ABSTRACT INTRODUCTION:
Bone marrow stromal cells (MSCs) have the capability under specific conditions of differentiating into various cell types such as osteocytes, chondrocytes, and adipocytes. They also can differentiate into neural lineages, but very few actually differentiate into functional neurons.

Neural progenitors isolated from the adult central nervous system differentiate into neuron and glia after transplantation into brain, and differentiate into oligodendrocytes and astrocytes after transplantation into spinal cord. It is necessary to improve motor function that transplanted cells differentiate neurons. We showed previously that von Hippel-Lindau (VHL) protein has potential to transform neural stem cell derived from the fetal rat cerebrum into neurons in vitro (1).
Recently, we succeeded that VHL peptide-transferred bone marrow stromal cells differentiated neurons in vitro.

In this study, we transplanted VHL peptide-transferred bone marrow stromal cells into the injured spinal cord of the rat and investigated functional recovery of the motor function.

METHODS:
Neuronal differentiation of MSCs: Mouse MSCs were provided by Dr. Okada (Pittsburgh University). EGFR gene had been transferred to the cells. Treatment with beta-macaptoethanol was followed by VHL-peptide transfer.

Spinal cord injury model: A total of 36 young adult male Wistar rats weighing between 200 and 250 g were used in the current study. Rats were equally divided into a control group, in which a sham operation was performed, and an injury group.

Rats were anesthetized with isofofuran before a laminectomy that consisted of removal only one spinal vertebra was performed at the 10th thoracic vertebra(T10). Spinal cord injury was performed using a spinal cord injury device(Pneumatic Injury Device PD-1000, Physio-Tech). This device creates a reliable contusion injury to the exposed spinal cord by rapidly applying a force-defined impact with a stainless steel-tipped impounder. The impounder tip was visually to approximately 50 mm above the laminectomy site. Injuries were applied by driving the rack downward at 1.20-1.30 m/sec until a preset force threshold was sensed by the load cell. The instrument was set to retract the impact tip immediately following the pre-determined injury level. The muscle and skin were closed in layers. After surgery, animals were injected with 33.3 mg/kg Cefazolin i.m. before being placed on a heating pad during recover. The bladder was manually expressed twice daily after spinal cord injury until automatic bladder control recovered, usually by 10 days following injury.

On day 7 after spinal cord injury, protein transduction domain connected VHL peptide-transferred bone marrow stromal cells pre-labeled in vitro with BrdU were transplanted with 25µl Hamilton syringe into the spinal cord. Control rats received intraspinal injections of culture medium alone as vehicle.

Locomotor function was evaluated using the Basso-Beattie-Bresnahan(BBB) Locomotor Rating Scale(Basso et al., 1995). Briefly, the BBB scale ranges from 0(no hindlimb movement) to 21(normal movement-coordinated gait with parallel paw placement). BBB scores were measured every day.

10days, 14days, 7weeks after spinal cord injury, the animals were given an overdose of sodium pentobarbital (60-100 mg/kg intraperitoneally) and were transcardically exsanguinated with 50 to 100ml physiological saline (0.9%) followed by 300ml of ice-cold 4% paraformaldehyde in 0.1 M PBS ( pH 7.4). The spinal cord was then removed and post-fixed for 24 hours in the same fixative at 4°C. A 20 mm length of the spinal segment centered on the injury and transplant sites was dissected and processed in paraﬁn wax.

The spinal segments were incubated for 1 hour at room temperature with primary antibodies. Antibodies against neurofilament-200 (Sigma) diluted 1:200, MAP-2 (Sigma) diluted 1:100, GFAP (Sigma), Nestin (PharMingen, San Diego, CA, USA), and BrdU (Boehringer Mannheim, Indianapolis, Indiana) were used. The spinal segments were rinsed in PBS and then incubated for 1 hour in either rhodamine-conjugated anti-mouse IgG or anti-rabbit IgG (Sigma). Counterstaining was achieved with DAPI (Molecular Probes), and observations were made with a fluorescence microscope system (Olympus, Tokyo, Japan).

RESULTS:
One day after VHL-peptide transfer, most of cells differentiated to neurons. Rats transplanted with VHL peptide-transferred bone marrow stromal cells showed improved functional recovery. 7weeks after spinal cord injury, there was a difference of three points on the BBB scale between groups; 15.1±0.7, sham operated;12.5±0.6, rats transplanted with VHL peptide-transferred bone marrow stromal cells. The latter score indicated weight supported ambulation, on the other hand, the former score indicated occasional weight supported plantar steps.

DISCUSSION:
We showed in a previous study that Lavendustin A enhances axon elongation in VHL gene-transfected neural stem cells (2). Axonal elongation is necessary for neural regeneration in the injured spinal cord in order to connect rostral and caudal sides of the injured cord when cells are transplanted. We think that treatment with lavendustin A before transplanted VHL peptide transfected MSCs would be useful.

Our study demonstrates that rats transplanted VHL peptide-transferred bone marrow stromal cells showed functional recovery. MSCs offer the possibility of autograft transplantation therapy, and our present study showed a clear functional recovery in the short term, we believe that further studies are necessary in order to allow clinical of this technique.