Targeting VEGF-sustained signaling to restore homeostasis in the outer retina

Mary Elizabeth Hartnett, MD
Salt Lake City, UT

Haibo Wang, MD PhD, Aniket Ramshekar, Eric Kunz

Purpose:
Anti-VEGF agents treat about 50% of neovascular age-related macular degeneration (AMD) by reducing permeability, proliferation and migration of choroidal endothelial cells (CECs). In order to improve AMD treatments, we focused on regulating Rac1GTPase, which is essential for CEC migration, and activated by inflammation, oxidation, or VEGF. Since Rac1 is important in physiologic events, we targeted the Rac1 binding site of the multi-domain protein, IQGAP1, and tested the hypothesis that IQGAP1 is important in active Rac1-mediated choroidal neovascularization (CNV).

Methods:
Iqgap1-/- and littermate Iqgap1+/+ mice were treated with laser and assayed for lectin-stained CNV volumes or sectioned for labeling 7 days after laser. Human sections of neovascular AMD were labeled with anti-IQGAP1. Human CECs were transfected with siRNA targeting human Iqgap1, treated with control or VEGF and assayed for active Rac1 (Rac1GTP) by co-immunoprecipitation (co-IP) with Rac1GTP antibody and western blot of total Rac1 or co-IP of IQGAP1 and Rac1GTP. Primary CECs were transfected with the mutant construct to the Rac1 binding domain of IQGAP1 (GFP-IQ-MK24) or wild type (GFP-IQ-WT) exposed to VEGF at 0, 15, 30 or 120 min, and analyzed for co-IP of Rac1GTP/Rac1 or Rac1GTP/IQGAP1.

Results:
In sections from human eyes with neovascular AMD and from murine laser-induced CNV, IQGAP1 was expressed in human CNV lesions and in lectin-labeled murine CNV lesions. Following laser-induced injury, Iqgap1-/- mice, compared to littermate controls, had smaller CNV volumes (p=0.022, n=15 mice), and did not demonstrate Rac1GTP colocalization with lectin labeled CNV. In cultured CECs, knockdown of IQGAP1 inhibited VEGF-induced activation of VEGF receptor 2, Rac1GTP and of CEC migration (p=0.026, n=6). Transfection of CECs with a GFP-IQ-MK24 mitigated VEGF-induced Rac1GTP over 120 min compared to GFP-IQ-WT.

Conclusions:
IQGAP1 localizes in CNV lesions from human AMD and colocalizes with active Rac1 in experimental CNV of Iqgap1+/+ but not Iqgap1-/- mice. Rac1/IQGAP1 interactions are important in CEC migration and CNV formation. Targeting the Rac1GTP binding domain of IQGAP1 reduces VEGF-induced sustained signaling of active Rac1 and may be considered in future studies as a therapeutic target to maintain homeostasis despite multiple stresses that lead to pathology in AMD.