Human amniotic fluid stem cell therapy for urethral sphincter regeneration

So Young Chun1, Eun Hye Song1, Hei Jeong Noh1, Jong Kil Lee2, Bum Soo Kim4, Jae-sung Bae2, Yun-Hee Shon1, Jeong Ok Lim1, James J Yoo1,3, Tae Gyun Kwon1,4

1 Joint Institute for Regenerative Medicine, Kyungpook National University Hospital, Daegu, Korea, 2 Dept of Physiology, School of Medicine, Kyungpook National University, Daegu, Korea, 3 Wake Forest Institute for Regenerative Medicine, Wake Forest University Health Sciences, Winston-Salem, NC, USA, 4 Dept of Urology, Kyungpook National University Hospital, Daegu, Korea

Introduction

Recently, several studies have been shown that human amniotic fluid stem cells (hAFSCs) can be an useful cell source for cell therapy. However, the role in urethral sphincter regeneration for cure of urinary incontinence is unknown. In this study, we investigated the potential role of hAFSCs in cell therapy for urethral sphincter regeneration.

Materials and Methods

hAFSCs were harvested from human amnion fluid, and isolated with stem cell marker (C-kit) and subcultured. For in vivo experiment, the cultured hAFSCs (10^6 cells) were injected to the pudendal-neurectomy female mouse model. Four experimental groups were established: a control group had a sham-operation without nerve transection (Ctrl+); a pudendal nerve transection group with cell-free saline injections (Ctrl-); a nerve transection group with periurethral hAFSC injections (Cell); and a nerve transection group with periurethral hAFSC injections combined with hydrogel (Cell/Gel). The urodynamic studies including leak point pressure (LPP) and closing pressure (CP) were examined at 1, 2, 4 and 8 weeks after treatment. And the urethra was harvested after urodynamic study. The injected hAFSCs were identified by immunohistochemical stain (IHC) using anti-human nuclei antibody (HuNu). The muscle tissue characterization was done by IHC using anti-desmin, anti-α-SM actin antibody. And myogenic differentiation was evaluated using real-time PCR for various genes related to myogenic pathway.

Results

The LPP of Ctrl+, Ctrl–, Cell and Cell/Gel groups were 30.25±1.9, 16.55±2.1, 17.6±0.49 and 26.25±2.03 cmH2O, respectively. The CP was 19.45±1.8, 9.13±0.87, 9.88±5.34 and 14.36±2.41 cmH2O, respectively. The presence of abundant positive human nuclei, nestin, MyoD, α-SM actin demonstrated that a large number of injected AFSC can survive, proliferate, differentiate into the urethral sphincter tissue (Fig. 1). The Ctrl+ group consisted of thick packed muscle layers. The Ctrl–group showed shrinked urethral sphincter tissue. The Cell and Cell/Gel injected group showed circular smooth and striated muscle tissue regeneration at the injection site. In real-time PCR analysis, cell injected group showed enhanced expression of both human and mouse gene related to myogenic pathway (Fig. 2). These results suggest that hAFSCs can give influence to the host cell differentiation through paracrine way.

Discussion and Conclusions

The injection of hAFSCs with hydrogel into the urethral sphincter of pudendal nerve trassected mouse promoted morphologically and functionally competent urethral sphincter regeneration. These results suggest the feasibility of hAFSC therapy in treating stress urinary incontinence; emphasize the importance of proper Cell/Gel combine for treatment efficacy; paracrine effect of injected hAFSCs for myogenic differentiation lineage drive of host cells.

Reference


Acknowledgments

This study was supported by a grant of the Korea Healthcare technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea. (A091224)