DNA-PKcs,[26] Ku70,[20] Ku80[21]) exhibit similar but milder phenotypes with respect to neuronal apoptosis and survive during the postnatal period. In these NHEJ-deficient mice, it is thought that their phenotypes result from unrepaired DSBs caused by NHEJ deficiency.[45] However, since the phenotypes of our Polβ-null mice considerably differ from those of the NHEJ-deficient mice, we favor the view that unrepaired SSBs cause neuronal apoptosis and lethality associated with Polβ deficiency.[7,40]

Double-mutant mice deficient in Polβ and DNA-PKcs showed more severe phenotypes than Pol$\beta^{-/-}$ mice as well as DNA-PKcs$^{scid/scid}$ mice (Figures 1–4). What is the reason for this difference? In the absence of Polβ, the majority of BER intermediates mentioned above might be repaired slowly by long-patch BER or other mechanisms. However, it is possible that at least some intermediates may be changed to DSBs by collision with DNA replication forks progressing during S phase.[46,47] Furthermore, AP sites on the genome may act as poison in the presence of DNA topoisomerase II, remain unligated and change into DSBs during replication or transcription.[48] In wild-type mice, these DSBs could be repaired by either NHEJ or homologous recombination (HR). In mice deficient in DNA-PKcs, the NHEJ pathway is abolished, so that the remaining HR pathway would be employed to repair these lesions but unable to repair all of them. In Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ mice, both an excess amount of unrepaired SSBs and a small, but significant, amount of unrepaired DSBs would accumulate within cells, interfere synergistically with their genomic integrity and, thus, the double-mutant mice would reveal even more severe phenotypes than either single mutant. This may be the cause of serious defects observed in embryogenesis and neurogenesis of the double-mutant mice.

Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$p53$^{-/-}$ mice showed that p53 deficiency rescues neuronal apoptosis but not embryonic lethality associated with a double deficiency of Polβ and DNA-PKcs (Figure 5d, e). These results indicate that the apoptosis is mediated by the p53-dependent pathway. We have recently shown that Pol$\beta^{-/-}$p53$^{-/-}$ mice do not exhibit neuronal apoptosis but die shortly after birth, and display cytoarchitectural abnormalities in the CNS.[40] These results indicate that Polβ is critical for embryonic development, especially for neuronal development. In contrast, p53 deficiency effectively rescues embryonic lethality of both LigIV- and XRCC4-deficient mice, and these mice, like DNA-PKcs$^{scid/scid}$p53$^{-/-}$ mice,[49] survive even in the postnatal stage but develop lymphomas at shorter latency than p53$^{-/-}$ mice.[38,42] In these double-mutant mice, deficiency in an NHEJ factor and p53 may cause genomic instability and increase chromosomal rearrangements such as deletions and translocation, leading to tumorigenesis during lymphocyte development.[50,51] These results suggest that NHEJ may not be indispensable for the development of mouse embryos but instead, greatly increases the risk of tumors. It should be noted that all E10.5 Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$p53$^{-/-}$ mice exhibit defects in neural tube closure, like exencephaly (Figure 5). Such defects have been reported even in a subset of p53$^{-/-}$ embryos, suggesting a crucial role of p53 protein in the process of neural tube closure.[52,53] Similarly, mice deficient in Pax-3, a transcription factor that is expressed in the neural tube and neural crest,[54] exhibit similar neural tube defects with apoptosis and die at midgestation; importantly, this deficiency increases p53 protein levels in the embryos. Since p53 deficiency rescues the above defect in Pax-3-deficient embryos, it is suggested that Pax-3 regulates neurulation by inhibiting p53-dependent apoptosis. In addition, a considerable fraction of mice deficient in both p53 and XPC, a nucleotide excision repair protein, shows a spectrum of neural tube defects including excencephaly.[55] These results suggest that the p53 level in developing neural tissues is controlled very precisely and that this allows for the normal process of neural tube closure. It is possible that both Polβ and DNA-PKcs deficiencies lead to elevated p53 levels and disrupt a balance between cell cycle arrest/DNA repair and apoptosis, causing apoptosis in specific neural cell types. Thus, even if p53 deficiency abolishes the apoptosis, Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$p53$^{-/-}$ mice may suffer severe, unregulated growth of neural cells. In other words, Polβ and DNA-PKcs, coupled with p53, are likely to play an important role in the process of neurulation. Further studies will be necessary to elucidate the mechanism of developmental abnormalities caused by deficiency in DNA repair factors.

Materials and Methods

Animals

Generation and characterization of Pol$\beta^{-/-}$ mice have been described.[7,40] Male Fox Chase C.B-17/ICR-ScidJcl (SCID) mice were purchased from CLEA Japan (Tokyo, Japan). p53$^{+/-}$ mice (C57BL/6J-Trp53 tmlTyj) were purchased from The Jackson Laboratory (West Grove, PA, USA).

Genotypings of Polβ, DNA-PKcs, and p53 were performed by PCR analysis as previously described.[7,22] All mice were maintained in a pathogen-free environment.

Generation of Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ and Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$p53$^{-/-}$ mice

Pol$\beta^{+/-}$ mice were mated to SCID mice and the resulting Pol$\beta^{+/-}$DNA-PKcs$^{scid/+}$ mice were intercrossed to generate Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$ mice. Also Pol$\beta^{+/-}$DNA-PKcs$^{scid/scid}$ mice were mated to p53$^{+/-}$ mice to generate Pol$\beta^{+/-}$DNA-PKcs$^{scid/+}$p53$^{+/-}$ mice, which were then intercrossed to generate Pol$\beta^{-/-}$DNA-PKcs$^{scid/scid}$p53$^{-/-}$ mice.

Histological analyses

Whole embryos were fixed, embedded, and sectioned (10 μm), as described.[7,40] The sections were immunostained as described,[7,40] except for p53 staining where TSA Biotin System (PerkinElmer Life Sciences) as anti-p53 antibody was used.

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