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Brief communication

Mutant mice with small amounts of BubR1 display accelerated age-related gliosis

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Abstract

Aging is an intricate biological process thought to involve multiple molecular pathways. The spindle assembly checkpoint protein BubR1 has recently been implicated in aging since mutant mice that have small amounts of this protein (BubR1$^{H/H}$ mice) develop several early aging-associated phenotypes. The phenotype within the brain of BubR1$^{H/H}$ mice has not yet been established. Here we show that BubR1$^{H/H}$ mice exhibit features of age-related cerebral degeneration. We found that glial fibrillary acidic protein (GFAP), a marker of reactive astrogliosis, was expressed at increased levels in the cortex and thalamus of BubR1$^{H/H}$ mice as early as 1 month of age. Furthermore, CD11b, a marker of microgliosis, was markedly elevated in the cortex and hippocampus of BubR1$^{H/H}$ mice at 5 months of age. Levels of both GFAP and CD11b further increased with age. Our results demonstrate that BubR1 acts to prevent cerebral gliosis of both astrocytes and microglial cells, and suggest a role for BubR1 in the aging process of the brain.

Keywords: BubR1; Gliosis; GFAP; CD11b; Astrocyte; Microglia

1. Introduction

Age-associated gliosis is well documented in many species, including mice, rats, monkeys and humans. Reactive astrocytosis is perhaps the most consistently observed finding and is known to occur as part of the normal aging process [2,7,11,12,14,18,23,24,26]. Astrocytes are believed to protect neurons by acting as metabolic buffers, electrical isolators, and detoxifiers and outnumber neurons by 10-fold [24]. Glial fibrillary acidic protein is a major cytoskeleton protein that is specific for astrocytes and is frequently used as an immunohistochemical marker to demonstrate hyperplasia and hypertrophy of astrocytes in aging [18,26]. Microglia are the major phagocytes in the central nervous system and are responsible for protecting the brain from infection as well as removal of cellular debris [11,14]. It has also been shown that microglia-associated antigens are elevated in normal aging [16] and age-associated neurodegeneration [14], and are frequently aggregated around dying neurons.

The molecular mechanisms governing age-related cellular deterioration are complex and poorly understood. Studies of genetically modified mouse models have led to the identification of several potential regulators of the aging process [8]. A recently identified regulator of aging in mice is the mitotic checkpoint protein BubR1 [1]. Faithful segregation of replicated chromosomes during mitosis is essential for maintenance of genetic stability and is monitored by several mitotic checkpoint proteins, including BubR1, Bub1, Bub3, Mad1 and Mad2. Mice that express only about 10% of

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normal levels of BubR1, so-called BubR1$^{H/H}$ mice, undergo chromosome missegregation at high frequency and become increasingly aneuploid as they age [1]. The lifespan of these mice is on average four- to five-fold shorter than that of wild-type mice of the same genetic background. At an early age, BubR1$^{H/H}$ mice develop various progeroid features, including dwarfism, cataracts, lordokyphosis, cachexia, loss of subcutaneous fat, impaired wound healing, and infertility. Several early aging-associated phenotypes that characterize some of the other mouse models of aging are not seen in BubR1$^{H/H}$ mice, including glucose intolerance, loss of hematopoietic stem cells reserves, hair graying and alopecia, osteoporosis and atherosclerosis [4,6,8,15,19–22]. Consistent with the development of early aging-associated phenotypes, murine embryonic fibroblasts (MEFs) from BubR1$^{H/H}$ senesce prematurely and express increased levels of the senescence-associated markers p53, p21, p16, and p19. Notably, as normal mice age, BubR1 expression has been shown to decline in multiple tissues, including testis, ovary, and spleen, further supporting the theory that BubR1 might be a key regulator of certain aging processes. It has not yet been determined whether BubR1$^{H/H}$ mice or other genetically modified mouse models of aging undergo accelerated brain aging or develop specific pathologies associated with age-related neurodegenerative diseases. Here we report on a screening of brains from BubR1$^{H/H}$ mice for such phenotypes.

2. Materials and methods

2.1. Mice

BubR1$^{H/H}$ mice were generated as previously reported [1]. All mice were of a 129/C57BL/6 mixed background. They were housed in pathogen-free barrier and were given standard diet and water ad libitum. Mice were kept at 22 °C on a 12-h light/dark cycle.

2.2. Histology

BubR1$^{H/H}$ mice were sacrificed for histological evaluations at 1 ($n = 2$ females), 2 ($n = 1$ male and 1 female), 5 ($n = 1$ male and 2 females), and 12 ($n = 2$ females) months of age. BubR1$^{+/+}$ mice were sacrificed for histological evaluations at 1 ($n = 2$ males), 2 ($n = 1$ male and 1 female), 5–7 ($n = 1$ male and 1 female), 12 ($n = 2$ females), and 35 ($n = 1$ male) months of age. Following an overdose of sodium pentobarbital (200 mg/kg, IP), they were perfused transcardially with 15 ml of 10 mM sodium phosphate, 138 mM sodium chloride, pH 7.4 (PBS) followed by 15 ml of 10% formalin in 100 mM sodium phosphate, pH 7.4. The brains were dissected out and further fixed in formalin overnight. The following day the brains were transferred to 10% sucrose, 100 mM sodium phosphate, pH 7.4 followed by 30% sucrose, 100 mM sodium phosphate, pH 7.4, for 24 h each for cryoprotection. Frozen, coronal sections (30 μm) were cut with a sliding microtome throughout the full extent of the cerebral cortex. All sections were saved and stored in 2 mM sodium azide in PBS at 4 °C until mounted or stained. Representative sections throughout the brain were mounted on gelatin-subbed slides and dried overnight at 37 °C for cresyl violet staining of Nissl substance and thioflavin S staining for possible amyloid deposits. For Nissl staining, sections from each animal were stained for 15 min with 0.1% cresyl violet. The sections were rinsed, dehydrated, and cleared through graded ethanols and xylene and then coverslipped with a xylene-based mounting media. For thioflavin S staining, the sections were stained with fresh, filtered, aqueous 1% thioflavin S (Sigma, St. Louis, MO) for 5 min after staining for 3 min with Mayer's hematoxylin to block nuclear autofluorescence. The sections were rinsed in two changes of distilled water and dehydrated in two changes of 70% ethanol. The slides were then coverslipped with Vectashield (Vector Laboratories, Burlingame, CA) to prevent fading.

2.3. Immunohistochemistry

Other sets of sections from each animal underwent immunohistochemical (IH) processing with antibodies raised against several age-related markers ($n \geq 4$ brain sections of each animal were used in each staining). These markers included: amyloid β (Aβ) peptide, α-synuclein, phosphorylated tau, several oxidative stress/apoptosis-related markers (Caspase-3, Jun-terminal (JNK) stress-activated kinase, p38 MAP kinase, neuronal nitric oxide synthase, nitrotyrosine), ubiquitin, and astrocytic (GFAP) and microglial (CD11b) markers: mouse anti-Aβ (residues 17–24; clone 4G8; 1:1000; #9220; Signet Laboratories, Dedham, MA), mouse anti-alpha-synuclein (clone LB509, 1:200; Zymed, South San Francisco, CA), mouse anti-phospho-tau (Ser202; clone AT8, 1:1000; Innogenetics, Alpharetta, GA), mouse anti-ubiquitin (clone Ubi-1; 1:250; #MAB1510; Chemicon, Temecula, CA), rabbit anti-GFAP (glial fibrillary acidic protein; 1:1000; #AB5040; Chemicon, Temecula, CA), rat anti-mouse CD11b (MAC 1; 1:100; #CBL1313; Chemicon), rabbit anti-active Caspase-3 (1:200; #G748; Promega, Madison, WI), rabbit anti-phospho-SAPK/JNK (stress activated protein kinase/Jun-terminal kinase; 1:500; #9251; Cell Signaling Technology, Beverly, MA), rabbit anti-phospho-p38 MAP kinase (1:200; #9211; Cell Signaling Technology), rabbit anti-neuronal nitric oxide synthase (nNOS; 1:500; #AB1552; Chemicon), rabbit anti-nitrotyrosine (3NT; 1:200; #06-284; Upstate, Charlottesville, VA). Free-floating sections were stained by standard immunoperoxidase methods using Vectastain Elite ABC kits (Vector Laboratories). The sections were first rinsed and permeabilized in PBS containing 0.3% Triton X-100 (PBST) for 5 min at room temperature. The endogenous peroxidase activity in the sections was blocked by reacting with 0.5% H_2O_2 in PBST for 30 min. The sec-