In Vitro Visualization of Mouse Hair-Follicle-Bulge-Derived Stem Cells with Various Imaging Modalities

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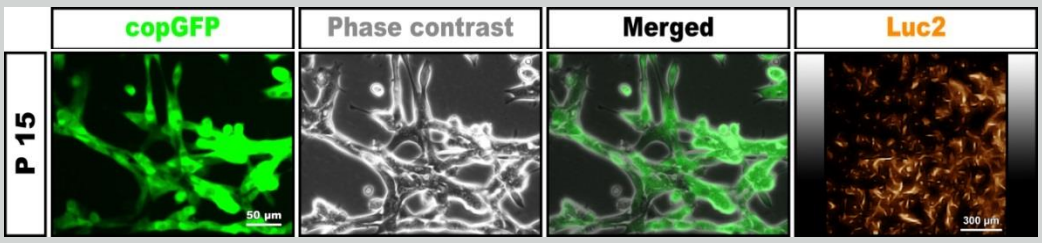
Introduction

Multipotent hair-follicle-bulge-derived stem cells (HFBSCs) are promising candidates for evoking regeneration of the cochlear nerve following sensorineural hearing loss. However, one limitation of cell-based inner ear therapy would be the need to monitor the location and viability of the grafted cells as well as their fate within the cochlea. An approach combining in vivo bioluminescence imaging and magnetic resonance imaging (MRI) may therefore be advantageous. 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 aim of this study was to investigate if HFBSCs can be used for such a combined approach.

Experimental design

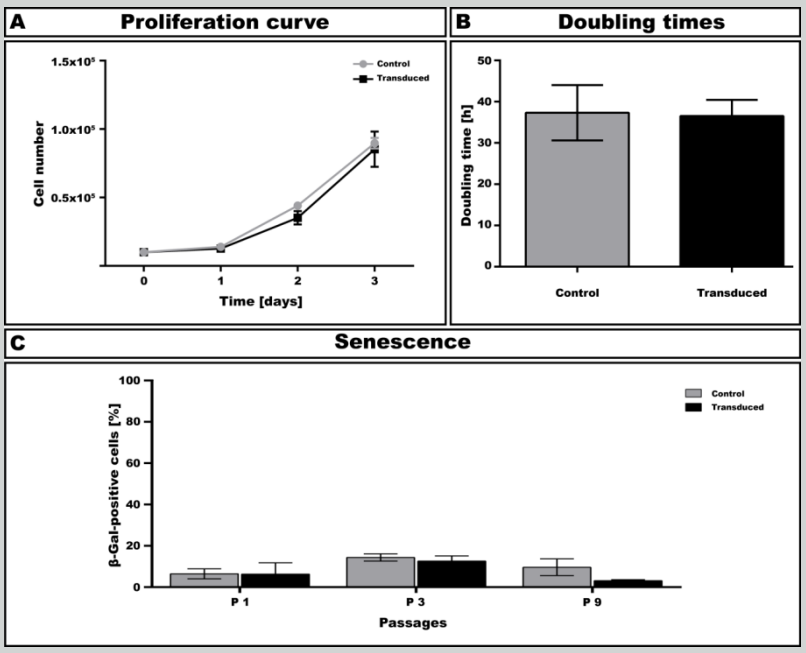
HFBSCs were transduced with a lentiviral construct containing the genes that code for copepod green fluorescent protein (copGFP) and codon-optimized firefly luciferase (Luc2) and allowing equimolar expression of both reporter molecules. We studied transduction efficiency, proliferation rate and cellular senescence. In a second series of experiments, transduced HFBSCs were loaded with magnetic, red-fluorescent nanoparticles followed by determination of cell viability, long-term retention of nanoparticles and ascertaining the possible cytotoxic effects. A multi-layered cell-gradient agarose phantom was produced – mimicking in vivo transplantation – and HFBSCs were visualized by means of fluorescence imaging and MRI.

1. Co-expression of reporter molecules



After lentiviral transduction with the Luc2-copGFP reporter gene construct, the majority of the cells exhibit green fluorescence indicative of copGFP expression. The fluorescent signal persists for at least 15 passages (P15). Addition of the substrate D-luciferin resulted in a bioluminescent signal due to luciferase activity at P10 and P15, confirming co-expression of Luc2. Scale bar = 50 µm (fluorescence) or 300 µm (bioluminescence).

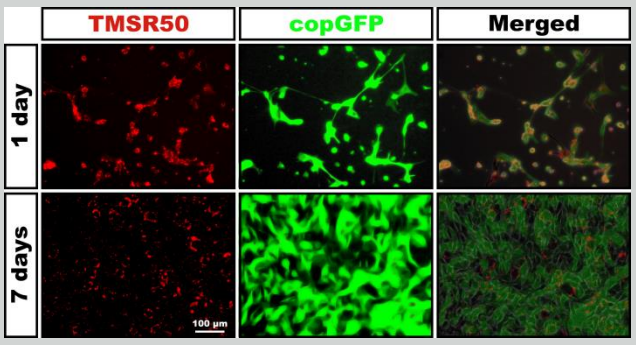
2. Proliferation of transduced and control HFBSCs



(A) The proliferation rate of transduced HFBSCs does not significantly differ from that of non-transduced (control) cells after 1 day, 2 and 3 days (error bars: SEM, n=12 per data point). **(B)** Comparison of the doubling times of transduced and control cells does not reveal statistically significant differences ($p=0.894$, two-tailed, unpaired *t* test, 95% confidence interval; error bars: SEM, n=9).

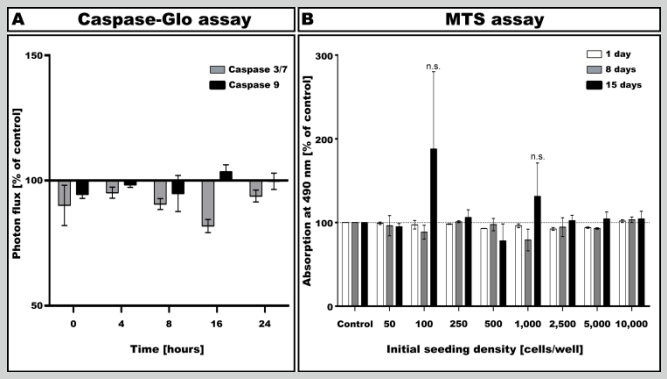
(C) Enzyme histochemical staining for lysosomal β-galactosidase reveals that transduced and control cells become senescent alike. The percentage of blue-stained cells remains stable up to P9 for both the control (mean: 10.2%) and transduced cells (mean: 7.4%).

3. Loading and retention of TMSR50 in transduced cells

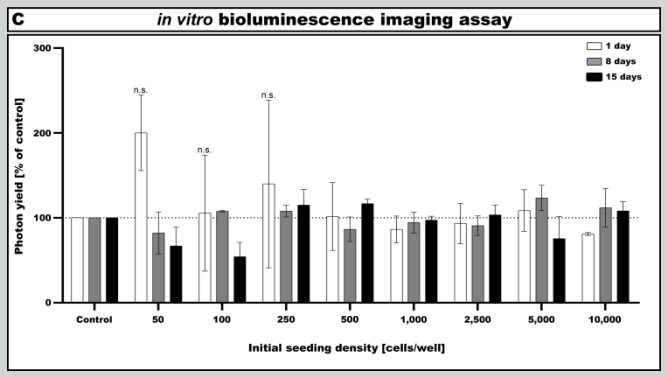


Retention of red-fluorescent TMSR50 nanoparticles was monitored in transduced HFBSCs at a seeding density of $7.5x10^4$ cells/well for up to 7 days. From the merged images, it is evident that copGFP and TMSR50 co-exist in the cells. Loss of red fluorescence was observed in cultures over time. However, red-fluorescent signal was retained in cultures seeded at 7.5x104 cells/well for up to 7 days. Scale bar = 100 µm.

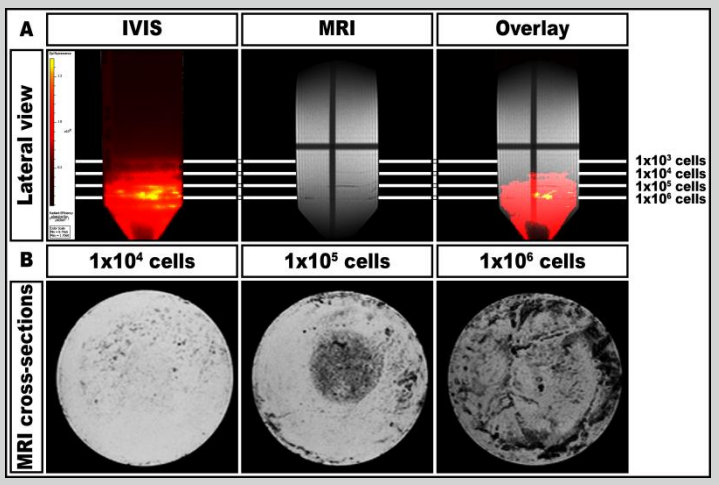
4. Caspase activity during TSMR50-loading and long-term viability



(A) Loading with TMSR50 nanoparticles does not result in significant increases in caspase 3/7 or caspase 9 activities in HFBSCs over a period of up to 24 hours. Bioluminescence of the cells is expressed as a percentage of the photon flux normalized to that of non-loaded transduced cells (100% line; error bars: SEM).
(B) MTS assay demonstrates that viability of transduced HFBSCs is not significantly reduced by TMSR50 nanoparticles after 1 day, or after 8 and 15 days. Data are expressed as percentage of absorption at 490 nm and normalized to that of non-loaded transduced cells (control: dotted line; error bars: SEM).
(C) Results with in vitro bioluminescence imaging are similar to those obtained with the MTS assay. Data are expressed as percentage of photon yield normalized to the non-loaded transduced cells (control: dotted line; error bars: SEM).



5. MRI of agarose phantom containing TMSR50-loaded cells



(A) Images of the exterior (lateral view) of an 0.5% agarose phantom show layers containing different amounts of TMSR50-loaded HFBSCs ($1x10^3$ - $1x10^6$). From the left to the right: cells, visualized by fluorescence imaging in an In Vivo Imaging System (IVIS), by MRI and the overlay of both. Excitation at 535 nm for 1 second reveals bright fluorescence of the layers with $1x10^6$ and $1x10^5$ cells and two distinct layers containing red-fluorescent nanoparticles ($1x10^4$ and $1x10^3$ cells) at 580 nm. MRI displays three distinct layers ($1x10^6$, $1x10^5$ and $1x10^4$ cells), while the layer containing $1x10^3$ cells was not visible.

(B) MRI cross-sections of layers of HFBSCs, loaded with iron oxide nanoparticles (TMSR50). For every cross-section, three MRI images of the respective layer were merged. While $1x10^3$ cells/layer could be visualised by fluorescence imaging, this amount was too low to enable detection using MRI. A layer containing $1x10^4$ cells gave a faint contrast, whereas $1x10^5$ cells and especially $1x10^6$ cells were clearly above detection level.

Conclusion

We conclude that HFBSCs can be transduced with the lentiviral Luc2-copGFP construct without inhibiting cell proliferation and subsequently be loaded with nanoparticles without affecting viability. The finding that these cells after nanoparticle loading can be visualized using MRI, implies that an approach combining bioluminescence imaging and MRI may enable in vivo localization of grafted HFBSCs within the cochlea of deafened animals, allowing long-term monitoring of cochlear nerve regeneration.

Acknowledgement

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