( ) R1 ( ) R2 ( ) R3 (X) PG0 ( ) PG1 ( ) Estagiário ( ) Tecnólogo ( )
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Service (sector) Cornea and External Disease - REFRACTIVE SURGERY Nº CEP

**VEGF is Involved in bFGF-Induced Corneal Neovascularization**

Hailton B. Oliveira, MD, Joel A. D. Javier, MD, Elias Jarade, MD, Jae Bum Lee, MD, PhD, Jin-Hong Chang, PhD, Dimitri T. Azar, MD **PURPOSE.** To characterize bFGF induced VEGF production in corneal keratocytes in vivo and in vitro. **METHODS.** Uniformly sized hydron pellets containing 80ng of bFGF, and control pellets were surgically implanted into wild type C57BL/6, VEGF Lac Z, and VEGF GFP mice corneas. The corneas were observed and photographed at 4 hours, 1, 4, 7, 10, 14 & 21 days post implantation, and the percentage of corneal surface occupied by new vessels was calculated using NIH image program. Wild-type mouse corneas implanted with control and bFGF containing pellets were harvested at 4 hours, 1, 4, 7, 10, 14, and 21 days after pellet implantation. The harvested wild type corneas were evaluated for the localization of CD-31 and VEGF using immuno-confocal microscopy. Western blot analysis of the 1, 4, and 7 day specimens was performed to comparison VEGF expression in the central and peripheral corneal region. The VEGF Lac Z mice received tail vein injections of an endothelial-specific fluorescein-conjugated lectin at day 0, 1, 4 and 7 after pellet implantation, to determine the colocalization of VEGF and neovascularizacao. Immunolocalization of bFGF receptors on immortalized keratocytes cell line and In vitro experiments were used to determine the effect of bFGF on keratocytes cultures from VEGF-GFP mice. **RESULTS.** Neovascularization of the corneal stroma began on day 4 and was sustained through day 21 following bFGF pellet implantation. In the corneal area adjacent to the limbus, the onset of VEGF stromal immunolocalization occurred 24 hours after bFGF pellet implantation and was maintained throughout the 21 day period. CD-31 localization lagged behind VEGF expression by approximately 4 day. In the more central zone (adjacent to the pellet), the onset of VEGF stromal immunolocalization occured at day 1 and peaked at days 4-7. The lag period of CD-31 expression in this zone was 2-5 days. Progression of the vascular endothelial cells, visualized by fluoreceinated tomato lectin, correlated with increased VEGF Lac Z expression. Western blot analysis showed the increased VEGF expression in the zone of corneal neovascularization. VEGF expression increased after implantation of bFGF pellet in VEGF-GFP mice. Enhanced GFP expression was visualized following bFGF stimulation in cultured keratocytes harvested from VEGF-GFP mice. **CONCLUSIONS.** bFGF-induced corneal neovascularization mediated via a VEGF-dependent pathway. Keratocytes express VEGF via bFGF stimulation. Corneal keratocytes by themselves do not express VEGF in the quiescent status; however, upon stimulation with bFGF, the keratocytes were found to express VEGF.