

# Propranolol Specifically Suppresses the Viability of Tumorous Schwann Cells Derived from Plexiform Neurofibromas *In Vitro*

ZIANG ZOU<sup>1</sup>, LINNA GUO<sup>2</sup>, VICTOR MAUTNER<sup>1</sup>, RALF SMEETS<sup>2</sup>,  
LAN KIUWE<sup>1,2</sup> and REINHARD E. FRIEDRICH<sup>2</sup>

<sup>1</sup>Department of Neurology, University Hospital Hamburg-Eppendorf, Hamburg, Germany;

<sup>2</sup>Department of Oral and Maxillofacial Surgery, University Hospital Hamburg-Eppendorf, Hamburg, Germany

**Abstract.** *Background/Aim:* Plexiform neurofibromas (PNFs) are benign tumors of the peripheral nerves sheath, which can damage neighboring organs, impair functions, cause pain and serious maxillofacial disfigurement, and have a high risk of malignant transformation. Complete resection is usually not possible since PNFs often extend through multiple layers of tissue. Therefore, it is necessary and beneficial to find a reasonable drug treatment for PNFs. Propranolol-treatment is the first-line therapy for infantile hemangiomas and the side effects are reversible and mostly benign. The present study aimed to examine the possible effect of propranolol for suppressing PNFs *in vitro*. *Materials and Methods:* Paired primary Schwann-cell-rich cultures and fibroblast-rich cultures were obtained from 4 PNFs of unrelated patients. Human Schwann cells (HSCs) were used as the control. These cultures were treated with propranolol for 7 days at concentrations up to 150  $\mu$ M. Cells were then measured for their viability and immune-stained with S100 to label the tumorous Schwann cells. *Results:* Propranolol inhibited the viability of the tumorous Schwann cells in a dose-dependent manner, while did not substantially suppress viability of the non-tumorous fibroblasts derived from the same PNFs. *Conclusion:* Propranolol may provide a treatment option for suppressing the growth of PNFs.

Plexiform neurofibromas (PNFs) are benign tumors of the peripheral nerve sheath and are associated mostly with neurofibromatosis type 1 (NF1), which is an autosomal

dominant, multisystem disorder and a tumor suppressor gene syndrome (1). Almost 30%-50% of the NF1 patients will develop PNFs (2-4). These benign tumors may cause pain and disfigurement, and functional impairment depends on their size, location, and type of growth (5). Approximately 10% of PNFs can transform into malignant peripheral nerve sheath tumors (MPNSTs), which is the leading cause of NF1-related death (6, 7). Since PNFs often extend through multiple layers of tissue, and the boundaries with the surrounding organizations are unclear, complete resection is usually not possible without damaging surrounding structures and functions (8, 9). Systemic medical therapy is therefore highly desirable (10). However, since PNFs are slow-growing benign tumors, complete regression is not necessarily the goal in all cases. Often, suppressing tumor growth would readily mean a success, whereas controlling side effects is a key issue.

Propranolol is a lipophilic beta-blocker primarily used for the treatment of hypertension and related indications such as hypertrophic cardiomyopathy, hyperthyroidism, and migraine. Recently, propranolol has been proven to be highly effective in treating infantile hemangiomas (11). Importantly, the side effects of propranolol are reversible and benign in the majority of cases. Propranolol is known to inhibit VEGF and other vascular endothelial factors, and consequently may have the effect of inhibiting abnormal tumor growth (12-15).

Schwann cells in PNFs are postulated to have dysregulated growth factors such as VEGF, which may contribute to the growth and progression of the tumors (16). The activation of beta-receptor on Schwann cells in PNFs was reported to increase the expression of VEGF (17). A recent study found that propranolol decreases expression of VEGF in human umbilical vein endothelial cells and promotes their apoptosis (18). These findings raise the question as to whether or not propranolol can inhibit the expression of VEGF by competitively inhibiting the beta-receptor on the tumorous Schwann cells, thereby achieving the purpose of inhibiting growth and progression of PNFs.

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*Correspondence to:* Ziang Zou, Laboratory for Tumor Genetics and Regenerative Medicine, Building O48, 4<sup>th</sup> floor, University Medical Center Eppendorf, Martinistr. 52, 20246 Hamburg, Germany. Tel: +49 40741058267, Fax: +49 40741059665, e-mail: xiangya.zou@gmail.com

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The present study was designed to address the issue of whether or not propranolol suppresses viability of PNFs cells *in vitro*, and if yes, whether or not this suppressing effect is more specific for the tumorous Schwann cells. For this purpose, paired Schwann-cell-rich and fibroblast-rich cultures were derived from 4 PNFs of unrelated patients and subjected to treatment with propranolol.

## Materials and Methods

Tumor tissues were obtained from patients who underwent tumor-resection surgery due to medical indication at the University Medical Center Hamburg-Eppendorf. Diagnosis of NF1 was conducted according to the modified National Institutes of Health criteria (19). All patients provided written informed consent for their tissues to be used in this study, which was approved by the Institutional Review Board (approval no. OB-061/05).

Culturing cells, enriching Schwann cells and staining Schwann cells with an S100 antibody were carried out as previously described (20). Cells grown under conditions for enriching Schwann cells (laminar-coating, supplementing with glia growth factor, IBMX, insulin, and forskolin), which contained more than 60% Schwann cells, were termed as "Schwann-cell-rich culture". Cells grown under standard conditions contained mostly fibroblasts and were termed as "fibroblast-rich culture".

Human Schwann cells (HSCs) are normal cells located in the peripheral nerves. HSCs were purchased from ScienCell Research Laboratories (catalog no., 1700; Carlsbad, CA, USA). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen®; Thermo Fisher Scientific, Inc., Waltham, MA, USA), supplemented with 10% fetal bovine serum (FBS; Invitrogen; Thermo Fisher Scientific, Inc.), 0.1% penicillin and streptomycin (Invitrogen; Thermo Fisher Scientific, Inc.), and 1% human Schwann cell supplement (ScienCell Research Laboratories).

A total of  $10^3$  cells of normal human Schwann-cells, Schwann-cell-rich culture and fibroblast-rich-culture were seeded into 96-well plates and treated with propranolol at 0, 5, 25, 75 and 150  $\mu$ M for 7 days. At the end of the treatment, a mitochondrial activity assay (MTS assay) was performed to measure cell viability (21). In addition, the Schwann-cell-rich cultures were stained with S100 to determine the proportion of tumorous Schwann cells. A number of S100 positive Schwann cells and the number of DAPI-stained nuclei were counted using Image J software. At each concentration of propranolol, the viability of real Schwann cells and that of fibroblasts in the Schwann-cell-rich culture were calculated as follows:

[Proportion of Schwann cells]=[S100 positive (green) cells]/[DAPI-stained nuclei (blue)]

[Viability of Schwann cells]=[Viability of total cells (from the MTS-assay)] $\times$ [proportion of S100 positive Schwann cells]

[Viability of fibroblasts]=[Viability of total cells]-[Viability of Schwann cells]

Viability and  $IC_{50}$ , the drug concentration for 50% inhibition, were checked for their normality using a skewness-kurtosis method. Afterward, data were analyzed using *t*-test in case of a normal distribution or non-parametric Kruskal-Wallis tests for skewed data. Data were presented as mean $\pm$ standard deviation. The hypothesis was two-tailed and the statistical significance was set at  $<0.05$ .

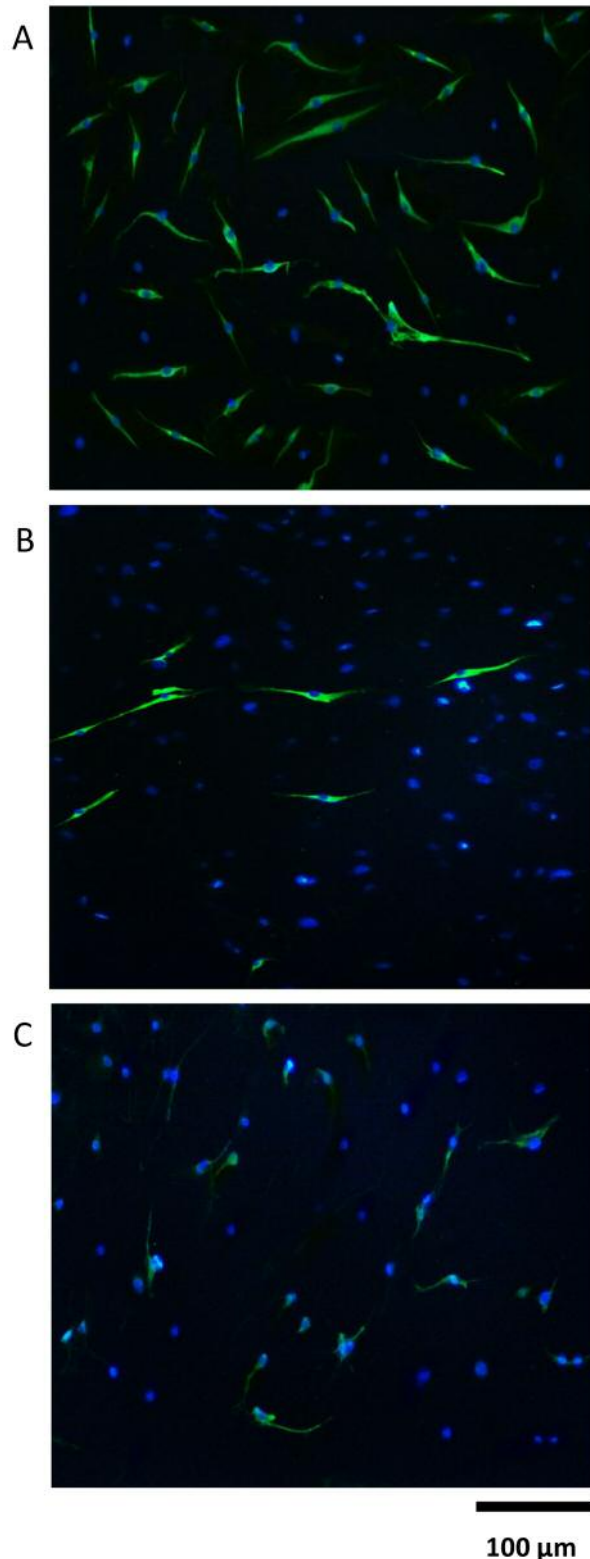


Figure 1. S100 positive (green) Schwann cells in a pair of Schwann-cell-rich (A) and fibroblast-rich (B) cultures derived from a PNF. Treatment with 75  $\mu$ M propranolol for 7 days significantly reduced the proportion of Schwann cells (C). Nuclei were stained in blue with DAPI.

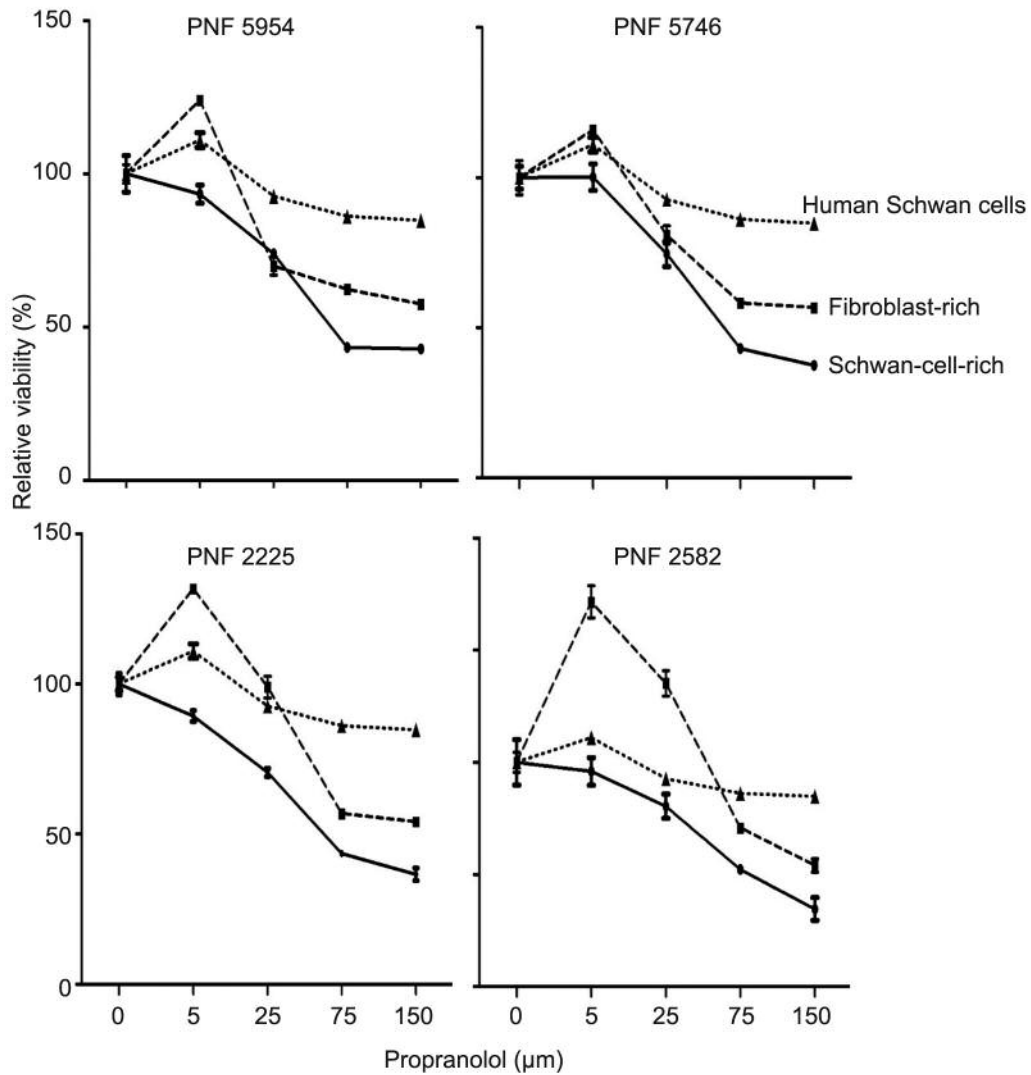


Figure 2. Dose-dependent inhibition of the viability of the 4 pairs of Schwann-cell-rich (solid lines) and fibroblast-rich (dotted lines with squares) cultures following treatment with propranolol. The effect of propranolol on the inhibition of normal human Schwann-cell was slight (dotted lines with triangles).

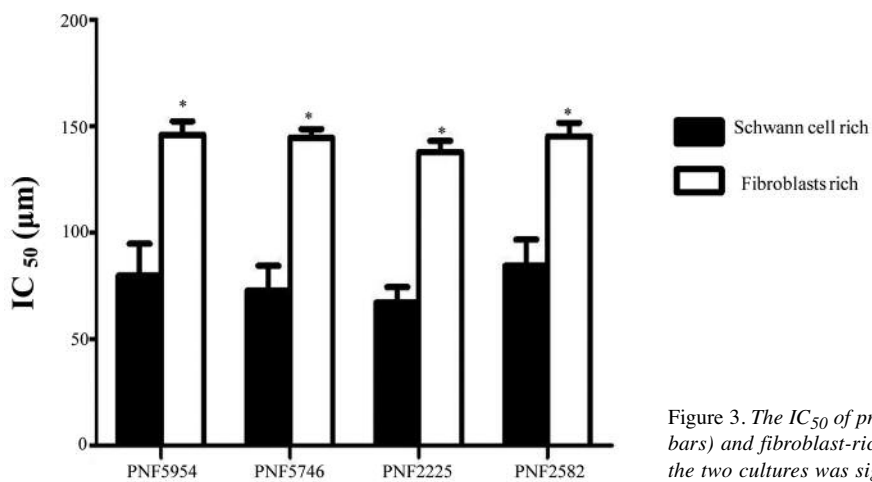


Figure 3. The  $\text{IC}_{50}$  of propranolol in 4 pairs of Schwann-cell-rich (black bars) and fibroblast-rich (white bars) cultures. The difference between the two cultures was significant ( $p < 0.05$ ) in all 4 pairs.

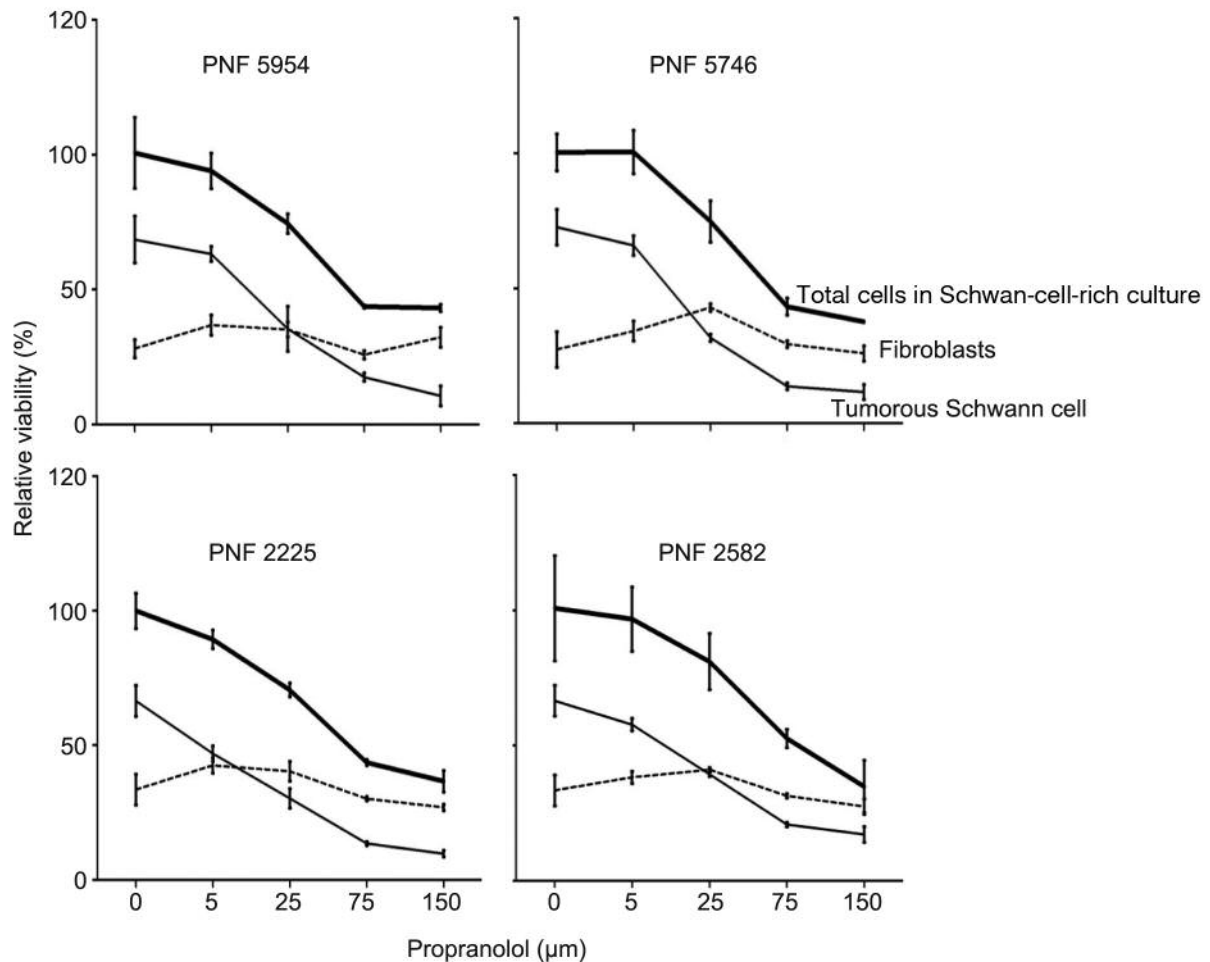


Figure 4. *Propranolol specifically inhibits Schwann cells. Viability of total cells (bolded black line) in a Schwann-cell-rich culture was separated into the viability of Schwann cells (black lines) and the viability of fibroblasts (dotted lines). The former decreased with increasing concentrations of propranolol, while the latter remained rather unchanged.*

## Results

**NF1-derived cells.** Schwann-cell-rich cultures, including 60–80% of Schwann cells, were successfully obtained from the 4 PNFs, as revealed by immunostaining with S100 (Figure 1). Cells from the 4 PNFs also grew under standard conditions and were mostly fibroblasts.

**Propranolol treatment.** Propranolol inhibited the viability of cells of all 4 Schwann-cell-rich cultures in a dose-dependent manner (Figure 2, solid lines). Interestingly, for the fibroblast-rich cultures, low concentration (5 μM) of propranolol slightly enhanced the viability of the cells (Figure 2, dotted lines with squares). At higher propranolol concentrations, the viability of cells of fibroblast-rich cultures was also inhibited, however, less prominent than the cells of the Schwann-cell-rich cultures. There was a slight inhibitory effect of propranolol on normal

human Schwann-cell (Figure 3, dotted lines with triangles). The  $IC_{50}$  values of propranolol was significantly lower for the Schwann-cell-rich culture compared to the corresponding fibroblast-rich culture in all 4 cases ( $p < 0.05$ ), indicating specific inhibition of propranolol on tumorous Schwann cells (Figure 3). In concordance, the proportion of S100 positive Schwann cells decreased upon treatment with propranolol (Figure 1). Then, the viability of all cells in the Schwann-cell-rich culture was calculated and compared to the viability of Schwann cells and fibroblasts. The viability of Schwann cells decreased dose-dependently while that of fibroblasts remained rather constant regardless of the dose of propranolol (Figure 4).

## Discussion

Propranolol exhibited a substantial inhibitory effect on PNFs cells *in vitro*. Moreover, several lines of evidence suggest that

this inhibitory effect is more specific for the tumorous Schwann cells than for the non-tumorous fibroblasts. 1) The inhibitory effect was significantly stronger for the Schwann-cell-rich culture than for the fibroblast-rich culture. 2) The proportion of Schwann cells in the culture treated with propranolol was obviously lower than that in the culture not treated with the drug. 3) When the viability of total cells in the mixed cultures was separated into the viability of the tumorous Schwann cells and the viability of the non-tumorous fibroblasts, the former decreased with increasing concentrations of propranolol while the latter remained unchanged.

Chemotherapeutics are generally expensive and have serious side effects. For example, tyrosine kinase inhibitors such as imatinib may cause bone marrow suppression, liver, and kidney damage. By contrast, propranolol, a beta-blocker for various cardiovascular indications, is among the most effective, safe and inexpensive medications. Propranolol is the first-line drug for infantile hemangiomas with a lower incidence of adverse reactions (22). A typical daily dose of propranolol is 3 mg/Kg/day, corresponding to the peak serum concentration of approximately 56  $\mu$ M. In the present study, the  $IC_{50}$  of propranolol of 75-90  $\mu$ M for the viability of Schwann cells was close to the physiological dose range. Since PNFs are benign tumors for which total resection is not possible for most cases, suppressing their growth by 30% would readily mean a valuable achievement in treatment. Retrospective studies regarding PNF growth and progression in NF1 patients taking propranolol for NF1-unrelated indications may help to elucidate the possible effect of the drug in suppressing PNFs.

In conclusion, propranolol inhibits the viability of tumorous Schwann cells while does not affect the non-tumorous fibroblasts derived from the same PNFs. Our *in vitro* results suggest that propranolol may provide a treatment option for suppressing the growth of PNFs.

## Conflicts of Interest

The Authors report no conflicts of interest regarding this study.

## Authors' Contributions

ZZ: Acquisition of data, analysis, and interpretation of data; drafting the article; revising the manuscript critically for important intellectual content; final approval of the version to be published. LG: Analysis, and interpretation of data; VF and LK, RS: substantial contributions to conception and design; revising the manuscript critically for important intellectual content; final approval of the version to be published. RF: Final approval of the version to be published

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