**Reviewer's report**

**Title:** Human amniotic fluid stem cell injection therapy for urethral sphincter regeneration in animal model

**Version:** 1  **Date:** 8 March 2012

**Reviewer:** Hiromitsu Mimata

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**Summary**
Authors investigated whether periurethral injection of hAFSCs can restore competent urethral sphincter in a mouse model transected bilateral pudendal nerve. They showed that periurethral injection of human amniotic fluid stem cells (hAFSCs) in an animal model of stress urinary incontinence (SUI) resulted histologically and functionally similar to normal urethral sphincter restoration, without immunogenicity and tumorigenicity.

**General comments**
Recently, regeneration of the urethral rhabdosphincter seems to be a promising new therapy for urinary stress incontinence and to be achieved by autologous transplantation of mesenchymal stem cells (i.e. bone –marrow derived stem cells, adipogenic stem cells, and muscle derived stem cells).

Likewise, authors has established an ideal cell source from human amniotic fluid cells, named hAFSCs, which have shown the potential to differentiate into lineages of myogenic stem cells. In the present study, they examined the effects of hAFSCs transplantation on periurethral tissue regeneration and sphincteric function.

The idea was good, but I think that there was a major problem in this study design.

**Major remarks**

1. Authors underwent the transaction of bilateral pudendal nerve to make SUI models in mice. Damage of this nerve causes to severe SUI due to degeneration of not only nerve but also urethral rhabdosphincter. Damaser et al. examined the time course of neuroanatomical and functional recovery after bilateral pudendal nerve injury in rats1). However, they performed not nerve transection but nerve crush injury. The most important issue is to investigate neuroregeneration as well as myogenic differentiation of rhabdosphincter by hAFSCs. I cannot believe only regeneration of dysfunctioned rhabdosphincter contributed recovery of SUI.

2. In histological analysis, I could not find urethral rhabdosphincter. Authors should perform immunostaining using not only MyoD but also myosin heavy chain (a marker of myogenic differentiation) or sarcometic actin (a marker of striated muscle). Moreover, it is better to add the description of length from
external urethral orifice, since the thickness of rhabdosphincter is dependent of where sections of urethra are cut.

Minor remarks
1. When time-dependent change is examined, one-way ANOVA should be used in statistical analysis.
2. In LPP test, I wonder why LPP did not improve like control, although histological analysis improved similar to control. I assume that neurodysfunction still had continued.

References

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.