Role of neurotrophic factors in corneal stromal wound healing

Kowtharapu B.S.[A], Stahnke T.[A], Wree A.[B], Guthoff R.F.[A], Stachs O.[A]

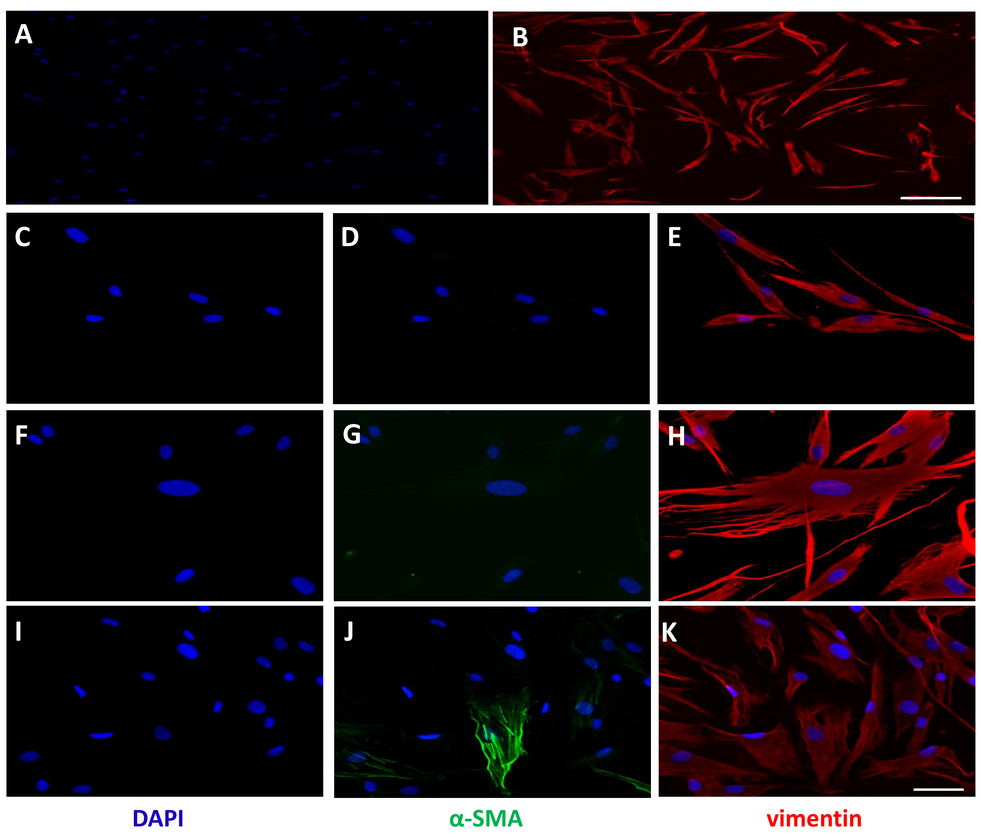
Purpose

Corneal keratocytes have a significant capability of healing the wounded cornea throughout the life. During corneal wound repair, coordinated interactions and bi-directional communication between epithelial cells and stromal keratocytes contributes to tissue reorganisation and plays an important role in wound healing. Previously, we have shown that the expression of substance P (SP) increased when corneal epithelial cells were cultured in the presence of trigeminal neurons[1]. In this context the present study is designed to investigate the effects of neurotrophic factors like nerve growth factor (NGF) and SP in stromal wound healing.

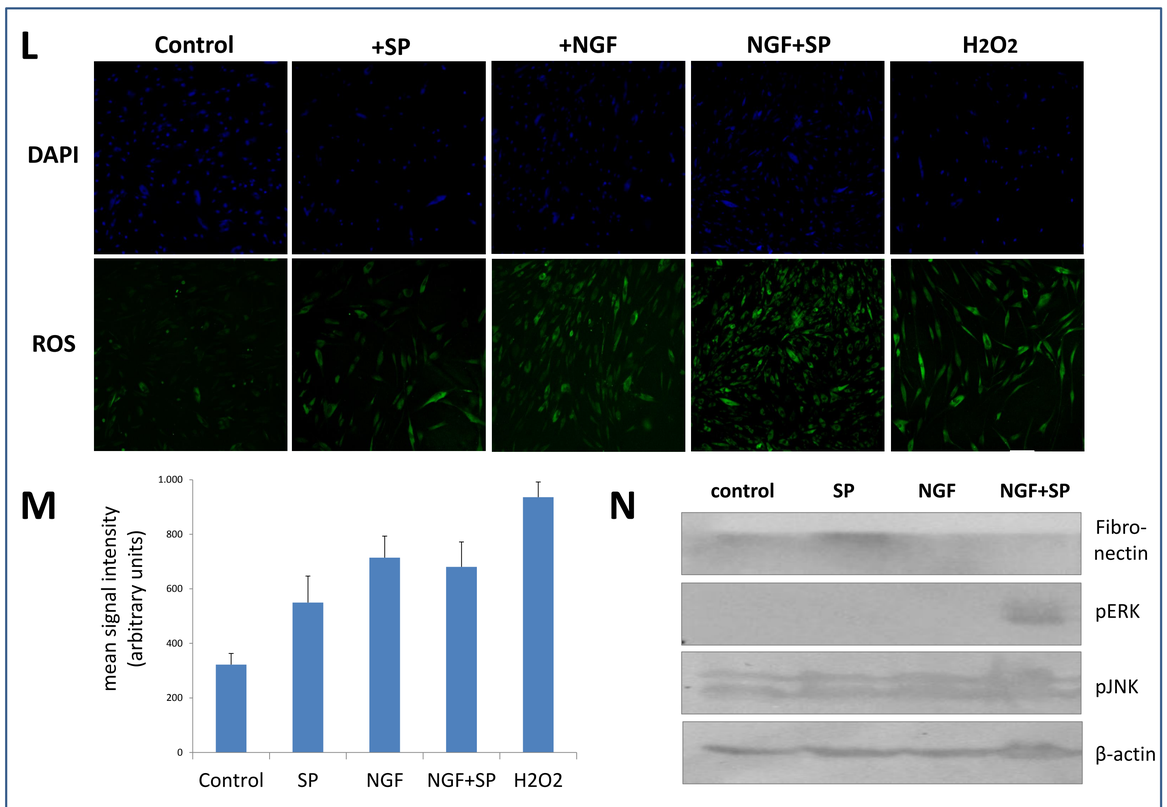
Methods

Primary human corneal fibroblasts (CF) were cultured by an explant culture method from the donor cornea in the presence of DMEM supplemented with 10% fetal calf serum. When outgrowing primary fibroblasts reached a confluent monolayer, cells were trypsinized and subcultured. For experimental analyses primary fibroblasts of third passage were used. The purity of CFs in culture was confirmed by vimentin antibody immune staining whereas the transformation of keratocytes into "repair-phenotype" was analyzed by alpha-smooth muscle actin (α-SMA) immune staining. Reactive oxygen species (ROS) generation in the presence of SP and NGF was measured by using CellROX® oxidative stress detection dyes and western blotting analyses were performed on the respective cell lysates to study the differences in the expression of extracellular matrix components and activation of signaling pathways.

Effect of neurotrophic factors on CFs



A-B: Immune staining images of CF with vimentin: (A) Nuclei stained with DAPI (blue); (B) CFs stained with vimentin antibody (red); Scale bar: 100 µm.
C-K: Immune staining images of CF with anti-α-SMA (green) and anti-vimentin (red) antibodies, respectively; Nuclei stained with DAPI (blue); Scale bar: 50 µm.
C-E: Control CFs; **F-H**: CFs treated with SP (0.5 µM) for 72 hours; **I-K**: CFs treated with TGF-β1 (5 ng/ml) for 72 hours;



L: Primary human CFs were cultured in the presence of neurotrophic factors SP (0.5 µM), NGF (100 ng/ml) and NGF+SP (100 ng/ml+0.5 µM) for 3 days in the abscence of serum. After 72 hours, CellROX® Green oxidative stress reagent was added to the cells, incubated at 37°C for 30 min and fixed. Immediately, after washing with PBS, nuclei were stained with DAPI and the cells were photographed for further analysis. **M:** Mean fluorescence signal intensity, which is an indication of the generated reactive oxygen species (ROS), was measured from the photographs by using ImageJ software. **N:** Western blot analysis of the extracellular matrix components and activated signaling pathways in the presence of neurotrophic factors.

Conclusions

- Treatment of primary CFs with SP alone could not induce the "repair-phenotype" as observed by α-SMA immunocytochemistry. Nevertheless, a significant change in the appearance of CFs was observed as evidenced by elongated cell morphology and cytoskeleton.
- Differences in the generation of ROS and in the expression of fibronectin, phospho ERK were observed after treatment of CFs with SP and NGF. These changes in the extracellular matrix components and activated signaling pathways may contribute to the proliferation of CFs during stromal wound healing.
- The observed alterations in the activated signaling pathways may suggest a bi-directional communication and a paracrine consequence of the released neurotrophic factors from the corneal epithelial cells on stromal keratocytes during wound healing.

1. Kowtharapu BS, Stahnke T, Wree A, Guthoff RF, Stachs O. Corneal epithelial and neuronal interactions: Role in wound healing. Exp Eye Res. 2014 Aug;125:53-61.

A | Department of Ophthalmology | **University of Rostock** | Rostock | Germany
B | Institute of Anatomy| **University of Rostock** | Rostock | Germany

Potentielle Interessenkonflikte: nein