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# *Brd2* is required for cell cycle exit and neuronal differentiation through the E2F1 pathway in mouse neuroepithelial cells

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ABSTRACT

To understand genetic programs controlling mammalian central nervous system (CNS) development, we have identified one transgene-inserted mutation, which showed embryonic lethality during neurulation. Determination of the transgene integration site and rescue experiments revealed that the *Brd2* gene, whose products specifically bind acetylated histone H4 and can mediate transcription, was the cause of this mutation. Expression studies with specific markers demonstrated that cell cycle progression was accelerated and neuronal differentiation as well as cell cycle exit were impaired in *Brd2*-deficient neruoepithelial cells. To investigate whether *Brd2* regulates neuronal differentiation through a E2F1 transcriptional factor, which directly binds Brd2 and controls genes expression for cell cycle progression and exit, we analyzed *Brd2;E2F1* double mutant phenotypes and, consequently found that abnormalities in neuronal differentiation and cell cycle progression due to *Brd2*-deficiency were restored by removing the *E2F1* gene. These findings suggest that *Brd2* is required for cell cycle exit and neuronal differentiation of neuroepithelial cells through the E2F1 pathway during mouse CNS development.


## 1. Introduction

Control of proliferation and differentiation of neuroepithelial cells associating with the cell cycle plays crucial roles in mammalian central nervous system (CNS) development. Initially, neuroepithelial cells expand by symmetric proliferative cell division and, thereafter, they produce one neuroepithelial cell and one post-mitotic neuron via neuron-generating asymmetric cell division, forming a neuronal layer [1,2]. For cell proliferation, neuroepithelial cells progress into the late G1 phase and pass the G1/S phase transition, while for neuronal differentiation, they exit the cell cycle at the early G1 phase and enter into the G0 phase [3]. Notably, cell cycle progression or exit is controlled by the E2F/Rb pathway in mammals [4,5]; when Rb is hyper-phosphorylated, released E2F from the E2F/Rb complex can transactivate E2F-target genes for cell cycle progression, and when Rb is hypo-phosphorylated, the complex can repress the E2F function to arrest the cell cycle generally. Consistently, loss or gain of mutations of the E2F/Rb complex has been shown to lead to impaired proliferation and neuronal differentiation [4]; i.e. E2F1 deficiency reduces proliferation of neural precursors while Rb deficiency up-regulates ectopic proliferation of neural precursors. Although proliferation and neuronal differentiation appear to be intimately linked to cell cycle progression and exit, molecular mechanisms of neuronal differentiation linking to the cell cycle progression and exit in neuroepithelial cells are not fully understood.

*Brd2* has been suggested to be involved in cell cycle progression and exit in cultured cell lines [6]. *Brd2* belongs to the BET family, which contains double bromodomains in the N-terminal side and an extraterminal (ET) domain in the C-terminal side [6]. These BET family proteins, consisting of Brd2, Brd3, Brd4 and Brdt, have been considered to recruit transcription factors to promoters by translating the histone code [6]. Particularly, Brd2 binds to acetylated lysine residues of histone H4, a transcriptionally active mark [7], through the double bromodomain [6]. Notably, in mammalian cell lines, ectopic expression of Brd2 inhibits S phase progression and induces G1 cell cycle arrest or exit [8,9], while knockdown of the *Brd2* expression promotes the S phase entry [9]. Consistent with these findings, *Brd2* is one of the transcriptional targets of the Rb complex along with RBP2, also named JARID1A, a H3K4

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histone demethylase, upon cellular differentiation [10]. These reports suggest the crucial role of *Brd2* in cell cycle exit. Conversely, Brd2 trans-activates target genes of E2F transcriptional factors by direct association with E2F1 and E2F2 [11], and *Brd2*-deficient embryonic fibroblast cells proliferate more slowly than those of the wild-type [12]. These latter findings rather suggest the involvement of *Brd2* in cell cycle progression. Thus, the role of *Brd2* in cell cycle progression or exit is still controversial. Moreover, it has been shown that *Brd2*-deficient mice display embryonic lethality and cranial neural tube closure defects (NTDs) [12,13], in where expression of genes involving neuronal development is decreased by microarray analysis [13]. However, it remains uncertain if *Brd2* plays crucial roles in proliferation and neuronal differentiation of mouse neuroepithelial cells.

In this study, we have identified a transgene-inserted *Brd2* mutant allele and characterized neuroepithelial deficits. Precise expression studies with specific markers revealed that *Brd2* is required for cell cycle exit and neuronal differentiation through the E2F1 transcription factor in mouse neuroepithelial cells.

## 2. Materials and methods

### 2.1. Experimental animals and genotyping

For the animal experiments, mice were housed under the guidelines of the Osaka Medical Center and Research Institute for Maternal and Child Health. To construct the *EGFP*-fused *Brd2-BAC* transgene, the *EGFP* cDNA (0.7 kb) was inserted into the translation initiation site of *Brd2* of the BAC clone (RP23–309K10) with a Counter-Selection BAC Modification Kit (Gene Bridges). *E2F1*$^{-/-}$ mice were obtained from Jackson Laboratory (002785). The PCR primers used to genotype mice were described in Supplementary Table 1.

### 2.2. In situ hybridization

*In situ* hybridization involving digoxigenin-labeled riboprobes was performed as described [14,15]. The 1330 bp fragment of *Brd2* cDNA (BC138656), which corresponds to 540–1870, was used for anti-sense probe. To avoid cross hybridization between family genes, specific anti-sense probes of *E2F1*, *E2F2* and *E2F3* were generated [16].

### 2.3. Immunohistochemistry, EdU incorporation assay and cell count

Immunohistochemistry was conducted as described [17]. The primary antibodies were described in Supplementary Table 2. For the EdU incorporation assay, EdU (40 μg/g of body weight; Invitrogen) was injected into pregnant dams intraperitoneally. Pregnant mothers were sacrificed after 15 min and embryos were collected and sectioned at 8 μm. EdU signaling was detected with a Click-iT EdU Alexa Fluor 488 Imaging Kit (Invitrogen). For quantitative analysis, cells were counted within the entire unilateral neuroepithelial and neuronal regions of two transverse sections at the levels of the eye to rhombencephalon and Rathke's pouch to rhombencephalon, and more than three independent embryos were examined for wild-type and mutant embryos. Statistical significance was determined using *Student's t*-test.

## 3. Results

### 3.1. A novel null allele of Brd2 causes neuroepithelial defects during neurulation

We identified one transgenic mouse line that displayed embryonic lethality (Table 1). This line originally arose from heterozygous transgenic mice produced by microinjection of the *F5placZ* transgene for other expression studies [18,19]. To examine abnormalities of mutant embryos more precisely, we have collected progeny from heterozygous parent crossings and found that homozygous mutants are died until E13.5 (Table 1). Although the external mutant phenotypes were varied at E10.5 ($n$ = 171), the majority of the mutant embryos displayed cranial neural tube defects (NTDs) and craniofacial malformations (88.9%) (Fig. 1B), while the remaining 11.1% of mutant embryos displayed severe growth retardation. To investigate neuroepithelial phenotypes during CNS development, thereafter we have analyzed highly penetrant phenotypes, showing cranial NTDs and craniofacial malformations, with the exception of growth retardation in this study. Histologic examination of mutant embryos demonstrated that mutant neuroepithelial layers were irregularly thickened and severely distorted at the level of the rostral rhombencephalon (Fig. 1A–F).

To determine a causative gene of the mutation, with fluorescence *in situ* hybridization (FISH) the chromosomal location of transgene insertion was cytogenetically mapped on chromosome 17q B1–B3 (Supplementary Fig. 1A–C). Analyses of an inverse PCR together with Southern blotting indicated that a 33.9 kb region including the 3rd to last exons of the *Brd2* gene, was deleted (Supplementary Fig. 1C). Consistently, *Brd2* transcripts were undetectable in mutant embryos (Supplementary Fig. 1D). Thereafter, the mutant allele was designated as the *Brd2 F5placZ* (*Brd2 tg*). To assess whether *Brd2* deficiency is responsible for mutant phenotypes, we generated *Brd2* cDNA-overexpressing transgenic mice (*CAG-EGFP-Brd2*) and explored whether the *Brd2* cDNA alone can restore the embryonic abnormalities observed in *Brd2*$^{tg/tg}$ embryos. By crossing *Brd2*$^{tg/+}$; *CAG-EGFP-Brd2* mice with *Brd2*$^{tg/+}$ mice, consequently, we found that *Brd2*$^{tg/tg}$; *CAG-EGFP-Brd2* mutant embryos were able to develop normally (Supplementary Fig. 1E–I). Taken together, these above findings indicated that the embryonic abnormalities induced by the *Brd2*$^{tg/tg}$ mutation certainly accounted for the loss of *Brd2* expression.

To establish the involvement of *Brd2* in the affected neuroepithelium, *Brd2* expression was analyzed more precisely. Indeed, *Brd2* transcripts were broadly found in the developing prosencephalon, mesencephalon, rhombencephalon and spinal cord in wild-type embryos (Fig. 1G–J). Additionally, we also generated

**Table 1**
Frequencies of genotypes among embryos crossing *Brd2* heterozygous transgenic mice.

| Embryonic stages (days) | Genotype | | | Absorbed (%) | No.total Embryos (%) |
|---|---|---|---|---|---|
| | +/+ (%) | *tg*/+ (%) | *tg*/*tg* (%) | | |
| 8.5 | 73(22.4) | 162(49.9) | 78(24.0) | 12(3.7) | 325(100) |
| 9.5 | 67(22.4) | 144(48.2) | 77(25.7) | 11(3.7) | 299(100) |
| 10.5 | 117(24.1) | 224(46.2) | 119(24.5) | 25(5.2) | 485(100) |
| 11.5 | 9(18.0) | 21(42.0) | 10(20.0) | 10(20.0) | 50(100) |
| 12.5 | 11(28.2) | 16(41.0) | 6(15.4) | 6(15.4) | 39(100) |
| 13.5 | 4(14.8) | 17(63.0) | 1(3.7) | 5(18.5) | 27(100) |

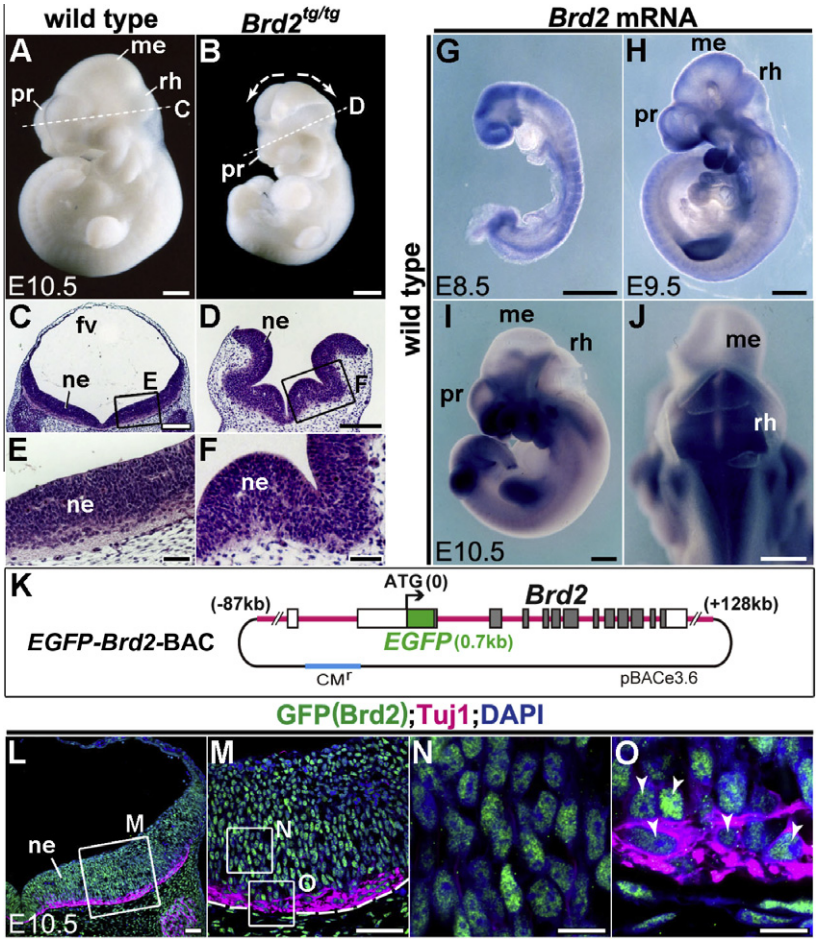


**Fig. 1.** Characterization of *Brd2*-deficient embryos and *Brd2* expression. (A, B) External lateral views of wild-type (A) and *Brd2*$^{tg/tg}$ (B) embryos at E10.5. White dashed arrow indicates opened neural tube (B). (C–F) Transverse HE section of wild-type (C, E) and *Brd2*$^{tg/tg}$ (D, F) embryos at the levels of dashed lines in the upper panels (A, B). Higher magnified views (E, F). (G–J) Whole mount *in situ* hybridization analysis of *Brd2* transcripts in wild-type embryos. (K) Schematic diagram of the *EGFP* knock-in *Brd2-BAC* transgene (*EGFP-Brd2-BAC*). (L–O) Immunohistochemistry of EGFP-fused Brd2 merged with DAPI (nucleus) and Tuj1 in *BAC* transgenic embryos. Magnified images are shown right. White dashed line in (M) indicates basal membrane. Tuj1-positive neurons express Brd2 (O, arrowheads). Scale bars: A, B, G–J, 500 μm; C, D, 200 μm; E, F, L, M, 50 μm; N, O, 10 μm. *Abbreviations*: fv, fourth ventricle; me, mesencephalon; ne, neuroepithelium; pr, prosencephalon; rh, rhombencephalon.

transgenic mice carrying the *EGFP*-fused *Brd2-BAC* transgene, in which the *EGFP* cDNA was inserted into the N-terminal region of the translational initiation site of the *Brd2* gene (Fig. 1K). As expected, *Brd2*$^{tg/tg}$ embryos carrying this *BAC* transgene, which encompasses the −87 kb to +128 kb region of *Brd2*, developed normally and were viable and fertile. Then, we examined expression of the *EGFP*-fused Brd2 products in *BAC* transgenic embryos with immunohistochemistry for GFP (Fig. 1L–O). The Brd2-GFP proteins were expressed in the nucleus of neuroepithelial cells as well as post-mitotic neurons, marked by Tuj1 (Fig. 1N,O); also supporting that mutant neuroepithelial abnormalities are dependent on *Brd2* expression.

### *3.2. Brd2-deficient neuroepithelial cells fail to exit the cell cycle and differentiate into neurons*

Since the above histologic examinations suggested the imbalance between proliferation and neurogenesis (Fig. 1), we tested if *Brd2*-deficient neuroepithelial cells can proliferate and differentiate into neurons normally (Fig. 2). As neuronal differentiation usually begins later than E9.5 at the level of the mes-rhombencephalon, we analyzed proliferation and differentiation markers at E10.5 (Supplementary Fig. 2A). In the wild-type embryos, Tuj1 expression, a marker for post-mitotic neurons, was robustly detectable surrounding multiple lines of neurons in the basal side (Fig. 2A, E). In contrast, Tuj1 expression was severely reduced in *Brd2*$^{tg/tg}$ embryos (Fig. 2B, F). Actually, the ratio of the number of Tuj1-positive neurons to the total cell number (marked by nuclear TOTO-3 staining) was severely decreased (Fig. 2I; wild-type, 26.7%; *Brd2*$^{tg/tg}$, 11.9%). Concurrently, Western blotting confirmed that the total amount of Tuj1 products was also reduced in mutant embryos (Supplementary Fig. 2B). These findings demonstrated that neuronal differentiation was severely impaired in *Brd2*-deficient embryos.

Since cell cycle exit is generally an indispensable process for terminal differentiation (Supplementary Fig. 2A), we next analyzed expression of p27$^{Kip1}$, a cell cycle exit (G0 phase) marker (Fig. 2C, D, G, H). In the wild-type, p27$^{Kip1}$-positive cells were detected as multiple lines of nuclei in the basal side (Fig. 2C, G). However, in *Brd2*$^{tg/tg}$ embryos, p27$^{Kip1}$-positive cells were hardly detectable and consequently, the ratio of p27$^{Kip1}$-positive *cells* was quantitatively reduced (Fig. 2D, H, I; wild-type, 30.3%; *Brd2*$^{tg/tg}$, 10.7%). The above studies revealed that the cell cycle exit was also severely impaired in mutant embryos.

Next, we analyzed the expression of Ki67, M-phase and interphase markers, which was restricted to the apical and central portion of the neuroepithelium of the wild-type (Fig. 2J, N). However, in *Brd2*$^{tg/tg}$ embryos, Ki67 was up-regulated throughout the apical to basal neuroepithelial cells and most prominently in the basal portion (Fig. 2K, O). Consequently, the ratio of the number of

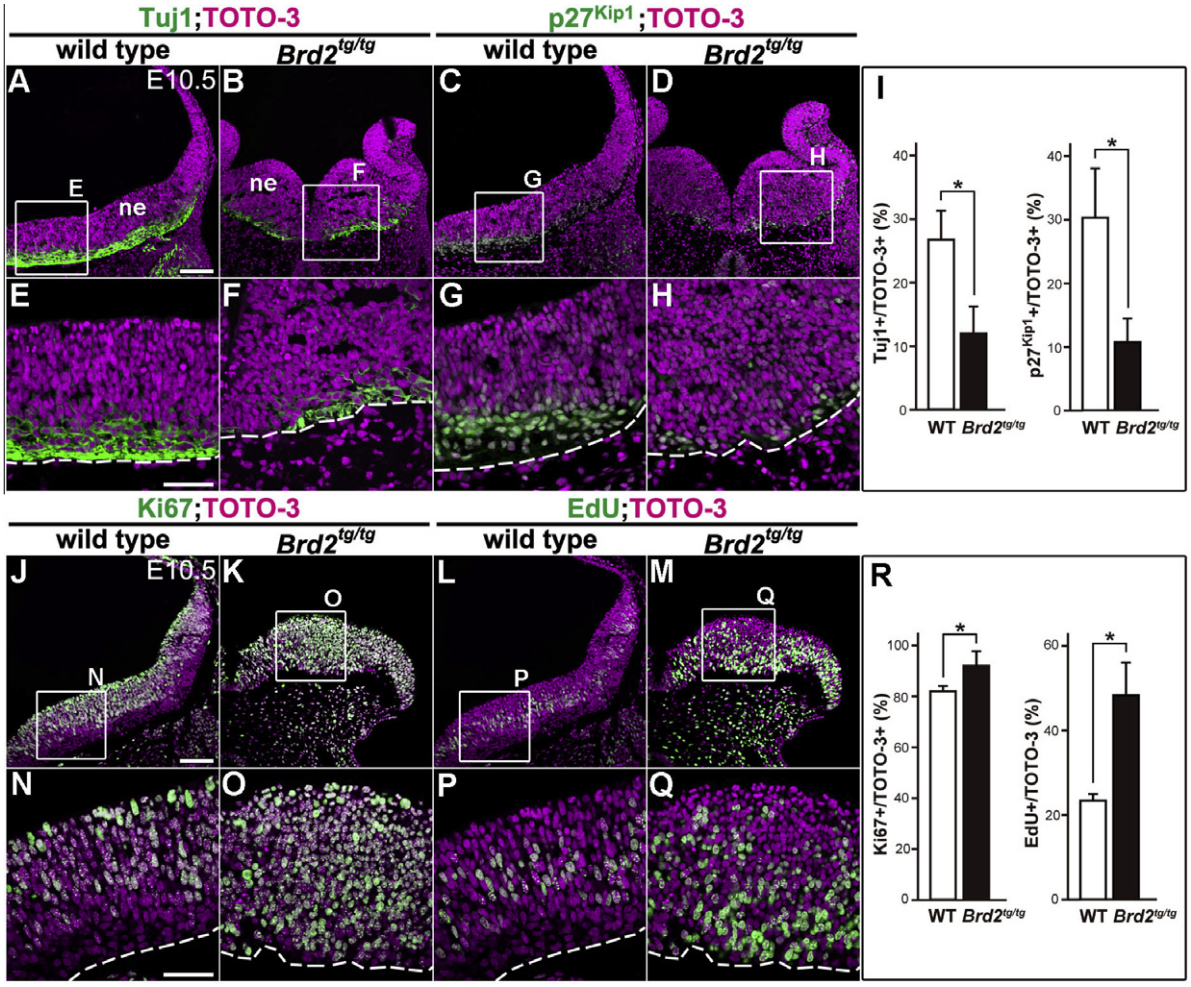


**Fig. 2.** Defects of proliferation and neuronal differentiation in *Brd2*-deficient embryos. (A–H, J–O) Immunohistochemistry of Tuj1 (A, B, E, F), $p27^{Kip1}$ (C, D, G, H) and Ki67 (J, K, N, O) merged with TOTO-3 (nucleus) in wild-type (A, C, E, G, J, N) and $Brd2^{tg/tg}$ embryos (B, D, F, H, K, O). (L, M, P, Q) EdU detection in wild-type (L, P) and $Brd2^{tg/tg}$ embryos (M, Q). Magnified images are in the lower panels (E–H, N–Q). Transverse sections are at the level of the eye toward rostral rhombencephalon. White dashed lines indicate basal membranes. (I, R) The ratio of the numbers of Tuj1-, $p27^{Kip1}$-, Ki67-, and EdU-positive cells to TOTO-3 (nuclei, magenta)-positive cells. Error bars indicate the s.d. *$P<0.001$. Scale bars: A–D, J–M, 100 μm; E–H, N–Q, 50 μm.

Ki67-positive nuclei was significantly increased (Fig. 2R, wild-type, 81.9%; $Brd2^{tg/tg}$ 91.9%). These findings indicated that proliferating cells were aberrantly increased in the $Brd2^{tg/tg}$ neuroepithelium.

To ascertain whether S phase progression is accelerated in *Brd2*-deficient embryos, an EdU incorporation assay during DNA synthesis was conducted (Fig. 2L, M, P–R). Normally, EdU incorporation was evident in the proliferative cells of the central portion of the wild-type neuroepithelial layer (Fig. 2L, P). In $Brd2^{tg/tg}$ embryos, however, EdU incorporation was strongly detected in the basal portion of neuroepithelial cells in addition to the central portion (Fig. 2M, Q). Consequently, the ratio of EdU-positive cells was increased in the mutant neuroepithelium (Fig. 2R; wild-type, 23.3%; $Brd2^{tg/tg}$, 48.1%); clearly demonstrating that the cell cycle progression was accelerated in the *Brd2*-deficient neuroepithelium. Collectively, these aforementioned findings revealed that cell cycle progression was accelerated while cell cycle exit upon neuronal differentiation was impaired in mutant neuroepithelial cells.

### 3.3. Neuroepithelial impairment due to Brd2-deficiency is restored by removal of the E2F1 gene

Considering that BRD2 has been shown to associate with E2F1 and E2F2 directly [11] and the E2F-regulated cell cycle progression is crucial for proliferation and neuronal differentiation [4,20], it can be hypothesized that *Brd2* may contribute to cell cycle exit and neuronal differentiation by controlling the E2F-directed target genes transcription (Fig. 3A). To explore this possibility, we first examined the expression of three transcriptional activators, *E2F1*, *E2F2* and *E2F3* at E10.5 (Fig. 3B–G). We found that among these, *E2F1* expression was evident at the level of the mes-rhombencephalon and appeared to be largely overlapped with *Brd2* expression at that stage (Fig. 1I, J, Fig. 3B, E).

To verify whether *Brd2* can regulate cell cycle exit upon neuronal differentiation via E2F1, we removed two copies of the *E2F1* gene in the *Brd2*-null background and subsequently examined $Brd2^{tg/tg}$; $E2F1^{-/-}$ mutant phenotypes. The morphological analyses indicated that single $E2F1^{-/-}$ embryos had developed normally in terms of neurulation (Fig. 3H, I, L, M; [21]). However, histologic neuroepithelial abnormalities as well as gross appearances of $Brd2^{tg/tg}$; $E2F1^{-/-}$ embryos appeared to be restored toward the wild-type condition to some extent (Fig. 3J, K, N, O).

To access whether neuroepithelial defects observed in $Brd2^{tg/tg}$ embryos can be restored, specific markers were analyzed in $Brd2^{tg/tg}$; $E2F1^{-/-}$ embryos (Fig. 4). The Tuj1 expression of $Brd2^{tg/tg}$; $E2F1^{-/-}$ embryos appeared to be up-regulated than that of $Brd2^{tg/tg}$; $E2F1^{+/+}$ embryos (Fig. 4A–C). This finding indicates that

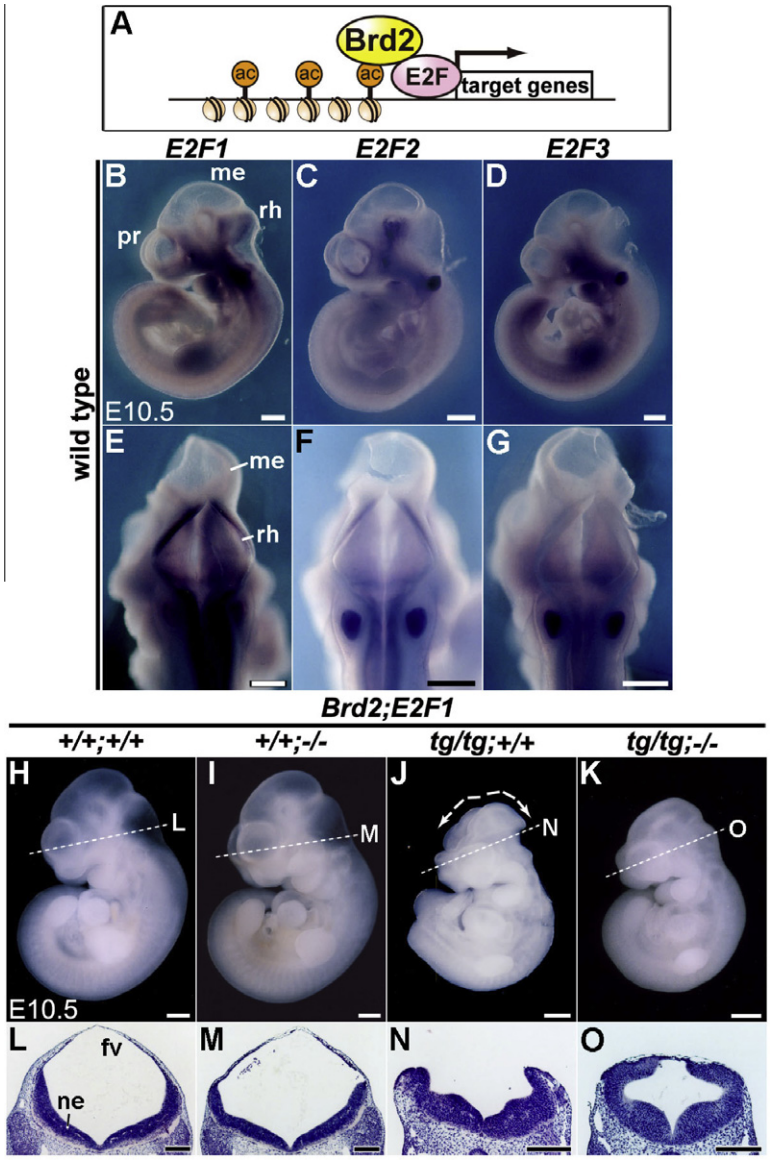


**Fig. 3.** Genetic interaction between *Brd2* and *E2F1* genes. (A) Hypothetical model for roles of Brd2 in E2F-directed transcription for cell cycle progression and exit. (B–G) Whole mount *in situ* hybridization analysis of *E2F1*, *E2F2* and *E2F3* transcripts in wild-type embryos at E10.5. Higher magnification of the dorsal views (E–G). (H–O) External lateral views and transverse HE sections at the level of rostral rhombencephalon of the $Brd2^{+/+}$; $E2F1^{+/+}$ [wild-type] (H, L), $Brd2^{+/+}$; $E2F1^{-/-}$ (I, M), $Brd2^{tg/tg}$; $E2F1^{+/+}$ (J, N) and $Brd2^{tg/tg}$; $E2F1^{-/-}$ (K, O) embryos at E10.5. White dashed arrow indicates opened neural tube (J). Scale bars: B–K, 500 μm; L–O, 200 μm.

failure in neuronal differentiation due to Brd2-deficiency was restored by removal of the *E2F1* gene. Additionally, the S phase progression of the cell cycle in $Brd2^{tg/tg}$; $E2F1^{-/-}$ neuroepithelial cells, as evidenced by the EdU incorporation, was down-regulated toward the normal condition by removing the *E2F1* gene (Fig. 4D–F). Moreover, ectopic cell death was consistently observed in $Brd2^{tg/tg}$; $E2F1^{+/+}$ embryos at E10.5 (Fig. 4H; [12]). However, cell death now appeared to be undetectable in $Brd2^{tg/tg}$; $E2F1^{-/-}$ neuroepithelial cells (Fig. 4I), indicating that apoptosis was also rescued by loss of the *E2F1* gene. To further confirm the neuronal differentiation state more quantitatively, the ratio of the number of Tuj1-positive neurons was counted and consequently it was clearly higher than that in $Brd2^{tg/tg}$; $E2F1^{+/+}$ embryos (Fig. 4J; wild-type 21.9%, $Brd2^{tg/tg}$; $E2F1^{+/+}$, 10.2%, $Brd2^{tg/tg}$; $E2F1^{-/-}$, 15.1%). These findings collectively indicated that abnormalities in *Brd2*-deficient neuroepithelial cells could be restored by removing the *E2F1* gene and moreover, suggested that *Brd2* is required for cell cycle exit and neuronal differentiation through the E2F1 pathway during mammalian CNS development.

## 4. Discussion

The current genetic study indicated that *Brd2* is required for cell cycle exit and neuronal differentiation of mouse neuroepithelial cells via modulating the activity of E2F1-directed cell cycle progression or exit (Fig. 2–4). Although E2F1 has traditionally been considered to act as a transcriptional activator, recent findings have suggested that E2F1 can act as a transcriptional repressor with Rb to prevent cell cycle progression and re-entrance into cell cycle from a differentiated state [22]. Thus, we can consider that without *Brd2*, E2F1 may neither promote to exit cell cycle for terminal differentiation nor arrest cell cycle for robust maintenance of the differentiated state. Consequently, E2F1 may act alone as a

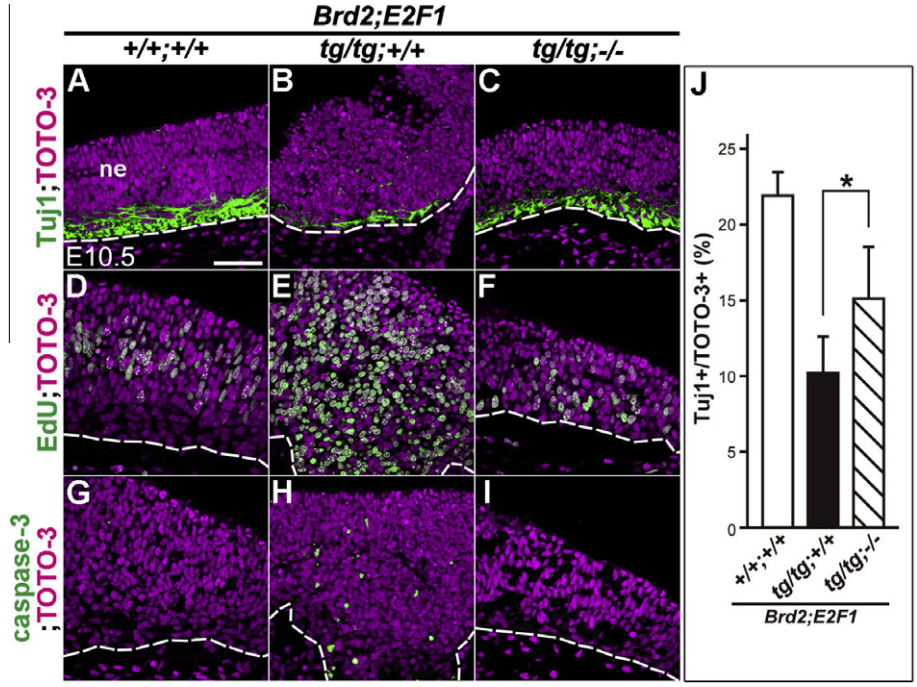


**Fig. 4.** Molecular markers analyses in *Brd2* and *E2F1* double mutant embryos. (A–I) Immunohistochemical studies with Tuj1 (A–C), EdU (D–F) and caspase-3 (G–I) with TOTO-3 (nucleus) merged views in $Brd2^{+/+}$; $E2F1^{+/+}$ [wild-type] (A, D, G), $Brd2^{tg/tg}$; $E2F1^{+/+}$ (B, E, H) and $Brd2^{tg/tg}$; $E2F1^{-/-}$ embryos (C, F, I). Transverse sections are at the level of the eye toward the rostral rhombencephalon. White dashed lines indicate basal membranes. (J) The ratio of the numbers of Tuj1-positive cells to TOTO-3 (nuclei, magenta). Error bars indicate the s.d. $^{*}P < 0.005$. Scale bars: A–I, 50 μm.

transcriptional activator, and thus cell cycle progression was accelerated aberrantly in *Brd2*-deficient embryos. However, without both *Brd2* and *E2F1* genes, since both *E2F1*-dependent transcriptional activation and repression can not take place, E2F1-directed cell cycle progression might not be enhanced.

In addition to E2F1, Brd2 has been shown to associate with histone modifying enzymes such as a histone deacetylase, a histone methyltransferase, and a chromatin remodeling component [6,23,24]. Given that chromatin remodeling SWI/SNF core components such as *Brg1* and histone deacetylases play crucial roles in proliferation and neural differentiation [25,26], to differentiate neurons Brd2 may recruit histone modification enzymes, transcriptional repressor complexes and chromatin remodeling factors into E2F1-target promoters, and thereby repress E2F1-directed transcription for cell cycle progression.

Despite transcriptional activation of E2F1-target genes and cell proliferation played by Brd2 [6,11], current results rather suggest that Brd2 appear to repress the activity of E2F1-directed cell cycle progression. This controversial issue provides the possibility that even if Brd2 can also contribute to cell cycle progression in association with E2F1, other redundant factors may complement this *Brd2* function in *Brd2*-deficient neuroepithelial cells. Alternatively, considering that Brd2 can associate with both co-activators and co-repressors [23], and BDF1, a yeast homolog of Brd2, appeared to repress the transcription of the target genes as well as activate them [27], if Brd2 can act as a transcriptional activator or repressor may be dependent on the experimental system employed, such as the various cell lines and tissues. Thus, we can consider that *Brd2* might be able to regulate transcription either with activators or with repressors in a context-dependent manner.

*Brd2*-deficient embryos have been shown to display cranial NTDs in the mouse (Fig. 1; [12,13]). In human, NTDs are the second-most prevalent and severe congenital birth defects, affecting about 0.5–2 out of every 1000 pregnancies worldwide [28]. However, the genetic programs governing the cellular events causing NTDs in the human embryo are not understood well. Current studies suggest that *Brd2*-deficient cranial NTDs may stem from the imbalance of cell proliferation and neurogenesis. Although several cellular processes have been considered to mediate cranial neurulation [29], other processes except cell proliferation and neurogenesis appeared to be not involved in mutant defects primarily (Supplementary Fig. 3). Additionally, human BRD2 has been closely associated with juvenile myoclonic epilepsy, including the photoparoxysmal response [30,31]. Indeed, *Brd2* heterozygous males have a decreased clonic seizure threshold, and *Brd2* heterozygous females a decreased tonic-clonic seizure threshold in the mouse [32]. Moreover, these *Brd2* heterozygous mutant mice show a reduced number of GABAergic neurons along the basal ganglia pathway [32]; being also good agreement with impairment of neuronal differentiation of *Brd2*-deficient neuroepithelial cells (Fig. 2). Considering that NTDs are one of the most common congenital diseases and the *BRD2* locus is associated with juvenile myoclonic epilepsy, the current study may imply that incorrect neuronal differentiation due to *BRD2* mutation causes congenital birth defects or neurological disorders in humans.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.07.149.

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