New Questions – New Answers

Optineurin and Its Mutants: Molecules Associated with Some Forms of Glaucoma

Chalasani et al., Hyderabad, India

Ophthalmic Res 2009;42:176–184

Optineurin is a gene associated with normal tension glaucoma and primary open-angle glaucoma. The aim of this study was to understand the functions of the protein optineurin in retinal ganglion cells, particularly when mutations occur in its sequence. To do so, the authors worked with the rat retinal ganglion cell line RGC5 and some others such as HeLa (human cancer cells), COS-1 (monkey kidney cells), IMR-32 (human neuroblastoma cells) and D407 (human retinal pigment epithelial cells). The cDNAs of wild-type OPTN and its mutants H26D, E50K, R454Q and H486R were transfected into each of these cell lines, and the effects were studied. Localization of the proteins was monitored by confocal microscopy and cell imaging. In addition, their interaction with other cellular proteins by yeast two-hybrid assay and co-immunoprecipitation was investigated. The authors found that optineurin interacts with several proteins that are involved in NF- κ B regulation, vesicular trafficking at recycling endosomes, immune response and transcription regulation. Some of these proteins showed altered/reduced interactions with E50K and H486R mutants. E50K, a severe phenotype-related mutant of optineurin, was found to selectively kill RGC5 cells through oxytosis and apoptosis, while none of the other mutants did so. The authors conclude that several independent mechanisms are likely to be involved in the pathogenesis of glaucoma caused by mutations in optineurin.

Development of Laser-Induced Choroidal Neovascularization in Rats after Retinal Damage by Sodium Iodate Injection

García-Layana et al., Pamplona, Spain

Ophthalmic Res 2009;42:205–212

The pathogenesis of CNV formation is only partly understood. The purpose of this study was to determine the role of the retinal pigment epithelium (RPE) in the development of laser-induced choroidal neovascularization by selective damage with sodium iodate (SI). To investigate the impact of this effect, the authors applied diode laser photocoagulation to the right eye of Long-Evans rats (group 1). In group 2, 40 mg/kg SI was adminis-

tered intravenously 2 days after photocoagulation. Group 3 received SI 2 days before the laser was applied. In group 4, SI was injected immediately after photocoagulation. The results show that CNV developed in 42 of 93 burns (45%) of the laser group and in 46 of 75 burns (61%) when SI was administered 2 days after laser ($p = 0.036$). No CNV developed in any of the other groups. The authors conclude that the angiogenic effect of the RPE is essential during the first hours after onset of the laser-induced CNV stimulation. When the RPE was damaged before or at the time of photocoagulation, no CNV was induced. The authors conclude that RPE integrity plays a major role in CNV formation.

Targeting of Integrin-Linked Kinase with a Small Interfering RNA Inhibits Endothelial Cell Migration, Proliferation and Tube Formation in vitro

Guo et al., Beijing, China

Ophthalmic Res 2009;42:213–220

The role of integrin-linked kinase (ILK) in endothelial cell migration and proliferation is still unclear. To investigate this question, cultured RF/6A cells were knocked down for ILK using small interfering RNA (siRNA). Cellular ILK expression was quantified by real-time quantitative PCR and Western blot analysis. Cell migration was measured by cell counting in modified Boyden chambers, while microfilament dynamics were assessed by immunofluorescence analysis. Cell cycling was determined by FACS flow cytometry, and the expression of cyclin D1 was measured by Western blot assay. Finally, the secretion of VEGF in culture medium samples was assessed by ELISA and capillary/tube-like network formation assayed using Matrigel. The results showed that both ILK mRNA and protein levels were virtually undetectable after transfection with ILK siRNA. In addition, there was a significant accumulation of cells in the G0-G1 phase and a marked decrease in cell numbers in the S phase in ILK siRNA-transfected cells; the expression of cyclin D1 was decreased after transfection. The knockdown of ILK significantly inhibited cell migration ability by disturbing F-actin assembly. In addition, VEGF secretion in conditioned medium was reduced by 33%. Furthermore, treatment with ILK siRNA suppressed tube formation in RF/6A cells and significantly reduced the overall length of the tubes. The authors conclude that knockdown of ILK with siRNA effectively inhibits endothelial cell migration, proliferation and tube formation in vitro.

Uwe Pleyer, Editor-in-Chief