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Astrocyte Dysfunction Associated with Cerebellar Attrition in a Nijmegen Breakage Syndrome Animal Model

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Abstract Nijmegen breakage syndrome (NBS) is a genomic instability disorder caused by hypomorphic mutations in the *Nbs1* gene. When *Nbs1* is conditionally inactivated in the central nervous system of mice (Nbs1-CNS-Δ), they suffer from severe cerebellar atrophy, ataxia, and white matter damage. Here, we show that conditional inactivation of the murine *Nbs1* gene has a profound effect on the integrity and the functionality of the glial cells, which suggests their crucial role in the pathogenesis of NBS. Interestingly, in Nbs1-CNS-Δ mice, the dramatic reduction in the numbers of Purkinje and granule cells was also linked to a reduction of microglial cells but not to astrocytes (GFAP+), suggesting an impairment in astrocytic functionality. *Nbs1* levels were dramatically reduced in adult astrocyte isolated from Nbs1-CNS-Δ mice, suggesting a major role in cerebellar pathology. In order to investigate the effect of Nbs1 deletion on astrocyte activity, we investigated glutamine synthetase levels in astrocyte and discovered 40% reduction as compared to WT. Furthermore, we found a significant reduction in the secretion of neurotrophic factors, such as brain-derived neurotrophic factor and neurotrophin 3. Understanding the contribution of malfunctioning astrocytes to the etiology of NBS can elucidate a hitherto unknown aspect of this disorder.

Keywords Nijmegen breakage syndrome · Asrocyte · Microglia · Glia · Cerebellum · Purkinje cells · BDNF · NT3

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Introduction

Ataxia telangiectasia (A-T) and Nijmegen breakage syndrome (NBS) are related genomic instability syndromes caused by null alleles in the *ATM* gene or hypomorphic mutations in the *Nbs1* gene, respectively. A-T's major features are progressive cerebellar degeneration, immunodeficiency, cancer predisposition, and extreme sensitivity to ionizing radiation (IR). NBS (nibrin) protein shares A-T's cardinal symptoms, but instead of cerebellar degeneration, NBS patients exhibit microcephaly and mental deficiency (Digweed and Sperling 2004). Loss or inactivation of *ATM* or hypomorphic mutations in the *MRE11* and *Nbs1* genes lead to severe genomic instability syndromes, A-T, an A-T like disease, which is a mild form of A-T (Taylor et al. 2004). Hypomorphic Nbs1 mutations underlie NBS, characterized by immunodeficiency, genomic instability, radiation sensitivity, and predisposition to lymphoid malignancies. Waltes et al. (2009) reported on a patient previously diagnosed as probably having NBS, with microcephaly, mental retardation, "bird-like" face, and a short stature. This patient is compound heterozygous for mutations in the Rad50 gene that give rise to low levels of unstable RAD50 protein. A

characterization of genes involved in these disorders illustrates the functional relationship between *Nbs1*, *Mre11, Rad50*, and *ATM*. *Nbs1* forms a complex with *Mre11* and *Rad50* (the MRN complex), which functions as a sensor of double-strand breaks (DSBs; Barzilai et al. 2008; Biton et al. 2008; Uziel et al. 2003). *Nbs1* was shown to serve as a scaffold protein, which specifically recruits *ATM* to the DNA damage sites (Falck et al. 2005). Knocking out the murine homolog of *Nbs1* (*Nbn*) led to embryonic lethality. When *Nbn* was conditionally inactivated in the central nervous system (CNS) using the nestin-Cre conditional gene targeting system, the animals (Nbs1-CNS-Δ) showed a dramatic neurological phenotype that combines the microcephaly typical of human NBS patients together with a proliferation arrest of granule cell progenitors and apoptosis of postmitotic neurons in the cerebellum, leading to severe ataxia (Assaf et al. 2008; Baranes et al. 2009; Frappart et al. 2005). These abnormalities are reminiscent of the clinical symptoms of NBS. The observed cerebellar degeneration, however, is more reminiscent of A-T rather than NBS.

The glial cell population is a heterogeneous mixture of cell sub-populations such as microglia and astrocytes that maintain the brain's plasticity and aid the brain to functionally recover from various insults and injuries (Hanisch and Kettenmann 2007; Kettenmann and Verkhratsky 2008). A dysfunction of glial cell receptors, which alters the glial cells' sense of their environment, can lead to the development of neurological diseases (Hanisch and Kettenmann 2007; Streit 2006). Emerging evidence in the literature suggests that changes in the dividing process of glial cells during aging might lead to their abnormality and may provide clues to the pathogenesis of devastating diseases. Moreover, a dysfunction of neurons interaction with microglia (Streit 2006) and astrocytes (Nimmerjahn 2009) in particular may promote the neurodegeneration observed in Alzheimer's disease (Farfara et al. 2008) and amyotrophic lateral sclerosis (ALS) (Ilieva et al. 2009).

Purkinje cells are critical components in the output circuit which conveys the information from the cerebellum to the cerebral cortex via the cerebellar deep nuclei. Bergmann glia, which are highly associated with Purkinje cell development and functionality, are also known to act as guideposts, directing the stellate interneuron process to their Purkinje neuron targets and coordinating the development of this precisely wired circuit.

Here, we show that conditional inactivation of the murine *Nbs1* gene has a profound effect on the integrity and the functionality of astrocytes, which most likely leads to neuronal attrition and severe cerebellar atrophy. This information provides evidence of an important regulatory mechanism for glial cells in a likely etiology of genomic instability disorders in humans, such as NBS.

Materials and Methods

Generation of Various Nbs1 Genotypes

Mice in this study have a SV129 background. Nbs1-CNS-Δ-deleted mice were generated by crossing Nbs1-floxed mice [harboring two floxP sites in Nbn exon 6 (Frappart et al. 2005)] with nestin-Cre+ mice. To measure the nuclear levels of Mre11 and Rad50 in adult neuronal cell, we crossbred Nbs1-CNS-Δ-deleted mice to P53−/− mice (Frappart et al. 2005) that delay neuronal death. Deletion of Nbs1 specifically in Purkinje cells was done by crossing Nbs1-floxed mice with L7/pcp—Cre transgenic mice. The mice were housed and maintained in the animal facility of Tel Aviv University, and all experiments were in compliance with protocols approved by the TAU animal care committee.

Immunohistochemical Analysis of Cerebellar Sections

Sections were fixed in 4% fixative (4% formaldehyde in phosphate-buffered saline (PBS)) for 10 min. and placed in PBS, sections were washed in PBS for 10 min. and then incubated with blocking solution containing 1% bovine serum albumin (BSA) (Sigma, St. Louis, MO) and 10% normal donkey serum (NDS; Jackson ImmunoResearch, Baltimore, MD) in PBS for 1 h at room temperature. The sections were incubated overnight with a primary antibody in 0.25% Triton X-100 at 4°C. The slides were washed three times with PBS and incubated with the appropriate secondary antibody: α-calbindin antibody (1:1,000, Sigma, St. Louis, Missouri, USA), α-gamma-aminobutyric acid (GABA)-α6 receptor antibody (1:500, Chemicon, Temecula USA), anti-Mre11 (1:500 Novus Biologicals, Littleton, USA), Rabbit α- glial fibrillary acidic protein (GFAP; 1:200, Sigma, St. Louis, Missouri, USA), α-CD11b (1:50, Serotec, Oxford, UK), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) (1:2,000, Abcam, Cambridge, Massachusetts, USA), anti-53BP1 rabbit polyclonal (NB100-34; Novus Biologicals; 1:500), and anti-γ-H2AX mouse monoclonal (clone JBW301; Millipore; 1:800). for 1 h at room temperature. After one wash with PBS and two in a buffer containing 10 mM Tris/1 mM ethylenediaminetetraacetic acid, the sections were incubated with the nucleic acid dye Sytox blue (Molecular Probes, Invitrogen, Carlsbad, Germany) for 30 min, slides were then washed three times with the same buffer and mounted with aqueous mounting medium containing anti-fading agents and 4′-6-diamidino-2-phenylindole (DAPI) for nuclei marker (VECTOR, Burlingame, CA). Observations and photography were carried out using a Zeiss (Oberkochen, Germany) LSM 510 META confocal microscope.

Immunoblotting Analysis

Tissues were washed with ice-cold PBS and homogenized in ice-cold homogenization buffer [150 mM NaCl, 10 mM Tris buffer pH 7.6, 1% Triton X-100, 0.5% DeoxyCholocacid, 0.1% SDS 1:50 phosphatase inhibitor cocktail (I and II Sigma), and 1:100 protease inhibitor cocktail]. Protein concentration was determined according to Bradford (1976) using BSA as a standard. Blots were prepared as described by Harlow and Lane (1988), using 10% polyacrylamide gels. Each lane was loaded with 50 μg of protein extract, and after the electrophoresis, the proteins were transferred to an immobilon polyvinyldiene disulfide membrane for at least 12 h at 220 mA. The following antibodies were used for Western blot analysis: P-95 1 (1:1,000, Cell Signaling, Danvers, MA, USA), GFAP (1:1,000, Sigma, St. Louis, Missouri, USA), CD11b (1:200, Serotec, Oxford, UK), BDNF (1:1,000, R&D, Minneapolis, MN, USA), and NT3 (1:200, Abcam, Cambridge, Massachusetts, USA). To normalize the level of protein expression, we used actin (MP Biochemicals, Solon, OH, USA 1:5,000).

Odyssey Analysis of Cerebellar Sections

The sections were fixed in 4% fixative (4% formaldehyde in PBS) for 10 min and placed in PBS. The sections were washed in PBS for 10 min. and then incubated with blocking solution containing 1% BSA (Sigma, St. Louis, MO) and 10% NDS (Jackson ImmunoResearch, Baltimore, MD) in PBS for 1 h at room temperature. The sections were incubated overnight with a primary antibody in 0.25% Triton X-100 at 4°C. The slides were washed three times with PBS and incubated with the appropriate secondary antibody (Li-Cor antibody) for 1 h at room temperature. After one wash with PBS, sections were mounted with an aqueous mounting medium containing anti-fading agents (Biomeda, Burlingame, CA). Observations and photography were carried out using a Li-Cor imaging system.

Isolation of Adult Glial Cells

Adult glial cells were isolated from the brains of 12-week-old wild-type (WT) or *Nbs1*-deficient mice using previously described protocols (Cardona et al. 2006; Hickman et al. 2008). In brief, microglial cells were labeled with magnetic CD11b MACS beads (Miltenyi Biotec Gmbh 120-000-300) for 15 min. at 4–8°C and separated with a MACS separator in a magnetic field (Cardona et al. 2006; Hickman et al. 2008). An astrocyte-enriched fraction (90%) was isolated following an additional stage to deplete endothelial cells (CD31+) using magnetic beads as described above.

Real-Time PCR Analysis

Total RNA was extracted from the cerebellum with male (n= 4) mice in each group. Reverse transcriptase polymerase chain reaction (PCR) assays were designed by Applied Biosystems (Foster City, CA) as described previously (Zhang et al. 2005). Reactions were performed according to the manufacturer's directions using an Applied Biosystems PRISM 7300 thermal cycler. The mice Actin gene, a housekeeping gene, was used to normalize each sample and each gene. Quantification analysis was performed by the ($2^{-\Delta\Delta Ct}$ method) and statistically analyzed by GraphPad Prism software.

Statistical Analysis

Data from each experiment are expressed as mean ± standard error of the mean (SEM). Two-tailed Student's t test was performed when two groups were compared. The one-way ANOVA followed by Bonferroni's multiple comparison tests for multiple samples. Statistical significance was determined at $P<0.05$.

Results

Conditional Nbs1 Inactivation Leads to Cerebellar Attrition and Disorganization

The *NBS* gene product, Nbs1 (also known as p95 or nibrin), is a part of the MRN complex, a central player associated with DSB recognition and repair. To insure Nbs1 inactivation in brain cells, we immunoreacted the cerebellar section with anti-Nbs1 antibody; however, no specific Nbs1 immunoreactivity was detected. As an alternative approach, we measured the nuclear levels of Mre11 which form the MRN complex with the Nbs1 and Rad50. Due to the physical association and functional cooperation between the components of this complex, even partial loss of one of them leads to abrogation of the complex's functions (Van Den Bosch et al. 2003). Fig. 1 shows the expression of Mre11 in Purkinje neurons derived from Nbs1-CNS-Δ mice as compared to control. Deletion of Nbs1 ablated the DNA damage response (DDR) in response to ionizing radiation (IR) as measured by the lack of γH2AX and 53BP1 nuclear focus formation in response to 15 Gy (30 min). As further shown in Fig. 1, WT DDR following IR is evident in WT Purkinje cells as compared to Nbs1-CNS-Δ mice. Furthermore, a cerebellar histopathology of Nbs1-CNS-Δ vs. WT mice revealed dystrophy of the cerebellar structure and significant loss of the Purkinje cell layer (Fig. 2a and c). Odessy analysis (Fig. 2b, c) of

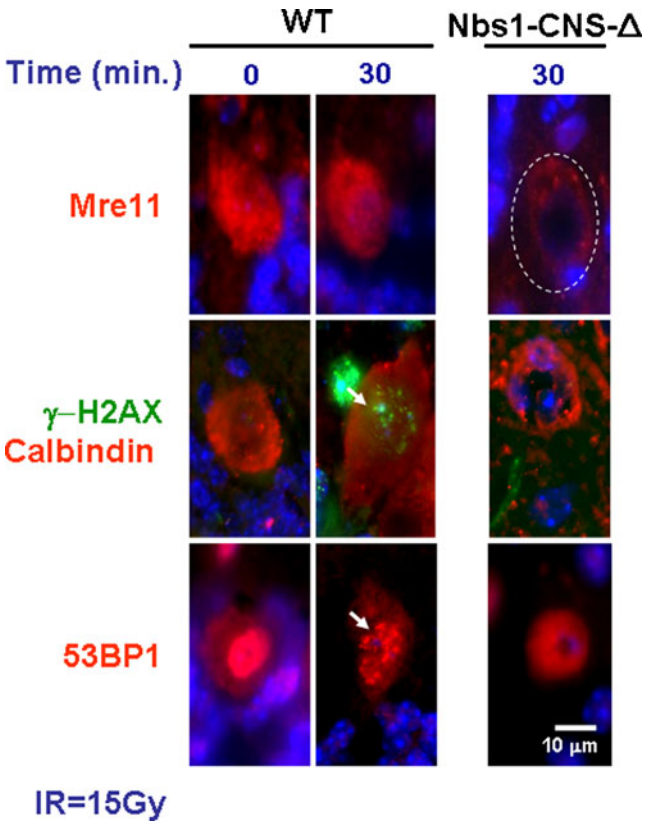

Fig. 1 Nbs1 deletion attenuates DDR in Purkinje neurons. Confocal micrographs of paraffin cerebellar sections with or without IR of WT or Nbs1-NCS-Δ mice immunoreacted with either α-Mre11(*red*) or γ-H2AX (*green*, marker of DNA DSBs), α-calbindin (*red*, Purkinje cell marker), α-53BP1 (*red*, marker of DNA DSBs), and DAPI (a marker of cell nuclei). The *white arrows* label γ-H2AX and 53BP1 nuclear foci whereas the *yellow arrow* labels γ-H2AX nuclear foci in non-Purkinje neurons

Purkinje cells (α-calbindin) and granule cells (GABA receptor-α6) showed marked cerebellar disorganization and a 70% reduction in granule neurons in Nbs1-CNS-Δ vs. WT (Fig. 2b, c).

Reduced Levels of Nbs1 in Astrocyte Affect Their Activity in Nbs1-CNS-Δ Mice

In order to determine the difference between WT and Nbs1-CNS-Δ in adult glia, we isolated adult microglia from the brains of 12-week-old WT or Nbs1-CNS-Δ mice as previously described (Hickman et al. 2008). In order to receive an astrocyte-enriched fraction, we also deleted the endothelial cells (CD31+) using a similar procedure as described above. Western blot analysis revealed a significant reduction of over 90% in Nbs1 levels in astrocytes derived from Nbs1-CNS-Δ as compared to WT (Fig. 3). Nevertheless, the microglia fraction showed only a slight but a significant (18%) decrease in Nbs1 levels. The difference between the Nbs1 levels in glia cells might be related to the source of the cells. While the origin of astrocytes is considered to be mostly CNS-oriented, microglia are likely to be of mesodermal (myeloid) origin, and their main source in the CNS is from the bone marrow-derived microglia. Indeed, there were no significant differences between Nbs1 levels in splenocyte between WT and Nbs1-CNS-Δ mice.

In order to investigate the effect of Nbs1 deletion on astrocytes' activity, we investigated glutamine synthetase (GS) levels in astrocytes. GS is an enzyme that converts glutamate into glutamine, thereby promoting glutamate uptake in the synaptic cleft. Therefore, a reduction in GS expression would entail glutamate toxicity for neurons and might contribute to lesion formation (Hardin-Pouzet et al. 1997). We discovered a significant (40%) reduction (Fig. 3b) in GS levels in astrocytes from Nbs1-CNS-Δ mice as compared to WT mice. These results show that Nbs1 inactivation affects astrocytic cell functionality in Nbs1-CNS-Δ mice.

Dysfunction in Astrocytes is Associated with Microglia Recruitment in Nbs1-CNS-Δ Mice

To further analyze the role of Nbs1 inactivation in astrocytes, we investigated the distribution and pattern of glial cells (e.g., astrocyte and microglia) along the folia of the cerebellum. Morphological analysis revealed that in WT animals, there is an expression of GFAP, an intermediate filament protein in the astrocyte cytoskeleton located within the Purkinje cell layer. While in WT animals, the morphology of the GFAP-positive cells is characterized by a long radial appearance, in Nbs1-CNS-Δ mice, the GFAP-positive cells are small and star-like (Fig. 4a). The dystrophy of the cell pattern correlated with the loss of the Purkinje cell layer (Fig. 4a, c). While there is a significant loss in cerebellar cells, quantified real-time PCR analysis revealed similar levels of cerebellar GFAP expression in WT and Nbs1-CNS-Δ mice (Fig. 4b). The results may point to a dysfunction of GFAP-positive cells that contributes to neurodegeneration. Indeed, only Nbs1-CNS-Δ mice displayed severe cerebellar dystrophy. We further investigated the appearance of CD11b, an integrin that is expressed in the CNS, specifically in microglia. In WT cerebella (Fig. 4a), the CD11b+ microglia were colocalized with the granular and molecular layers, and a marked reduction in CD11b+-positive cells was observed in Nbs1-CNS-Δ mice. This finding was further confirmed using RT-PCR, where a significant (60%) reduction in CD11b+ expression in Nbs1-CNS-Δ cerebella (Fig. 4b) was observed.

The continuous cross talk between astrocyte and microglia plays an important role in maintaining CNS integrity (Kettenmann and Verkhratsky 2008). Cysteine–cysteine

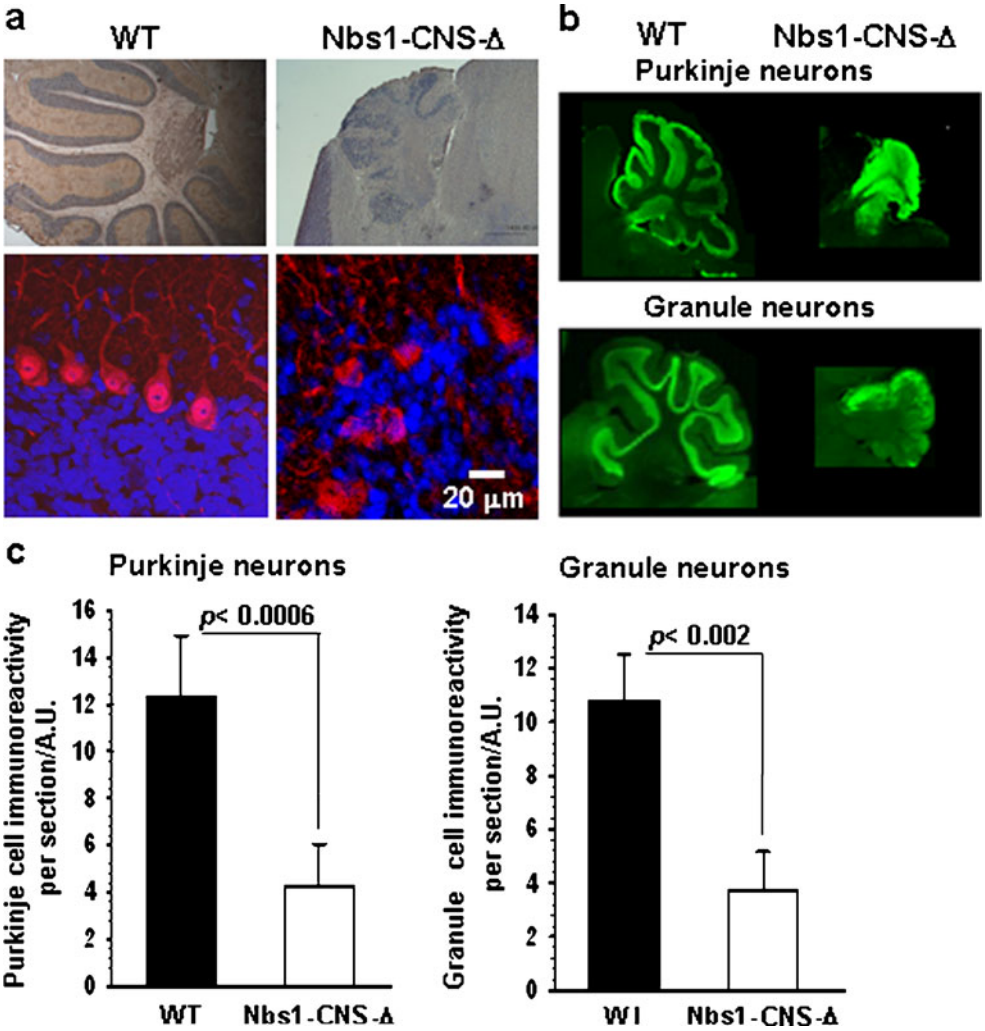

Fig. 2 Nbs1 inactivation associates with Purkinje and granule neuronal attrition in Nbs1-CNS-Δ mice. **a** (*Upper panel*) Cerebellar sections stained with Hematoxylin & Eosin. (*Lower panel*) Confocal micrographs of frozen cerebellar sections immunoreacted with mouse α-calbindin antibody (*red*, Purkinje cell marker) and Sytox blue (a marker of cell nuclei). *Bar* 20 μm. **b** Cerebellar sections derived from WT and Nbs1-CNS-Δ immunoreacted with Purkinje cells: α-calbindin antibody and granule cells: Rabbit α-GABA-α6 receptor antibody using Odyssey system and analyzed **c** using relative intensity arbitrary unit (A.U.; two-tailed Student's *t* test, $n=6$). *Nbs1* inactivation leads to marked cerebellar disorganization. *Error bars* represent SEM

chemokine Ligand 2 (CCL2) is the main chemokine attracting CD11b- positive cells and mediates the innate immune response in the CNS (Huang et al. 2001). We found a 56% reduction in the expression level of CCL2 in cerebella of Nbs1-CNS-Δ mice as compared to WT mice (Fig. 4c). Furthermore, the expression levels of the cysteine–cysteine chemokine receptor 2 (CCR2) was also significantly down-regulated in Nbs1-CNS-Δ cerebella compared with WT mice (Fig. 4c). Recent evidence indicates that a CCR2 deficiency accelerates the severity of neurodegenerative disease and markedly impairs microglial cell accumulation in the mouse brain (El Khoury et al. 2007).

Reduction in Glia Neurotrophic Mediators in Nbs1-CNS-Δ Mice

Neurotrophic factors play an important role in the maintenance of the CNS homeostasis. Astrocytes are known to produce and secrete their own repertoire of neurotrophic factors, including BDNF and NT3 (Ma et al. 2005). Recent data suggest that a dysfunction in glial cell activity may contribute to the pathogenic process that leads to neurodegenerative diseases (Ma et al. 2005). RT-PCR analysis revealed a significant reduction in the levels of BDNF and NT3 in the cerebellum of Nbs1-CNS-Δ mice (Fig. 5a, b). These results were in line with a reduction in the levels of the corresponding proteins (Fig. 5c). Furthermore, while in WT animals, BDNF-positive cells surrounded the Purkinje cell layer, a marked reduction of these cells in Nbs1-CNS-Δ mice was observed (Fig. 5a, c). This finding suggests that multiple deficiencies in glial cell activation might be linked to the neurodegenerative process in Nbs1-CNS-Δ mice. Collectively, these results suggest that astrocytic cell dysfunction in Nbs1-CNS-Δ mice plays a major role in CNS abnormalities that are reminiscent of the clinical symptoms of NBS.

Discussion

As shown in this study, Nbs1 depletion in Nbs1-CNS-Δ mice results in a marked reduction in astrocytic cell activity as

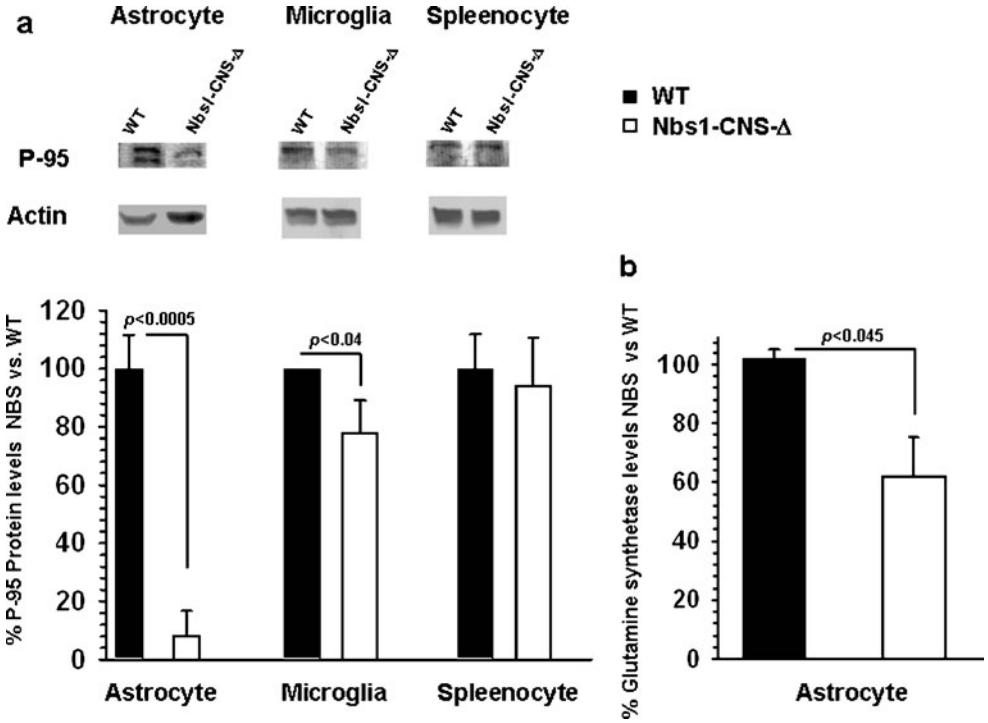

Fig. 3 Reduction in Nbs1 expression is accompanied with reduced GS activity in astrocytes derived from Nbs1-CNS-Δ mice. **a** Western blot analysis displaying reduction in Nbs1 (P-95; protein levels in astrocyte, microglia and spleenocyte in Nbs1-CNS-Δ mice (n=4)). While the levels of Nbs1 are significantly reduced in astrocyte in Nbs1-CNS-Δ mice, there is only slight but significant reduction in Nbs1 levels in microglia. **b** Western blot analysis displaying significant reduction in GS in astrocyte in Nbs1-CNS-Δ mice. *Error bars* represent SEM (statistical analysis was performed using two-tailed Student's t test)

shown by a reduction in the levels of GS and reduced release of chemocines and neurotrophic factors. Furthermore, one of the most striking features of this study is the linkage between Nbs1 deficiency in non-neuronal cells rather than in neuronal Purkinje cells that results in CNS abnormalities.

Glial cells are not only essential for maintaining a healthy well-functioning brain, but they also protect the brain and enhance the functional recovery from injuries. Moreover, glial cells play an essential role in synaptic transmission, thereby modulating neuronal activity (Giaume et al. 2007). The activation of glial cells in the CNS is the first defense mechanism against pathological abnormalities that occur in neurodegenerative diseases (Farfara et al. 2008). Bergmann glia forms an ornate and highly organized meshwork of radial processes in the cerebellar cortex. This striking architecture has long been recognized and recently shown to play a role in the development of cerebellar neural circuits (Bellamy 2006; Colon-Ramos and Shen 2008; Sudarov and Joyner 2007).

The current view of neurodegenerative diseases portrays them by impaired functionality of the brain. Brain functionality is influenced by parameters such as the number of cells in specific neuronal circuits, interactions among neuronal cells, interactions among different neuronal circuits, level of organization of each circuit, and functionality of the cells and their environmental in each circuit. Although traditionally viewed as diseases mainly affecting the most vulnerable neurons, in most instances of inherited disease, the causative genes may affect the activity of non-neuronal cells. Damage to a specific set of non-neuronal cells may account for the selective susceptibility of neuronal subtypes in many human neurodegenerative diseases such as ALS (Ilieva et al. 2009). It was reported that in ALS, mutations in the gene encoding Cu/Zn superoxide dismutase (SOD1) have been implicated in a fraction of familial cases of the disease (Gros-Louis et al. 2006). Transgenic mouse models with *SOD1* mutations develop pathology reminiscent of the disorder, including progressive death of motor neurons (Julien and Kriz 2006). Recent works in *SOD1* mutant mice suggest that damage in motor neurons is enhanced by damage incurred by non-neuronal neighboring cells expressing mutant *SOD1* (Ilieva et al. 2009). Furthermore, in the mouse model where glial cells don't express mutant *SOD1*, degeneration is delayed, and mutant-expressing motor neurons (Clement et al. 2003) demonstrate a significantly extended survival. These results are in line with our current findings.

Different signals can mediate and influence the fate of glial cell activation. It has been shown that in CCR2−/− mice, microglia failed to migrate in the CNS (El Khoury et al. 2007). CCR2, which alters microglia's ability to sense their environment, can lead to neurological diseases such as Alzheimer's disease (Hanisch and Kettenmann 2007). We have shown a significant reduction in CCR2 that correlates with the levels of microglial cells (CD11b+). Astrocytes have been implicated as the major source of CCL2/monocyte chemoattractant protein-1 in the CNS (Kalehua et al. 2004). Interestingly, in Nbs1-CNS-Δ mice, the dramatic reduction in the number of Purkinje and granule cells was also linked to a reduction of microglial cells (CD11b+) but not to

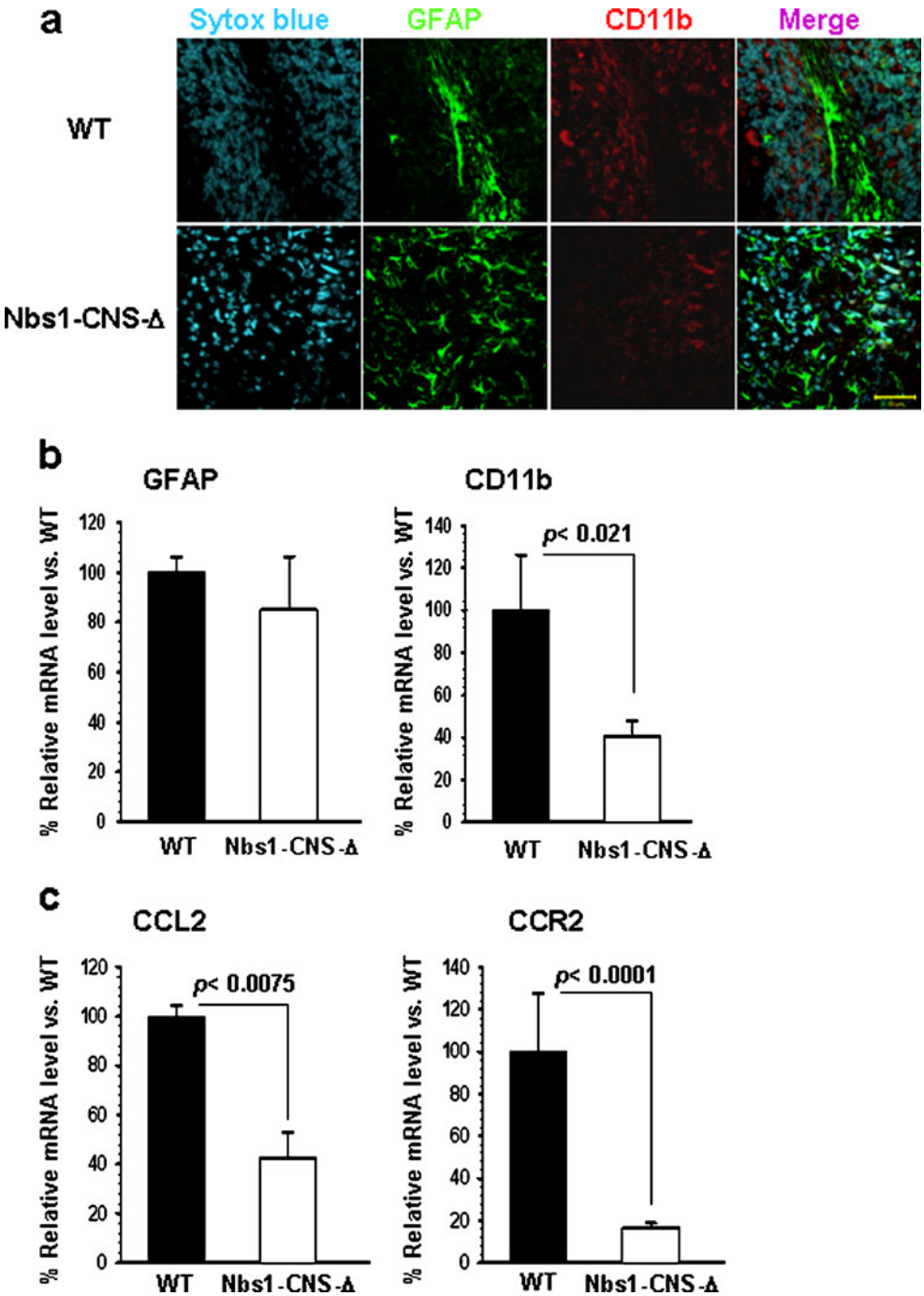

Fig. 4 Reduction in cerebellar microglia in Nbs1-CNS-Δ mice. **a** Immunofluorescent staining of cerebellar sections immunoreacted with Rabbit anti-GFAP (astrocyte marker, *green*) and α-CD11b (microglia marker, *red*) antibodies. While the levels of CD11b are reduced in Nbs1-CNS-Δ cerebella, GFAP levels are not altered in the mutant mice but display marked disorganization. *Bar*=20 μm. **b** RT-PCR analysis measuring the expression of GFAP and CD11b vs. housekeeping gene ACTB-full name (two-tailed Student's *t* test, n=4). *Error bars* represent SEM. **c** Reduction in CCL2 and CCR2 gene expressions vs. housekeeping gene ACTB in Nbs1-CNS-Δ mice as evident by quantitative RT-PCR analysis. *Error bars* represent SEM (two-tailed Student's *t* test, n=4)

astrocyte (GFAP+), suggesting an impairment in astrocytic functionality. Astrocytes are the most abundant cells in the CNS and most likely play an important role in the homeostasis and maintenance of the brain (Nimmerjahn 2009). They emerged as sophisticated cells participating in a large and diverse variety of functions vital for normal brain development and adult physiology and pathology (Nimmerjahn 2009). Swanson et al. suggest that astrocytes influence neuronal survival following stress (Swanson et al. 2004).

While glutamate is an important neurotransmitter, an excess of glutamate is thought to be toxic for neurons (Hardin-Pouzet et al. 1997). In Nbs1-CNS-Δ mice, a reduction in the expression of a glutamate-degrading enzyme such as GS might lead to a loss of the ability to modify local glutamate levels, which may contribute to neurodegeneration (Hardin-Pouzet et al. 1997). Our results are further supported by recent evidence that in the related syndrome A-T, the *ATM* gene is required for normal astrocytic cell growth (Kim and Wong 2009). In other words, mutations in the *ATM* gene may lead to astrocyte death or failure to propagate, resulting in neuronal pathology.

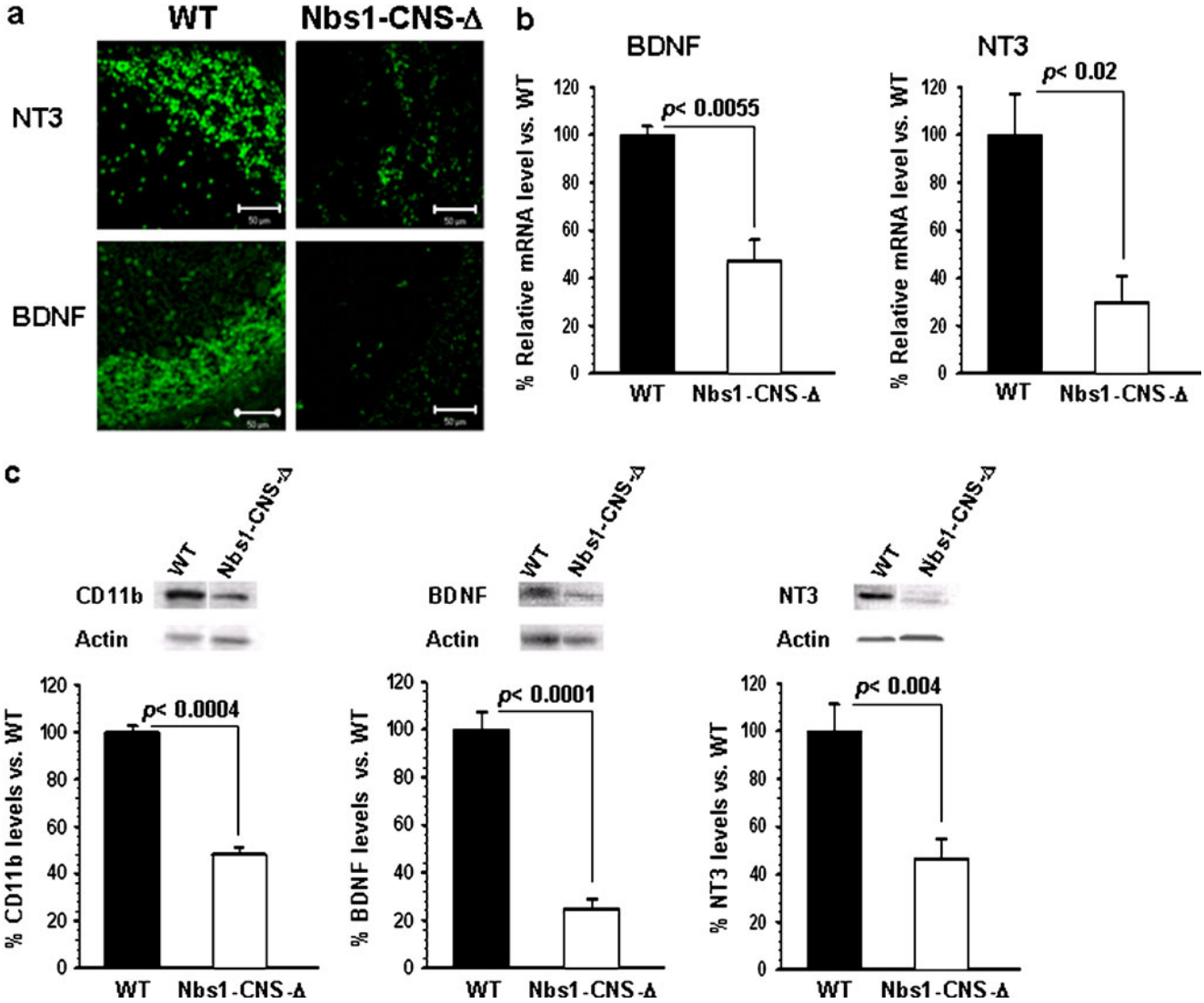

Fig. 5 Reduction in neurotrophic mediators in Nbs1-CNS-Δ mice. **a** Immunostaining showing the reduction in BDNF and NT-3 in the cerebellum of Nbs1-CNS-Δ mice vs. WT. *Bar*=50 μm. **b** Reduction in BDNF and NT3 gene expressions vs. housekeeping gene ACTB in Nbs1-CNS-Δ mice as evident by quantitative RT-PCR analysis. *Error bars* represent SEM (n=4). **c** Western blot analysis displaying reduction in CD11b, BDNF, and NT-3 (protein levels in Nbs1-CNS-Δ mice (n=3) and NT3 (n=3). *Error bars* represent SEM (statistical analysis was performed using two-tailed Student's t test)

In line with our results, it was reported that the deletion of Rad50, a component of the MRN complex, in post-mitotic Purkinje cells did not phenocopy the mild ataxia observed in Atm−/− mice (Adelman et al. 2009). This raises the possibility that events during neuronal development that may be linked to glial dysfunction may contribute to Purkinje cell loss in A-T and AT-like disorder patients. This interpretation is consistent with the report of ataxia in Nestin-Cre mice that lose Nbs1 during development of the nervous system (Frappart et al. 2005). Adelman et al. suggested that the MRN complex is not required for the viability of quiescence or postmitotic but is indispensable for the viability of proliferative cells (Adelman et al. 2009). Since glial cells are considered more proliferative cells compared to neurons, they are more prone to be affected by the inactivation of the MRN complex. The loss of MRN in glial cells should reduce their functionality, thereby affecting the environment in which neurons function.

We therefore suggest the following model for NBS disease as mediated by astrocytic functionality. In WT brains, both glia and neurons are healthy and capable of repairing DNA damage, thereby generating a fully supportive environment in which the neuronal cells are fully functional. In Nbs1-CNS-Δ brains, Nbs1-deleted glial cells are not fully functional, and as a consequence, they generate a "sick" environment, which severely affects the functionality of the remaining Nbs1-deleted neuron.

Our results indicate the crucial role of astrocytes in modulating neuronal survival. Indeed, glial cells produce a repertoire of neurotrophic factors, including BDNF, NT3, insulin-like growth factor-I (IGF-1), and vascular endothelial growth factor (Farfara et al. 2008). Here, we show that astrocytic impairment is linked to a reduction in CNS levels of BDNF and NT3. Loss of sensitivity to neurotrophic factors, such as IGF-I in ataxia telangiectasia, has been reported, and treatment with IGF-I has proven effective in animal models of cerebellar ataxia (Fernandez et al. 2005).

Here, we suggest an important regulatory mechanism of glial cells in the mouse brain and a likely important factor in the etiology of genomic instability disorders in humans, such as NBS, which severely affects the central nervous system. Glial cells make up the largest cell population in the brain and are continuously renewed over the course of a human's lifetime, which makes them an attractive target for therapeutic intervention in neurodegenerative disorders. Hence, modifying the activity of glial cells to enhance their neuroprotective features (such as BDNF and NT3 and recruitment of microglia) may hold promise as a new direction in the search for therapeutic interventions in genomic instability neurodegenerative disorders.

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