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Differentially promoted peripheral nerve regeneration by grafted Schwann cells over-expressing different FGF-2 isoforms

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Artificial nerve grafts are needed to reconstruct massive defects in the peripheral nervous system when autologous nerve grafts are not available in sufficient amounts. Nerve grafts containing Schwann cells display a suitable substrate for long-distance regeneration. We present here a comprehensive analysis of the in vivo effects of different isoforms of fibroblast growth factor-2 (FGF-2) on peripheral nerve regeneration across long gaps. FGF-2 isoforms were provided by grafted, genetically modified Schwann cells over-expressing 18-kDa-FGF-2 and 21-/23-kDa-FGF-2, respectively. Functional tests evaluated motor and sensory recovery. Additionally, morphometrical analyses of regenerated nerves were performed 3 and 6 months after grafting. Distinct regeneration promoting effects of the different FGF-2 isoforms were found. 18-kDa-FGF-2 mediated inhibitory effects on the grade of myelination of regenerating axons, whereas 21-/23-kDa-FGF-2 mediated early recovery of sensory functions and stimulation of long-distance myelination of regenerating axons. The results contribute to the development of new therapeutic strategies in peripheral nerve repair.

Keywords: Fibroblast growth factor-2; Isoforms; Peripheral nerve regeneration; Cell therapy; Neurotrophic therapy; Schwann cells; Genetic modification

Introduction

A complete nerve transection is the most severe peripheral nerve injury and is primarily seen in obstetrical and traumatic brachial plexus lesions, but can also be seen in advanced extremity injuries. Depending on the distance between the nerve stumps, treatment typically consists of either direct end-to-end anastomosis of the cut nerve ends or the use of an autologous nerve graft (Schmidt and Leach, 2003). The availability of autologous nerve transplants is especially limited when a large amount of grafting material is needed as in massive peripheral nerve lesions, because it requires sacrifices of healthy nerves (Lundborg, 2004). There are experimental and clinical approaches to use synthetic guidance channels in peripheral nerve regeneration (Nakamura et al., 2004). However, the golden standard is still transplantation of autologous nerve grafts as they provide a scaffold which contains Schwann cell basal laminae and growth factor constituting optimal growth substrate and environment for regrowing axons (Ansselin et al., 1997; Bunge, 1993; Lundborg, 2004). For development of an optimal artificial nerve graft as alternative to autologous ones, it is of high interest to combine synthetic nerve guides with preferentially autologous Schwann cell transplants and the respective biologically active molecules.

Artificial nerve grafts filled with physiological Schwann cells from neonatal (Hadlock et al., 2000; Mosahebi et al., 2002) and adult rats (Ansselin et al., 1997; Guenard et al., 1992) stimulated nerve fiber regeneration. Enhanced morphological peripheral nerve regeneration has also been seen after treatment with nerve growth factor (NGF) either slowly released from synthetic nerve guides (Fine et al., 2002) or from NGF-containing polymeric microspheres within synthetic nerve guides (Fine et al., 2002; Xu et al., 2003) or to a even better extent after treatment with glial-derived neurotrophic factor also slowly released from synthetic nerve guides (Fine et al., 2002). Furthermore, it has been shown previously that entrapment of the low molecular weight (18-kDa) isoform of fibroblast growth factor-2 (FGF-2) in synthetic nerve guidance channels is able to enhance growth of myelinated and unmyelinated axons across long gaps significantly (Aebischer et al., 1989). With regard to the low and high molecular weight FGF-2 isoforms, it has been shown that the isoforms are differentially regulated following peripheral nerve injury, indicating differential physiological functions during peripheral nerve regeneration (for review, see: Grothe and Nikkhah, 2001).

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Non-resorbable silicone tubes were introduced as an experimental model for tubulization in peripheral nerve repair. Sciatic nerve gaps exceeding 10 mm in rats resulted in no regeneration at all across the tube (Francel et al., 1997; Lundborg et al., 1982) and provide in this way optimal conditions to test new cell transplantation strategies in peripheral nerve repair across long gaps. Recently, we have shown that genetically modified Schwann cells are a useful tool to bridge long gaps (15 mm) after peripheral nerve injury (Timmer et al., 2003). Furthermore, over-expression of the 21- and 23-kDa-FGF-2 isoforms by the transplanted Schwann cells improved both lengths and number of regenerating myelinated axons in a short 4-week observation time (Timmer et al., 2003).

Knowledge of specific functions of FGF-2 isoforms and other growth factors within the regeneration scenario could contribute to the establishment of new therapeutic strategies after peripheral nerve lesion. In the present study, nerve guides filled with genetically modified Schwann cells over-expressing different FGF-2 isoforms were used to combine the necessary presence of Schwann cells in artificial nerve transplants with the effects of added growth factors. The objective of the present study was to investigate specific functions of the low and high molecular weight FGF-2 isoforms over-expressed by grafted Schwann cells on peripheral nerve repair across long gaps in a long time period (3 and 6 months). Grade and quality of peripheral nerve regeneration were determined by functional and morphometrical parameters as well as retrograde labeling of regenerating sensory and motor neurons.

Materials and methods

Animals and overview of experimental design

Adult female Sprague–Dawley rats weighing approximately 180 g (Central Animal Laboratory Medical School Hannover, Germany and Charles River Wiga, Germany) were kept under standard conditions (room temperature 22 ± 2°C, humidity 55 ± 5%, light/dark cycle LD 12:12) with food and water ad libitum. Animal care, housing, and surgery followed the guidelines of the German law on the protection of animals and were approved by the local animal care committee.

The animals were distributed into two groups concerning different observation periods, a 3-month and a 6-month group. Silicone tubes were implanted to the transected left sciatic nerve of each rat. The tubes were filled with different ingredients to build further experimental subgroups (Fig. 1): (1) Matrigel (Matrigel,

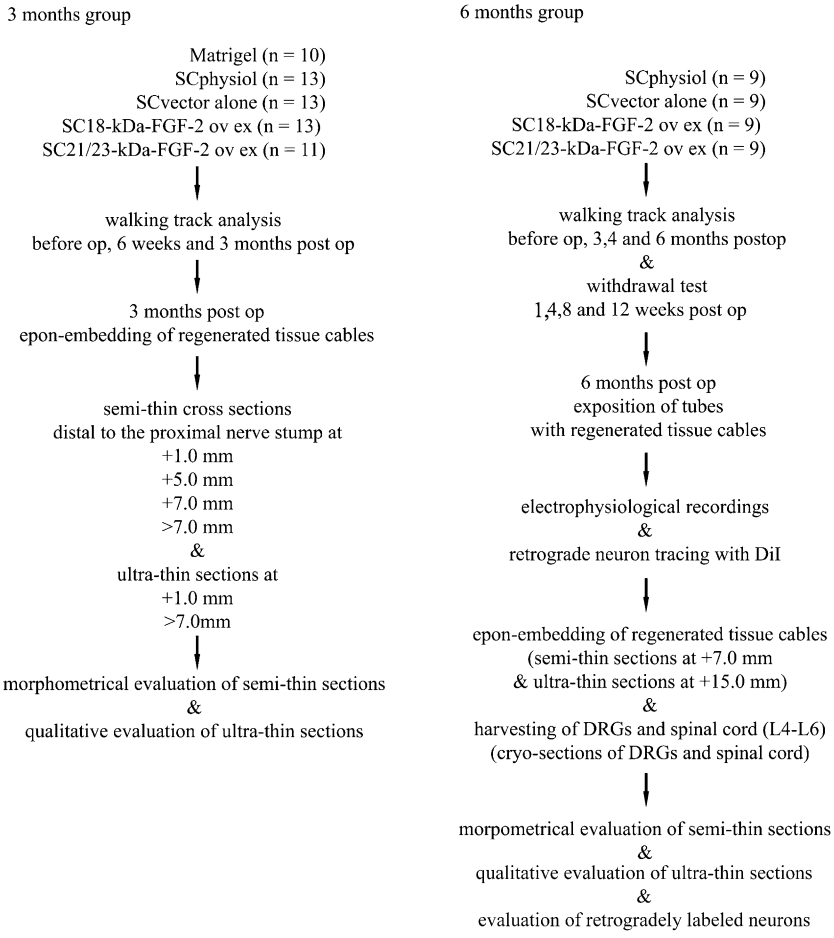

Fig. 1. The experimental groups and experimental design of the groups observed for 3 and 6 months. Division in experimental groups referred to the differently filled silicone tubes. Walking track analysis and electrophysiological tests were carried out to check for motor recovery and the withdrawal test to evaluate sensory recovery. Semi-thin and ultra-thin cross sections of the explanted, regenerated tissue cables were evaluated at several section points. Neuron tracing by DiI was performed to reveal the quality of regenerating neurons projecting into the regenerated nerves.