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Role of Raf-1 kinase in Diabetes-induced Accelerated Apoptosis of Retinal Capillary Cells

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Small molecular weight G-proteins serve as fundamental signaling switches that regulate cell fates by coupling receptor activation to downstream effector pathways. H-Ras, a small molecular weight G-protein, in its active form, recruits Raf. Activated Raf via a signaling transduction pathway regulates apoptosis. Our previous studies have shown that H-Ras has an important role in the loss of retinal capillary cells in diabetes. The purpose of this study is to investigate the role of Raf-1 in the development of diabetic retinopathy. Bovine retinal endothelial cells were incubated in 5 mM or 20 mM glucose in the presence of Raf-1 kinase inhibitor (10μM of GW5074), activator (200μM of ZM336374) or mitogen activated protein kinase inhibitor (30μM of PD098059) for five days. Apoptosis of endothelial cells was analyzed by ELISA and activation of Raf-1 and its downstream signaling proteins by determining genes and protein expressions. Inhibition of Raf-1 kinase repressed glucose-induced apoptosis of the cells by 75%, and this was accompanied by attenuation of activation of MAP kinase, ERK-1, nuclear transcriptional factor and caspase-3. In contrast, ZM336374 further increased glucose-induced apoptosis by 50%, and activated the signaling molecules and caspase 3 by over 30%. Further, PD098059 alone also attenuated glucose-induced apoptosis of retinal endothelial cells. These findings demonstrate that accelerated loss of retinal capillary cells in diabetes is mediated via Raf-1 kinase activation. Modulation of Raf-1 kinase activity could, in part, regulate apoptosis of retinal endothelial cells, which may ultimately contribute to the development of diabetic retinopathy..