ISLETS FROM RATS AND PIGS TRANSGENIC FOR PHOTGENIC PROTEINS.

Teratani T, Matsunari H, Kasahara N, Nagashima H, Kawarasaki T, Kobayashi E.

Abstract

Translational research is necessary for the development of efficient experimental animal models that can be used to develop innovative medical treatments, such as improvements in organ or tissue transplantation. We have developed animal models that produce photogenic proteins in their islet cells: rats models expressing the gene for luciferase or green fluorescent protein (GFP), and pig models expressing the gene for GFP or Kusabira-Orange. We also developed methods for preserving isolated islets in culture and showed that the fluorescence of the islets remains at usable levels for at least seven days. These models will enable transplanted islets to be visualized without the need for chemical reactions, and will be useful for research on the biology of islets as well as for the development of new transplantation methods.
The Lewis GFP Transgenic Rat Strain is a Useful Cell Donor for Neural Transplantation.

Krause M, Ganser C, Kobayashi E, Papazoglou A, Nikkiah G.

Abstract

Stem cell transplantation is a promising therapeutic approach in neurodegenerative diseases. Studying graft survival and development have important implications for the further development of experimental and clinical transplantation protocols. Cellular elements in neural transplants are sometimes difficult to identify the existing labeling methods cannot reliably provide stably labeled cells that can be detected in long-term experiments. Transgenic(tg) Lewis rats ubiquitously expressing Green fluorescent protein (GFP) provide an ideal donor source. The aim of this project was to investigate the potential of GFP-tg Lewis rats to serve as donor tissue for neural stem cell transplantation. Ventral mesencephalon (VM) GFPtg E14.5-derived cells were compared to wild-type (wt) in vitro and in vivo. Firstly, cells from GFP and non-GFP VM tissue were compared with regard to their proliferation and response towards 6-OHDA-toxicity in culture. Secondly, 6-OHDA-lesioned hemiparkinsonian Sprague-Dawley/Crl:CD(SD) rats received intrastratal grafts derived from VM of E14.5 GFP-tg rats. Due to the fact that donor and recipient belong to two different rat strains, we focused on graft survival in correlation with immunosuppression and graft GFP- and THexpression. In summary, in vitro tg cells exhibited 98% GFP-expression and did not differ from wt cells in any of the measured parameters. In vivo, all experimental groups showed a significant compensation in rotation behavior after transplantation. Furthermore, there was no difference on rotation behavior or graft morphology and survival pattern as well as GFP-expression between immunosuppressed and non-immunosuppressed animals. The
GFP-positive population of the graft was composed of 13.3% GFAP-positive, 56.1% NeuN-positive and 1.9% TH-positive cells. Analysis of graft subpopulations manifested that 70.6% of GFAP-positive, 86.9% of NeuN-positive and 80.1% of TH-positive cells, co-expressed GFP. In conclusion, our data show that the Lewis GFP-tg rats serve as an excellent cell source for studying primary neural precursor cells in the transplantation paradigm.


**GFP-Transgenic Animals for In Vivo Imaging: Rats, Rabbits, and Pigs.**

Murakami T, Kobayashi E.

Abstract

Specifically, gene-encoded biological probes serve as stable and high-performance tools to visualize cellular fate in living animals. The rat, as with the mouse, has offered important animal models for biology and medical research, and has provided a wealth of physiological and pharmacological data. The larger-body animals, in comparison to the mouse have allowed the application of various physiological and surgical manipulations that may prove to have biological significance. We have further extended the techniques of genetic engineering to rats, rabbits, and pigs, and have created corresponding GFP-transgenic animals. The GFP-positive organs of these animals provide valuable sensors in preclinical settings for cell therapy and transplantation studies. In this chapter, we highlight expression profiles in these animal resources and describe examples of preclinical applications.

The role of microstructured and interconnected pore channels in a collagen-based nerve guide on axonal regeneration in peripheral nerves.


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Abstract

The use of bioengineered nerve guides as alternatives for autologous nerve transplantation (ANT) is a promising strategy for the repair of peripheral nerve defects. In the present investigation, we present a collagen-based micro-structured nerve guide (Perimaix) for the repair of 2 cm rat sciatic nerve defects. Perimaix is an open-porous biodegradable nerve guide containing continuous, longitudinally orientated channels for orientated nerve growth. The effects of these nerve guides on axon regeneration by six weeks after implantation have been compared with those of ANT. Investigation of the regenerated sciatic nerve indicated that Perimaix strongly supported directed axon regeneration. When seeded with cultivated rat Schwann cells (SC), the Perimaix nerve guide was found to be almost as supportive of axon regeneration as ANT. The use of SC from transgenic green-fluorescent-protein (GFP) rats allowed us to detect the viability of donor SC at 1 week and 6 weeks after transplantation. The GFP-positive SC were aligned in a columnar fashion within the longitudinally orientated micro-channels. This cellular arrangement was not only observed prior to implantation, but also at one week and 6 weeks after implantation. It may be concluded that Perimaix nerve guides hold great promise for the repair of peripheral nerve defects.
Bone marrow-derived progenitor cells do not contribute to podocyte turnover in the puromycin aminoglycoside and renal ablation models in rats.


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Abstract

A key event in the progression of glomerular disease is podocyte loss that leads to focal and segmental glomerulosclerosis (FSGS). Because adult podocytes are postmitotic cells, podocyte replacement by bone marrow-derived progenitors could prevent podocytopenia and FSGS. This study uses double immunofluorescence for Wilms' tumor-1 and enhanced green fluorescent protein (eGFP) to examine whether an eGFP-positive bone marrow transplant can replace podocytes under normal circumstances and in 3 different rat models of FSGS: puromycin aminoglycoside nephropathy, subtotal nephrectomy, and uninephrectomy. Bone marrow engraftment was successful, with more than 70% eGFP-positive cells and virtually normal histologic findings. No bone marrow transplant-derived podocytes were found in four control rats after transplantation, in nine rats at up to 10 weeks after puromycin aminoglycoside nephropathy induction, in three rats 23 days after subtotal nephrectomy, and in six rats up to 21 days after uninephrectomy. A total of 2200 glomeruli with 14,474 podocytes were evaluated in all groups. Thus, podocyte replacement by bone marrow-derived cells does not contribute to podocyte turnover in rats, even in models of podocyte damage. This is in contrast to previous studies in mice, in which bone marrow-derived podocytes were found. Further studies will address this discrepancy, which could be explained by species differences or by predominant podocyte regeneration from a parietal epithelial cell niche.
Equivalent neurogenic potential of wild-type and GFP-labeled fetal-derived neural progenitor cells before and after transplantation into the rodent hippocampus.

Lepski G, Jannes CE, Wessolleck J, Kobayashi E, Nikkhah G.

Abstract

The hippocampal formation is a specific structure in the brain where neurogenesis occurs throughout adulthood and in which the neuronal cell loss causes various demential states. The main goal of this study was to verify whether fetal neural progenitor cells (NPCs) from transgenic rats expressing green fluorescent protein (GFP) retain the ability to differentiate into neuronal cells and to integrate into the hippocampal circuitry after transplantation. NPCs were isolated from E14 (gestational age: 14 days postconception) transgenic-Lewis and wild-type Sprague-Dawley rat embryos. Wild-type and transgenic cells were expanded and induced to differentiate into a neuronal lineage in vitro. Immunocytochemical and electrophysiological analysis were performed in both groups. GFP-expressing cells were implanted into the hippocampus and recorded electrophysiologically 3 months thereafter.

Immunohistochemical analysis confirmed neuronal differentiation, and the yield of neuronal cells was determined stereologically. NPCs derived from wild-type and transgenic animals are similar regarding their ability to generate neuronal cells in vitro. Neuronal maturity was confirmed by immunocytochemistry and electrophysiology, with demonstration of voltage-gated ionic currents, firing activity, and spontaneous synaptic currents. GFP-NPCs were also able to differentiate into mature neurons after implantation into the hippocampus, where they formed functional synaptic contacts. GFP-transgenic cells represent an important tool in transplantation studies. Herein, we demonstrate their ability to generate functional neurons both in vitro and in vivo conditions. Neurons derived from fetal NPCs were able to integrate into the normal hippocampal circuitry. The high yield of mature neurons generated render these cells important candidates for restorative approaches based on cell therapy.