Involvement of SDF-1 in recruitment of alpha smooth muscle actin-positive cells to the urinary bladder.

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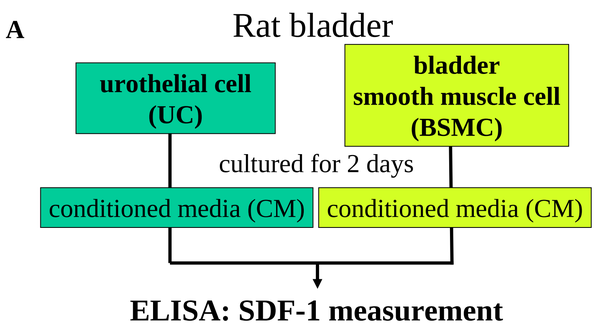
Abstract

Trials on the urinary bladder regeneration by acellular grafts have not always reported functional success because of suboptimal formation of smooth muscle layer[1]. We reported that marrow-derived stromal cells (MSC) migrated into acellular extracellular matrix grafts and expressed a smooth muscle cell-like phenotype in regenerated bladder tissue[2]. However, the mechanism causing migration of the MSC into regenerating bladder remains still unknown.

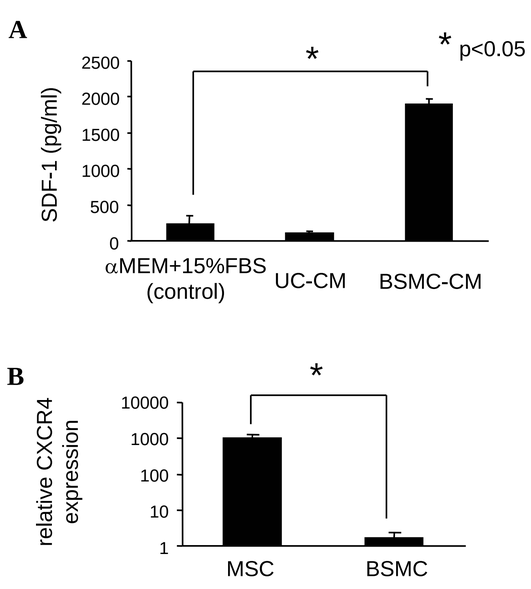
To elucidate the mechanism underlying cellular recruitment into the grafts, we investigated the involvement of stromal cell-derived factor 1 (SDF-1) in bladder muscle regeneration in in vitro culture systems and two in vivo animal models. In vitro culture system showed that SDF-1 secreted from bladder smooth muscle cells (BSMC) induced migration of MSC expressing alpha smooth muscle actin (SMA) but not calponin. In vivo bladder patch repair model with the acellular grafts showed SDF-1 expression and myofibrobalst-like cell migration in the whole graft including regenerating smooth muscle layer. Another in vivo model, where SDF-1 was released from gelatin hydrogels fixed on rat bladder walls, showed recruitment of myofibrobalst-like cells to the bladder. These data suggest that SDF-1 produced from regenerating BSMC recruit myofibroblast-like cells into the grafts, thereby contributing to the initial phase of bladder regeneration.

Detection of SDF-1 in conditioned media

Study design



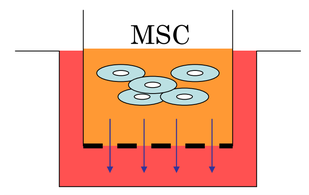
Results



SDF-1 was elevated in BSMC-CM when compared to control medium (A). Expression level of CXCR4, a specific receptor for SDF-1, was higher in MSC than in BSMC. (B).

Chemotaxis assay of MSC in media containing SDF-1

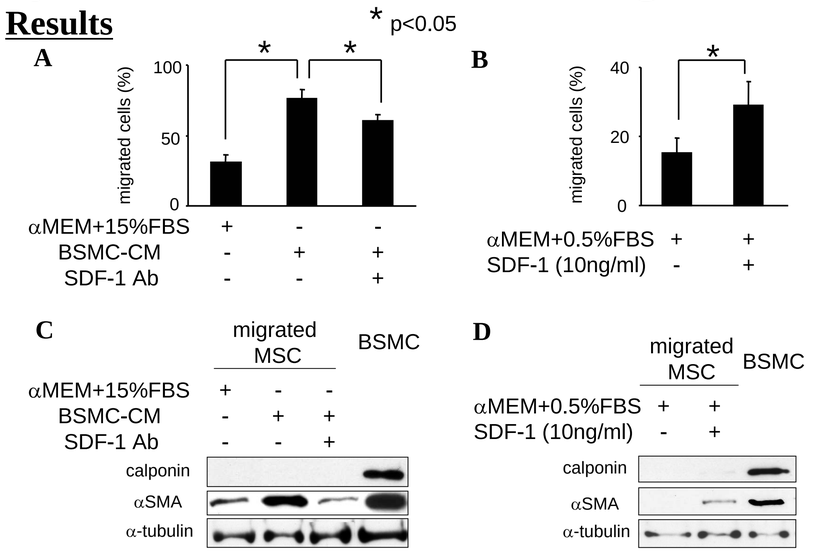
Study design



loss of function study: media
1. αMEM+15%FBS
2. BSMC-CM
3. BSMC-CM+SDF-1 Ab

gain of function study: media
1. αMEM+0.5%FBS
2. αMEM+0.5%FBS+SDF-1

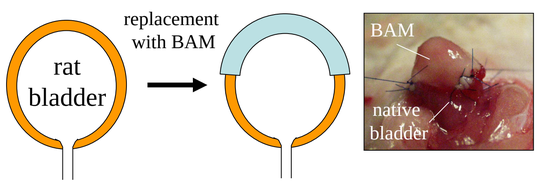
Results



BSMC-CM induced increase in migrated cell number which was inhibited with blockade of SDF-1 (A). SDF-1 treatment also induced increase in migrated cell number (B). Western blot showed that BSMC-CM induced αSMA upregulation in migrated MSC which was inhibited with blockade of SDF-1, but not calponin expression (C). SDF-1 treatment also induced αSMA upregulation in migrated MSC (D).

Bladder patch repair with bladder acellular matrix (BAM)

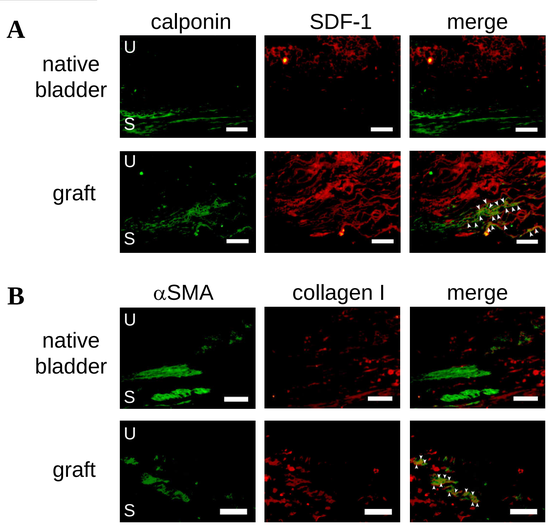
Study design



Immunofluorescence study

1. SDF-1 expression
2. myofibroblast-like cells detection (positive for αSMA & collagen I)

Results

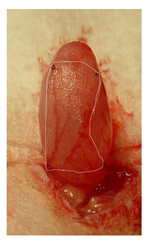


SDF-1 was expressed in urothelium of native bladder but in whole area of regenerating graft. Double-positive cells for calponin and SDF-1 were observed (arrows), indicating that proliferating smooth muscle cells expressed SDF-1 (A). Myofibroblast-like cells, double-positive for αSMA and collagen I, were observed in regenerating grafts (arrows) (B). U: urothelial side, S: serosal side, scale bars: 50 μm.

In vivo effect of SDF-1 on bladders using release carriers

Study design

gelatin hydorgel
as a release carrier of SDF-1



fixed over the bladder

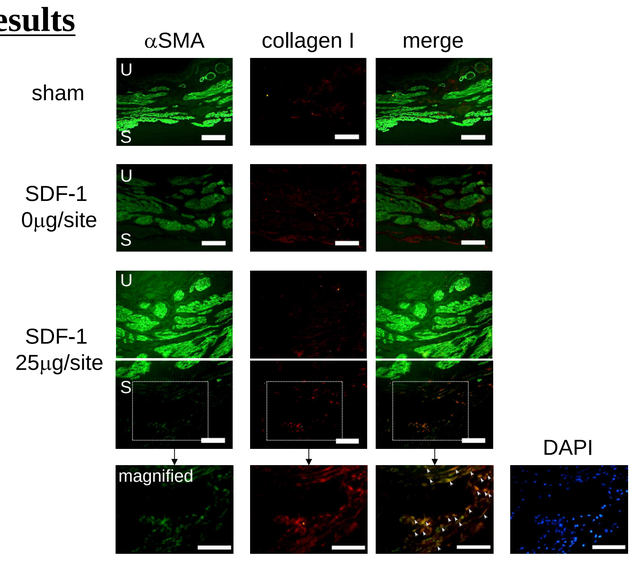
3 groups (n=4)
1 : sham op.
2 : 0 μg
3 : 25 μg
(SDF-1/site)

day 7

Immunofluorescence study

myofibroblast-like cells detection
(positive for αSMA & collagen I)

Results



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 (arrows) but not in sham-operated bladders or bladders without SDF-1.
U: urothelial side, S: serosal side, scale bars: 50 μm.

Summary/Conclusion

1. SDF-1 was produced by bladder cells including BSMC and recruited cells having features of myofibroblast during bladder regeneration.
2. Taking into account that myofibroblasts appear at the first inflammatory phase and play a role of tissue contraction in the secondary remodeling phase[3] , SDF-1 could be a pivotal signal for inducing the inflammatory phase of bladder regeneration through migration of myofibroblast-like cells into the graft.

References

1. Brown AL et al. 22 week assessment of bladder acellular matrix as a bladder augmentation material in a porcine model. *Biomaterials*.23:2179-90. 2002.
2. Kanematsu A et al. Induction of smooth muscle cell-like phenotype in marrow-derived cells among regenerating urinary bladder smooth muscle cells. *Am J Pathol*.166:565-73. 2005.
3. Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *J Clin Invest*.117:524-9. 2007.