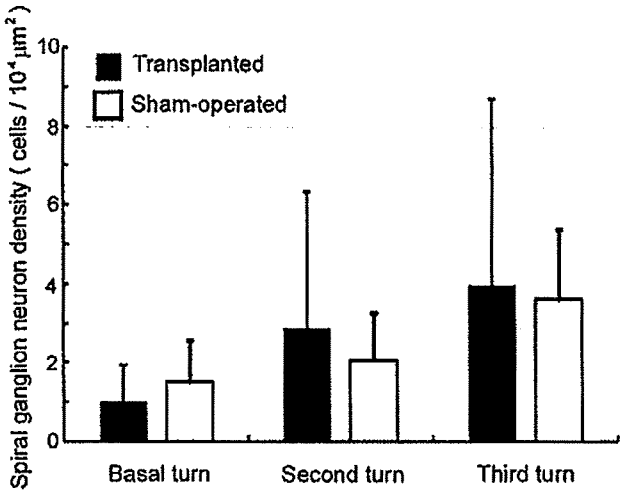

Fig. 5. Densities of remaining spiral ganglion neurons in the basal, second, or third turn of transplanted or sham-operated cochleae. There are no significant differences in the density of spiral ganglion neurons between transplanted and sham-operated cochleae.

of transplants in the cochlear modiolus and enabled functional evaluation using eABRs.[10,13] Moreover, our refined technique for cell introduction into the cochlear modiolus of guinea pigs caused no significant elevation of eABR thresholds.[10] Based on these previous findings, we used guinea pigs as experimental animals in the present study.

After transplantation of BMSC-derived spheres into the intact or damaged cochleae, BMSC-derived neurons were found in various portions of cochleae, including the cochlear modiolus. These findings indicate that BMSCs can be an alternative source of transplants for replacing SGNs. However, measurements of eABRs in the present study revealed no significant improvements of eABR thresholds after transplantation of BMSC-derived spheres. Previously, we demonstrated the recovery of eABR thresholds after transplantation of embryonic stem (ES) cell-derived neural progenitors in a different injury model.[13] There are several possible explanations for this lack of functional recovery following transplantation of BMSC-derived spheres. One is insufficient neurite elongation from BMSC-derived neurons to the central nervous system. Another possibility relates to different subtypes of neurons that are generated from BMSC-derived spheres. Previous studies have demonstrated that glutamatergic neurons are generated from both ES cells[14] and BMSCs,[15] meaning that both cell types have the capacity for differentiation into glutamatergic neurons. To achieve functional SGN restoration by transplantation of BMSC-derived spheres, additional treatments are required to enhance elongation of neurites from BMSC-derived neurons or to induce differentiation of BMSC-derived spheres into glutamatergic neurons.

Interestingly, the localization of transplants was different between the intact and damaged cochleae. In the intact cochleae, a number of transplants were found in the scala tympani. We injected BMSC-derived spheres through the scala tympani.[10] Therefore, transplants that were found in the scala tympani may have originated from the leakage of injected cell suspensions. In the intact cochlea, there are limited spaces in the cochlear modiolus, because host SGNs and auditory nerves are present, which may cause the leakage of injected cell suspensions into the scala tympani. On the other hand, the loss of host SGNs may result in an increase of spaces for transplants in the cochlear modiolus. Hence, in the damaged cochleae limited numbers of transplants were observed in the perilymphatic spaces including the scala tympani. In the damaged cochleae a number of transplants were found not only in the cochlear modiolus but also in the internal auditory meatus. Transplants in the internal auditory meatus may migrate from the cochlear modiolus, which is an injected site. The degeneration of SGNs could make a path from the cochlear modiolus of the basal portion to the internal auditory meatus, or stimulate production of chemotactic factors that promote the migration activity of BMSC-derived spheres. Future studies should be performed to determine the mechanisms of migration of BMSC-derived spheres into the internal auditory meatus.

CONCLUSION

The present findings demonstrate that BMSCs are a preferable source of neurospheres and that BMSC-derived spheres retain the ability for neural differentiation after transplantation into the cochlea. Functional restoration of damaged cochleae was not achieved by transplantation of BMSC-derived spheres, although a number of transplant-derived neurons settled in the cochlea and in the internal auditory meatus. To achieve functional restoration of SGNs by transplantation of BMSC-derived spheres, additional treatments including local application of neurotrophic or growth factors may be required.

BIBLIOGRAPHY

1. Coleman B, De Silva MG, Shephered RK. The potential of stem cells for auditory neuron generation and replacement. *Stem Cells* 2007;25:2685–2694.
2. Nakagawa T, Ito J. Cell therapy for inner ear diseases. *Curr Pharm Des* 2005;11:1203–1207.
3. Li H, Corrales CE, Edge A, Heller S. Stem cells as therapy for hearing loss. *Trends Mol Med* 2004;10:309–315.
4. Kitada M, Dezawa M. Induction system of neural and muscle lineage cells from bone marrow stromal cells; a new strategy for tissue reconstruction in degenerative diseases. *Histol Histopathol* 2009;24:631–642.
5. Liu ZJ, Zhuge Y, Velazquez OC. Trafficking and differentiation of mesenchymal stem cells. *J Cell Biochem* 2009;106: 984–991.
6. Naito Y, Nakamura T, Nakagawa T, et al. Transplantation of bone marrow stromal cells into the cochlea of chinchillas. *Neuroreport* 2004;15:1–4.
7. Matsuoka AJ, Kondo T, Miyamoto RT, Hashino E. In vivo and in vitro characterization of bone marrow-derived stem cells in the cochlea. *Laryngoscope* 2006;116:1363–1367.
8. Sharif S, Nakagawa T, Ohno T, et al. The potential use of bone marrow stromal cells for cochlear cell therapy. *Neuroreport* 2007;18:351–354.

ID: thambikkanue | Black Lining: [ON] | Time: 17:19 | Path: N:/3b2/LARY/Vol00000/090467/APPFile/C2LARY090467

9. Matsuoka AJ, Kondo T, Miyamoto RT, Hashino E. Enhanced survival of bone-marrow-derived pluripotent stem cells in an animal model of auditory neuropathy. *Laryngoscope* 2007;117:1629–1635.
10. Ogita H, Nakagawa T, Lee KY, et al. Surgical invasiveness of cell transplantation into the guinea pig cochlear modiolus. *ORL J Otorhinolaryngol Relat Spec* 2009;71:32–39.
11. Kada S, Nakagawa T, Ito J. A mouse model for degeneration of the spiral ligament. *J Assoc Res Otolaryngol* 2009; 10:161–172.
12. Tamura T, Nakagawa T, Iguchi F, et al. Transplantation of neural stem cells into the modiolus of mouse cochleae injured by cisplatin. *Acta Otolaryngol* 2004; (suppl 551): 65–68.
13. Okano T, Nakagawa T, Endo T, et al. Engraftment of embryonic stem cell-derived neurons into the cochlear modiolus. *Neuroreport* 2005;16:1919–1922.
14. Reyes JH, O'Shea KS, Wys NL, et al. Glutamatergic neuronal differentiation of mouse embryonic stem cells after transient expression of neurogenin 1 and treatment with BDNF and GDNF: in vitro and in vivo studies. *J Neurosci* 2008;28:12611–12631.
15. Kondo T, Johnson S, Yoder M, et al. Sonic hedgehog and retinoic acid synergistically promote sensory fate specification from bone marrow-derived pluripotent stem cells. *Proc Natl Acad Sci U S A* 2005;102:4789–4794.

ID: thambikkanue | Black Lining: [ON] | Time: 17:19 | Path: N:/3b2/LARY/Vol00000/090467/APPFile/C2LARY090467