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INVOLVEMENT OF SDF-1 IN RECRUITMENT OF ALPHA SMOOTH MUSCLE ACTIN-POSITIVE CELLS TO THE URINARY BLADDER.

Hypothesis / aims of study

Trials on the urinary bladder regeneration by acellular grafts have not always reported functional success because of suboptimal formation of smooth muscle layer, indicating understanding the process of muscle layer regeneration is essential [1]. We reported that marrow-derived stromal cells (MSC) migrated into smooth muscle layer of acellular grafts and expressed a smooth muscle cell-like phenotype [2], but the mechanism underlying MSC migration into regenerating bladder remains unknown. Meanwhile, stromal cell-derived factor 1 (SDF-1), which is well known to contribute to hematopoiesis, was reported to induce migration of non-hematopoietic stem cells for tissue regeneration [3]. Hence, we investigated the involvement of stromal cell-derived factor 1 (SDF-1) in bladder muscle regeneration in in vitro culture systems and two in vivo animal models.

Study design, materials and methods

1) *Detection of SDF-1 in conditioned media*: rat urothelial cell (UC) and bladder smooth muscle cell (BSMC) were incubated in αMEM and 15% fetal bovine serum (FBS) and those conditioned media (CM) were retrieved after 48h incubation. The concentration of SDF-1 in UC or BSMC-CM was evaluated with ELISA assay (n=3).
2) *Chemotaxis assay of MSC in media containing SDF-1:* Expression of CXCR4, a specific receptor for SDF-1 in MSC was confirmed by qPCR analysis. Chemotaxis of MSC was evaluated with the Boyden chamber migration method for 8 hours. For the experiment of adding SDF-1, MSC were treated with αMEM and 0.5% FBS in the presence or absence of 10 ng/ml human SDF-1 (n=3). For the experiment of blocking SDF-1, MSC were treated with αMEM and 15% FBS or BSMC-CM in the presence or absence of 5 μg/ml neutralizing anti-SDF-1 antibody (n=3).
3) *Bladder patch repair with Bladder acellular matrix (BAM)*: BAM was processed from wild-type SD rats. The upper one third portion of the bladder was resected and replaced with BAM graft (n = 5). Bladders were harvested 14 days after the operation. The expression of SDF-1 and characterization of the infiltrating cells was assessed by immunostaining.
4) *In vivo effect of SDF-1:* Acidic gelatin hydrogel was employed as a release carrier of SDF-1 (sham, 0, and 25 μg/site), and was fixed over rat bladder for 7 days (n=6). The expression of SDF-1 and characterization of the infiltrating cells was assessed by immunostaining.

Results

1) BSMC-CM showed significantly higher levels of SDF-1 than αMEM + 15%FBS or UC-CM.
2) The medium with SDF-1 significantly increased the number of migrated MSC than without SDF-1. BSMC-CM also increased the number of migrated MSC than control medium or UC-CM. Inhibition of SDF-1 in BSMC-CM with neutralizing antibody induced significant decrease in the number of migrated MSC.
3) SDF-1 was expressed in the whole graft area including a few cells positive for calponin, a SMC marker. Myofibroblast-like cells, double-positive for α□ smooth muscle actin (SMA) and collagen I, were observed in the grafts.
4) Granuloma formation was observed in the serosal region of the bladders treated with 25 μg/site of SDF-1. Myofibroblast-like cells, double-positive for αSMA and collagen I, were also observed in the newly formed granuloma tissue (Fig.).

Interpretation of results

SDF-1 was produced by bladder cells including BSMC and recruited cells having features of myofibroblast during regeneration.

Concluding message

SDF-1 could be a pivotal signal for inducing the inflammatory phase of bladder regeneration through migration of myofibroblast-like cells into the graft. This study may provide a molecular insight for new approaches to bladder regeneration using acellular grafts

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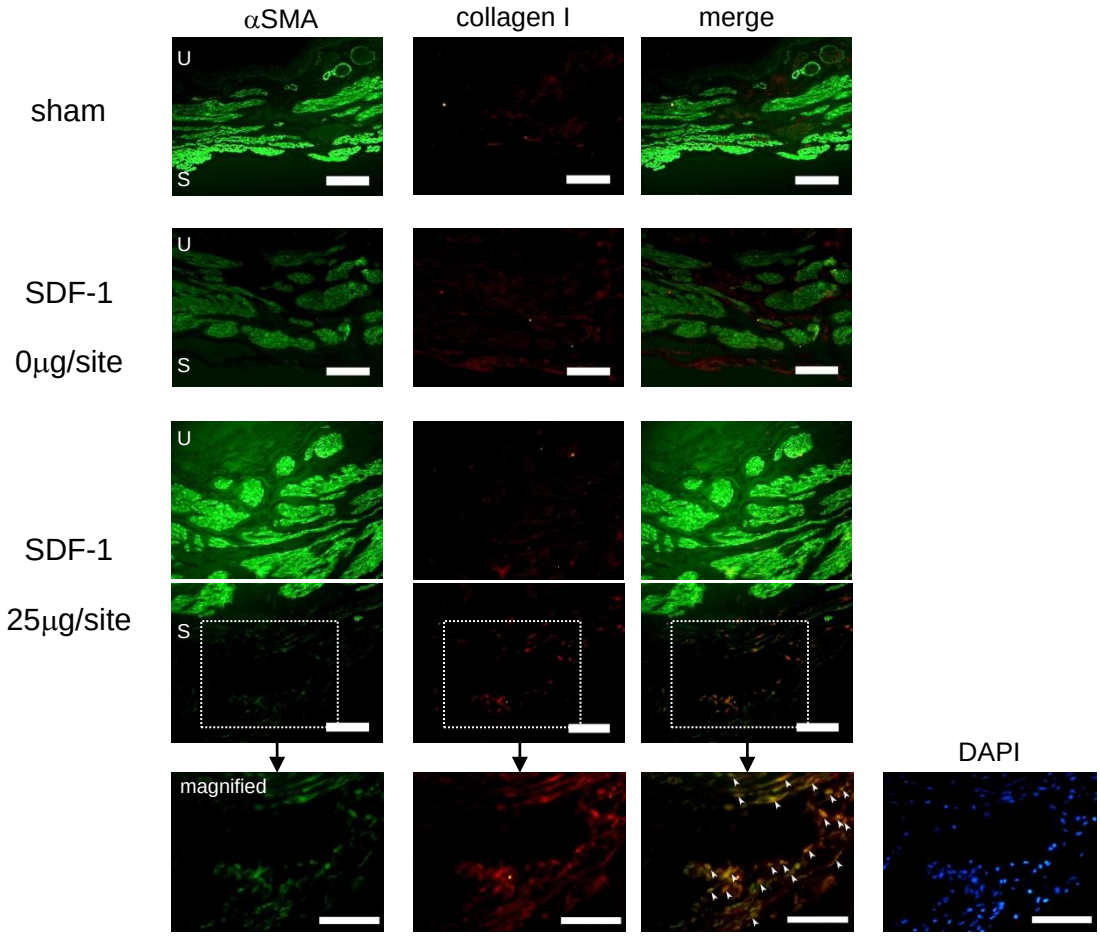

Fig.: Immunofluorescent study of rat bladders using gelatin hydrogels incorporating SDF-1 (n=6). In bladders treated with 25 μg/site of SDF-1, inflammatory tissue formation was observed in serosal layer. Cells positive for αSMA and collagen I were observed in these regions (arrows). No cell positive for αSMA and collagen I was observed in sham-operated bladders or bladders treated with 0 μg/site of SDF-1. Scale bars indicate 50 μm. U; urothelial layer side, S; serosal layer side.

References

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Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
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