Multimodal Visualization of Mouse Hair-Follicle-Bulge-Derived Stem Cells

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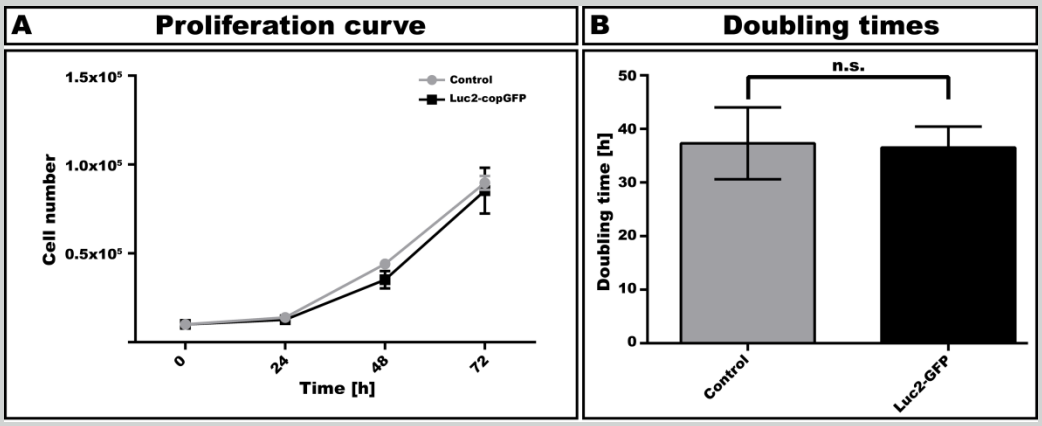
Introduction

Cell-based inner ear therapy may restore sensorineural hearing loss. Multipotent hair-follicle-bulge-derived stem cells (HFBSCs) are promising candidates for evoking cochlear nerve regeneration. However, cell-based inner ear therapy could be limited by the problem of ascertaining the viability of grafted cells and their location. An approach combining bioluminescence optical imaging and magnetic resonance imaging (MRI) may be advantageous to monitor grafted cells within the cochlea. The bioluminescent signal from cells expressing ATP-dependent luciferase is a proxy of their viability, whereas cells loaded with magnetic nanoparticles can be localized by means of MRI. The purpose of this study was to investigate if HFBSCs can be stably transduced with a lentiviral dual-reporter gene construct and loaded with magnetic nanoparticles without undesired effects on cell viability and proliferation.

Experimental Design

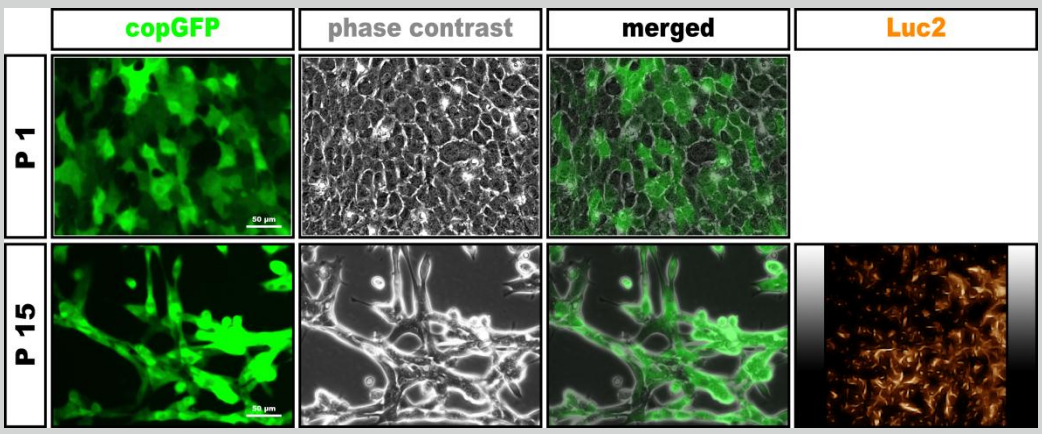
HFBSCs were transduced with a lentiviral dual-reporter gene construct allowing stable equimolar co-expression of the fluorescent reporter molecule (copepod green fluorescent protein; copGFP) and the bioluminescent reporter molecule (codon-optimized firefly luciferase; Luc2). Transduction efficiencies, proliferation rates, cell viability and persistence of reporter protein expression were studied during long-term culture (till P15). In addition, transduced HFBSCs were loaded with TMSR50 nanoparticles (containing a magnetic core and a red-fluorescent dye) and observed for 7 days. Loading efficiencies and the possible cytotoxic effect of TMSR50 nanoparticles upon proliferation and viability were investigated.

1. Proliferation of transduced and control HFBSCs



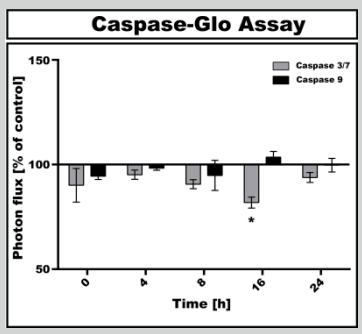
(A) Proliferation does not significantly differ in HFBSCs transduced with Luc2-copGFP as compared to non-transduced (control) HFBSCs. Initially, an amount of $1x10^4$ cells was seeded and cells were counted after 24, 48 and 72 hours, respectively (error bars: SEM, n=12 per data point). **(B)** There are no significant differences in doubling time between transduced and control cells (p=0.894, two-tailed, unpaired *t* test, 95% confidence interval; error bars: SEM, n=9).

2. Co-expression of reporter molecules



After transduction with the Luc2-copGFP construct, approximately 83.56% of the cells exhibit green fluorescence indicative of copGFP expression. The fluorescent signal persists for at least 15 passages (P 15). Addition of the substrate D-luciferin resulted in a bioluminescent signal due to luciferase activity at P15, confirming co-expression of Luc2.

3. TMSR50 loading and apoptosis-related caspase activity

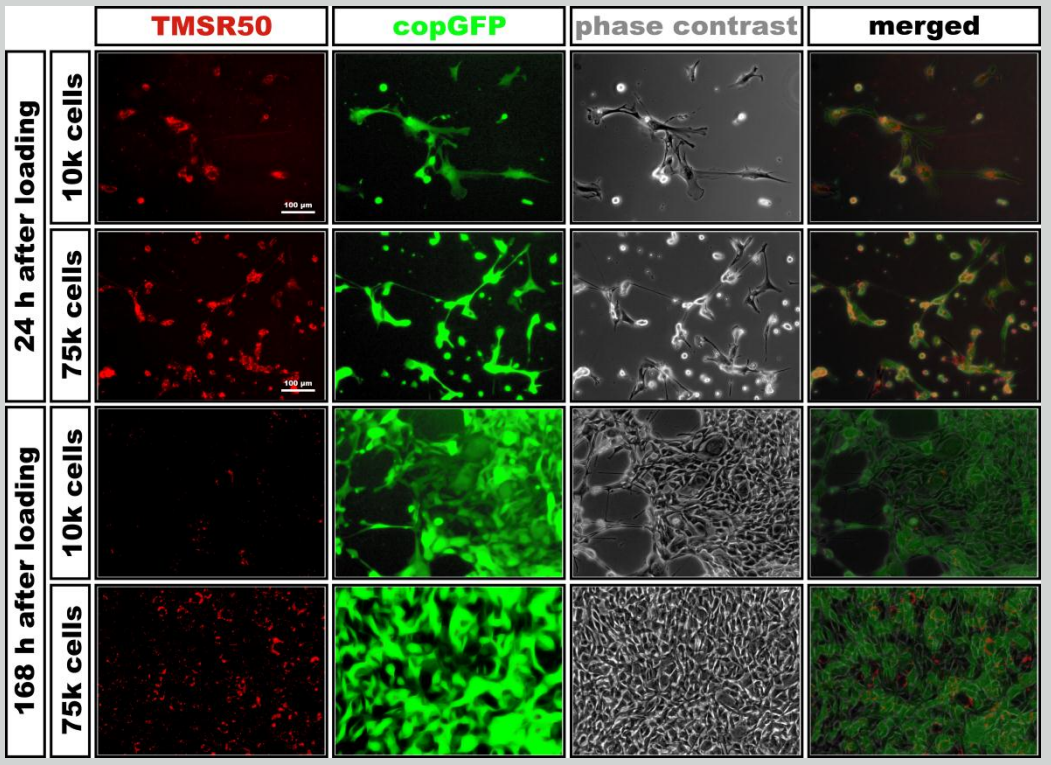


TMSR50 loading does not influence caspase 3/7-mediated or caspase 9-mediated apoptosis. Caspase activities were measured in transduced cells loaded with TMSR50 nanoparticles and transduced (control) cells at different time points over a period of 24 hours using a Caspase-Glo assay. Bioluminescence of the loaded cells is expressed as a percentage of the photon flux of the control cells (100%-line; error bars: SEM; n=3 per data point).

Acknowledgement

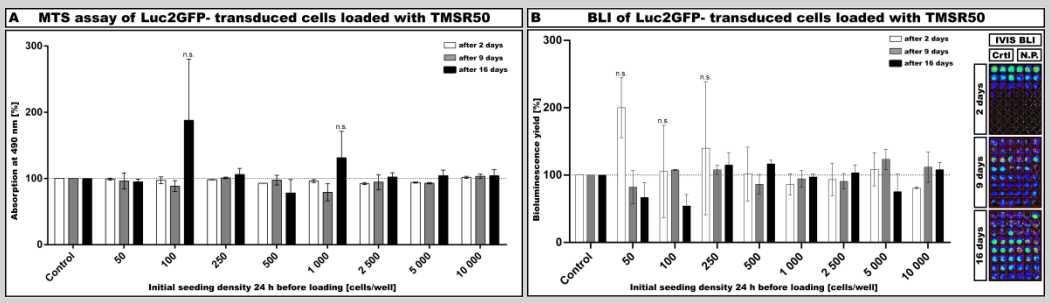
The authors thank Renate Buijink and Stephan Michel (Laboratory for Neurophysiology, LUMC, the Netherlands) for technical assistance and the use of their bioluminescence microscope.

4. Retention of TMSR50 nanoparticles in transduced cells



Transduced cells at seeding density of $7.5x10^4$ demonstrate a strong red fluorescent signal after 168 hours (i.e., 7 days) indicating retention of TMSR50 nanoparticles in the cells. Different amounts of transduced cells, $1x10^4$ (10k) and $7.5x10^4$ (75k), are loaded with nanoparticles (0.2 mg/ml TMSR50 in medium). Many GFP-expressing cells demonstrate red fluorescence indicating the presence of TMSR50 nanoparticles (merged images), which declines with every subsequent medium change.

5. Long-term viability of TMSR50-loaded cells



(A) MTS assay demonstrates that cell viability is not influenced by nanoparticle loading. Absorption of different amounts of cells is measured at 2, 9 and 16 days after loading with TMSR50 nanoparticles and expressed as a percentage of the non-loaded (control) cells (dotted 100%-line; error bars: SEM, n=6 per data point). **(B)** Bioluminescence imaging (BLI) results are similar to those obtained by means of the MTS assay. Bioluminescence yield of the transduced cells loaded with nanoparticles is expressed as a percentage of the non-loaded (control) cells (dotted 100%-line; error bars: SEM, n=6 per data point). These results indicate that TMSR50 loading does not influence long-term viability of transduced HFBSCs.

Conclusion

We conclude that HFBSCs can be efficiently and stably transduced with the Luc2-copGFP construct followed by subsequent nanoparticle loading, without affecting cell viability and proliferation. This implies that a combined approach using bioluminescence optical imaging and MRI may enable *in vivo* localization of grafted HFBSCs within the cochlea. Therefore, *in vivo* monitoring during stem cell therapy in the inner ear might be feasible.