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Tetsuya I[1], Yoshiaki K[1], Osamu I[1], Hiroo I[1], Yuji M[1], Naoki A[1], Satoshi S[1], Masako K[1], Yasuhiko I[1], Osamu N[1]
1. Department of Urology, Shinshu University School of Medicine

MOUSE BONE MARROW-DERIVED CELLS CAN RECONSTRUCT SMOOTH MUSCLE LAYER-LIKE STRUCTURES IN INJURED URINARY BLADDER WALLS

Hypothesis / aims of study
Urinary bladder dysfunction is often associated with irreversibly damaged smooth muscle layers. The best remediation would be to replenish or replace the damaged tissues. This study is a preliminary investigation to determine whether cells derived from bone marrow can reconstruct smooth muscle layers in damaged urinary bladder walls. One of the strategies to regenerate the smooth muscle layers is to use bone marrow stem cells that are capable of developmental plasticity. In this study, cells harvested from mouse femurs were cultured *in vitro*. The adherent proliferating cells were defined as bone marrow-derived cells. These cells were harvested and then implanted into urinary bladder walls in which the smooth muscle layers were destroyed by freezing.

Study design, materials and methods
The femurs of five week-old male ICR mice (n = 5) were extirpated, and the bone marrow cells were flushed out, centrifuged, and then inoculated into a collagen-coated dish. The cells were cultured for 7 days with Dulbecco's Modified Eagle Medium including 15% fetal bovine serum. During culture, the medium was completely replaced daily, and non-attached cells were discarded. After 5 days of culture the cells were transfected with green fluorescence protein (GFP) for identification in recipient tissues. Seven days after seeding, adherent proliferating bone marrow-derived cells were dissociated and resuspended at 2.0×10^6 cells/ml with culture medium for injection into the injured bladders. The urinary bladder walls of 30 female 5-week-old nude mice, six per experiment, were exposed through abdominal midline incisions. A dry ice chilled iron bar (24 x 3 x 2 mm) was applied to approximately half of the posterior bladder wall for 30 seconds to briefly freeze it. The bladders were immediately thawed by body temperature and returned to the pelvic cavity. Three days after injury, the bladders were re-exposed. The injured region was identified by the presence of a hematoma, and the resuspended cells (1.0×10^5 cells, 50 ul) were injected into the wall (n = 3) by a microsyringe with a 30G-needle. For controls (n = 3), only the medium was injected. The injections were performed under a stereoscope, and confirmed by the presence of a small swelling formed by the injected cells or medium. Three days after cold injury, uninjected bladders (n = 9) were used to assess the degree of damage in the wound site. Fourteen days after implantation, the implanted bladders (n = 3), the control bladder (n = 1), and the 3-day cold-injured bladders (n = 3) were removed and double-stained with anti-GFP antibody and smooth muscle specific protein markers (alpha smooth muscle actin, SMA; smooth muscle myosin heavy chain, MHC; desmin; and calponin l), uroplakin 3 (a marker for urothelium), or proliferating cell nuclear antigen, PCNA. In addition, other samples were analyzed by quantization of smooth muscle specific gene expression.

Results
After 7 days of culture, adherent proliferating cells were relatively homogenous in spindle-shaped appearance. Before implantation, the injured areas had no smooth muscle layers, although there was a normal urothelium. For mice receiving the bone marrow-derived cell injection, SMA-positive smooth muscle layer-like structures were reconstructed with GFP-labeled cells in the damaged tissues by fourteen days after cell implantation. The cells were also positive for anti-PCNA, and some had other smooth muscle markers. Many of the cells in the smooth muscle layer-like structures were in contact with one another. In contrast, for mice receiving the cell-free control injection, the bladder walls did not have smooth muscle layer-like structures. The approximately 70% control-mice died within 14 days after operation. Quantitative analysis of smooth muscle specific gene expression also supported these observations.

Interpretation of results
In vitro cultured, GFP-labeled, adherent proliferating bone marrow-derived cells reconstructed smooth muscle layer-like structures in injured bladder walls. In contrast, control bladders that were not injected with the cells did not have these structures. The high death rate of the control mice was probably due to urinary leakage into the peritoneal space through the wound site. The bone marrow-derived cells were easily cultured, and the surviving cells had the developmental plasticity of stem cells with the potential to replenish or replace the smooth muscle layers in damaged bladders.

Concluding message
The implantation of bone marrow-derived cells may be developed as a useful treatment for urinary bladder dysfunction and also other lower urinary tract dysfunctions that require smooth muscle cells and/or layers.

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