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Research report

Behavioral abnormalities in mice lacking mesenchyme-specific *Pten*

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HIGHLIGHTS

- *Pten* was deleted under control of the Fibroblast-specific protein 1 (FSP1) promoter.
- $Pten^{-/-}$ mice behaved remarkably normal in all behavioral tasks except the rotarod.
- $Pten^{-/-}$ mice had reduced hippocampal sizes.

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ABSTRACT

Phosphatase and tensin homolog (*Pten*) is a negative regulator of cell proliferation and growth. Using a *Cre*-recombinase approach with *Lox* sequences flanking the fibroblast-specific protein 1 (Fsp1 *aka* S100A4; a mesenchymal marker), we probed sites of expression using a β-galactosidase $Rosa26^{LoxP}$ reporter allele; the transgene driving deletion of *Pten* (exons 4–5) was found throughout the brain parenchyma and pituitary, suggesting that deletion of *Pten* in Fsp1-positive cells may influence behavior. Because CNS-specific deletion of *Pten* influences social and anxiety-like behaviors and S100A4 is expressed in astrocytes, we predicted that loss of *Pten* in Fsp1-expressing cells would result in deficits in social interaction and increased anxiety. We further predicted that environmental enrichment would compensate for genetic deficits in these behaviors. We conducted a battery of behavioral assays on $Fsp1\text{-}Cre;Pten^{LoxP/LoxP}$ male and female homozygous knockouts ($Pten^{-/-}$) and compared their behavior to $Pten^{LoxP/LoxP}$ ($Pten^{+/+}$) conspecifics. Despite extensive physical differences (including reduced hippocampal size) and deficits in sensorimotor function, $Pten^{-/-}$ mice behaved remarkably similar to control mice on nearly all behavioral tasks. These results suggest that the social and anxiety-like phenotypes observed in CNS-specific $Pten^{-/-}$ mice may depend on neuronal *Pten*, as lack of *Pten* in Fsp1-expressing cells of the CNS had little effect on these behaviors.

1. Introduction

Phosphatase and tensin homolog (*Pten*) is involved in regulating proliferation and apoptosis (reviewed in: [1]). *Pten* is mostly known as a tumor suppressor [2]. Tissue-specific deletion of *Pten* in brain leads to increased neuronal size and brain enlargement, causing seizures and ataxia [3]. CNS-specific *Pten* insufficiency is also associated with deficits in social behavior in mice, a phenotype that has been compared to autism spectrum disorder in humans [4–6]. Loss of *Pten* causes macrocephaly due to neural and astrocytic soma hypertrophy and increased astrocyte proliferation [7], and CNS-specific *Pten* is a dynamic regulator of brain growth through interactions with β-catenin signaling [8]. Although fibroblast-specific protein 1 (Fsp1 aka S100A4) is known mostly as a mesenchymal (mainly lymphatic and connective tissue precursor) marker [9,10] and having roles in driving metastasis [11], the S100A4 protein is also expressed in the spinal cord, nerves and brain white matter [12–15]. Indeed, S100A4 overexpression in human and rodent brain astrocytes is associated with brain damage and neurodegeneration [16]. A *Cre*-recombinase approach

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using the Fsp1 (S100A4) promoter was developed targeting *Pten* to study the effects of *Pten* deletion in stromal fibroblasts on tumor cell dynamics [17]. Upon examination of *Fsp1-Cre* expression sites using the β-galactosidase *Rosa26*^{LoxP} reporter allele in the *Fsp1-Cre;Pten*$^{LoxP/LoxP}$ (*Pten*$^{-/-}$) mice, the transgene was also found in the brain parenchyma and pituitary. This discovery raised the possibility that the Fsp1 promoter could be used for manipulation of *Pten* in the nervous system to influence behavior.

Therefore, we sought to evaluate the behavioral phenotype of mice deficient in *Pten* under the control of the Fsp1 (S100A4) promoter. We hypothesized that mesenchymal (and putatively white matter astrocyte) cell-specific deletion of *Pten* would alter behavior. Specifically, we predicted that social and anxiety-like behaviors would be altered in mice lacking *Pten* in *Fsp1*-expressing cells, and that enriched environments would ameliorate these behavioral alterations.

2. Methods

2.1. Generation and genotyping of Fsp1-Cre;Pten$^{LoxP/LoxP}$ mice

Generation of *Fsp1-Cre;Pten*$^{LoxP/LoxP}$ and *Pten*$^{LoxP/LoxP}$ mice, hereafter referred to as *Pten*$^{-/-}$and *Pten*$^{+/+}$respectively, has been previously described [17]. Briefly, exons 4–5 of *Pten* in the N-terminal region were deleted in Fsp1-expressing cells. Because the *Fsp1-Cre; Pten*$^{LoxP/LoxP}$ mice are infertile, we adopted the following strategy: "Fathers" were *Fsp1-Cre;Pten*$^{LoxP/WT}$ males crossed with *Pten*$^{LoxP/LoxP}$ females ("mothers"). The Fsp1-Cre transgene was inherited through the father side only. There was no maternal effect because female mice were *Pten*$^{LoxP/LoxP}$ mice. The *Fsp1-Cre;Pten*$^{LoxP/LoxP}$ (*Pten*$^{-/-}$) and the *Pten*$^{LoxP/LoxP}$ (*Pten*$^{+/+}$) mice used in the study were littermates. Animals were maintained and euthanized following The Ohio State University institutional guidelines. Tenth-generation congenic (N10) FVB/N animals were used for all mouse studies presented in this manuscript. The studies were approved by the OSU Institutional Animal Care and Use Committee.

2.2. Experimental design

Six- to eight-week-old *Pten*$^{-/-}$and *Pten*$^{+/+}$ mice of both sexes were maintained by sex (1–4 per cage) in filter-capped polypropylene cages (27.8 × 7.5 × 13 cm) with corncob bedding on a 14:10 light-dark cycle. Mice were supplied a cotton nestlet and provided *ad libitum* access to pelleted food (H8640; Harlan-Teklad) and filter-purified tap water. Room temperature remained at 22 ± 2 °C for the duration of the study.

After acclimating to the facility for ∼1 wk, mice were randomly assigned to groups (enriched vs. singly housed). Animals assigned to the 'enriched' group were housed (7–9 per cage) in a large rat cage (45 × 25 × 20 cm) with a running wheel, multiple colored translucent plastic platforms and tubes, and multiple cotton nestlets. Mice of both genotypes were mixed in enriched chambers, but sexes remained separated. Animals assigned to the 'singly housed' group were maintained alone in a standard cage (27.8 × 7.5 × 13 cm) and provided only with a single cotton nestlet. Due to persistent fighting and aggression within the male enrichment chamber, males were divided into 'singly housed' cages with running wheels after 2 wks.

2.3. Behavior

After approximately 5 wks in their respective groups, mice underwent a battery of standard behavioral phenotyping tests. These tests were administered over five days. Tests were ordered so that less stressful procedures (based on our previous experiences) were completed before more stressful tests. Sensorimotor (including rotarod) tests were completed first, followed by novel object testing, Y-maze, elevated plus maze, sociability, and then aggression. All tests, except the sensorimotor tasks, were completed during the early dark phase (0–3 h after "lights off") to coincide with the active phase in nocturnal mice. The novel object recognition test, elevated plus maze, Y-maze, social preference, and aggression tests were recorded on video tape and analyzed by a condition/genotype blind observer at a later time-point using Observer software (Noldus, Leesburg, VA). All other tasks were scored in real-time by an experimenter.

2.3.1. Initial assessment

Body weight (g), body length (cm), vibrissae condition, eye appearance, and muscle tone were assessed prior to behavioral testing. Body length was determined by measuring from the tip of the nose to the base of the tail. Vibrissae were assessed on a scale of 0–4. Mice with all whiskers present and in good condition (un-barbered) were given a score of 0. Mice with no whiskers were given a score of 4. Scores of 1–3 were assigned for mice with whisker condition lying between the two extremes. Eyes were examined for discrepancies in size, shape, and condition (0 = normal, 1 = mild abnormalities, 2 = severe abnormalities). These discrepancies included any accretion of material around the eye, deficits in eye opening, 'cloudy' eyes, or any otherwise 'non-normal' traits compared to Pten$^{+/+}$ mice of the same strain (FVB/N). Muscle tone was assessed while handling each animal and allowing it to climb on the examiner's hand using the 0–2 scale described above.

2.3.2. Sensorimotor reflexes

Sensorimotor function was assessed using visual placement, auditory orientation, contact placing, and olfactory discrimination tests as previously described [18]. Visual placement capability was determined by lowering the mouse slowly toward the edge of a table. A positive score was recorded if the animal extended its forepaws before touching Table on at least 2/3 trials. Ability to orient to an auditory cue was determined by sounding a 'clicker' ∼15 cm behind the mouse's head. A positive score was recorded if the animal turned its head toward the stimulus. Contact placement was determined by lowering the mouse toward the edge of a Table until its vibrissae touched the edge; this test was conducted in a room illuminated by dim red light (∼3 lx) to prevent the use of visual cues. The reaction of the animal to a touch on the vibrissae was scored on a scale of 0 (no response) to 3 (maximal response, indicated by a head turn in the direction of vibrissae contact). The olfactory discrimination test consisted of moving the mouse from its home cage for 1 min while a Froot Loop® cereal piece was hidden beneath 1 cm of home cage bedding. The mouse was then placed back in its cage, and the latency to locate the Froot Loop® was recorded. The test was terminated if the mouse could not locate the food item within 10 min.

2.3.3. Limb clasping

The mouse was suspended by its tail ∼30 cm above the surface of a table for 5 s on 3 trials. Normal mice typically extend limbs outwards in anticipation of being lowered back onto a horizontal surface [19]. A summed 'limb-clasping score' was given for all trials as follows: all limbs clasped or held against the body = 0; all limbs extended out = 3 (i.e., "normal"), and scores of 1 or 2 for stages between the two extremes.

2.3.4. Novel object recognition

To assess non-spatial memory function, mice were tested in the novel object paradigm [20] over the course of two days (as described in [21]).

Day 1: 0–2 h after lights were turned off, mice were placed into an empty 45 × 24 × 22 cm black chamber and allowed to freely