Introduction: Bach1 is a transcriptional repressor of heme oxygenase-1 gene. Heme oxygenase (HO)-1 is an inducible cytoprotective enzyme that degrades pro-oxidant heme to carbon monoxide and biliverdin/bilirubin, which are thought to mediate anti-inflammatory and anti-oxidant actions of HO-1 [1]. Previous reports demonstrated that the genetic ablation of Bach1 caused a marked reduction of tissue damage in myocardial infarction and arteriosclerosis in mouse model [2,3]. In the present study, we investigated the role of Bach1 in tissue protection after spinal cord injury in vivo using Bach1 knock-out (KO) mice and wild-type (WT) mice.

Materials and Methods: Animals Adult female Bach1 KO mice and WT mice between 8 and 10 weeks of age were used in this study. Congenic Bach1 KO mice were obtained by repeatedly backcrossing with C57BL/6J mice at least up to 12 generations.

Surgical procedures The T10 vertebra was laminectomized to expose the spinal cord. Spinal cord injury was induced with a modified NYU impactor.

Tissue preparations At 3 days and 42 days after spinal cord injury, mice were killed and the spinal cords at the lesion were fixed with 4% paraformaldehyde, embedded in paraffin. Serial 7μm transverse sections at 250μm intervals around the lesion center were mounted on slides.

Lesion size measurement The sections at 250μm intervals around the lesion center of the spinal cord at 42 days after spinal cord injury were stained with Luxol Fast Blue. The images of the stained specimen were captured by a digital photographic camera and the lesion area of the spinal cord was analyzed by Image J 1.37v software (National Institutes of Health).

NeuN-positive cell counts The tissue sections at the lesion center, 250μm caudal and rostral sides of the spinal cord at 42 days after spinal cord injury were incubated with mouse anti-NeuN antibody (1:100; Chemicon) and visualized by Alexa Fluor 488 goat anti-mouse IgG antibody (1:500; Molecular Probes). The number of NeuN-positive cells in each section was counted.

TUNEL-positive cell counts To investigate the apoptosis in the spinal cord at 3 days after spinal cord injury, terminal deoxynucleotidyl transferase-mediated dUDP nick end labeling (TUNEL) staining was performed with the In Situ Cell Death Detection Kit (Roche). The number of TUNEL-positive cells in the sections at the lesion center, 250μm caudal and rostral sides were counted.

Western blot analysis Mice were killed at 3 days after spinal cord injury, and their spinal cords were removed and homogenized in lysis buffer. Lysates were resolved by SDS–PAGE gels and transferred to a polyvinylidene difluoride membrane. To assess the level of HO-1 protein expression, immunoreactive bands were detected using rabbit anti-HO-1 antibody (1:1000; gift from Prof Taketani S). The expression of active caspase-3 protein was also analyzed using anti-active caspase-3 antibody (1:200; Santa Cruz Biotech.) to investigate the apoptosis in the spinal cord.

Behavioral analysis Motor function of the hindlimbs was evaluated by the locomotor rating test with the Basso mouse scale (BMS) for 6 weeks after spinal cord injury [4].

Results: Lesion size measurement The lesion area was significantly smaller in KO mice than in WT mice at 42 days after spinal cord injury (Fig.1).

NeuN-positive cells counts The numbers of NeuN-positive cells in KO mice were significantly higher than those in WT mice at 42 days after spinal cord injury (Fig. 2A).

TUNEL-positive cell counts The numbers of TUNEL-positive cells in WT mice were significantly higher than those in KO mice at 3 days after spinal cord injury (Fig. 2B).

Western blot analysis In KO mice, the expression of HO-1 protein was significantly increased compared to that in WT mice at 3 days after spinal cord injury. On the other hand, the expression of caspase-3 was significantly lower in KO mice than in WT mice at 3 days after spinal cord injury. Western blot analysis WT mice had significantly lower BMS score than KO mice from 3 days to 42 days after spinal cord injury (Fig. 3).

Discussion: In the present study, we have demonstrated that the genetic ablation of Bach1 caused marked reduction in neural tissue damage, apoptosis and impairment of locomotor function after spinal cord injury. The damage reducing effect was probably mediated, at least in part, by increased levels of HO-1 expression with the anti-inflammatory and anti-oxidant activity in Bach1 KO mice. We can conclude that Bach1 may represent a novel molecular target in the anti-inflammatory and anti-oxidant therapy to reduce the neural tissue damage, apoptosis and impairment of locomotor function after spinal cord injury.