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Expression of Protein Kinase CK2 in Astroglial Cells of Normal and Neovascularized Retina

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We previously documented protein kinase CK2 involvement in retinal neovascularization. Here we describe retinal CK2 expression and combined effects of CK2 inhibitors with the somatostatin analog octreotide in a mouse model of oxygen-induced retinopathy (OIR). CK2 expression in human and rodent retinas with and without retinopathy and in astrocytic and endothelial cultures was examined by immunohistochemistry, Western blotting, and reverse transcriptase-polymerase chain reaction. A combination of CK2 inhibitors, emodin or 4,5,6,7-tetrabromobenzotriazole, with octreotide was injected intraperitoneally from postnatal (P) day P11 to P17 to block mouse OIR. All CK2 subunits (α, α', β) were expressed in retina, and a novel CK2α splice variant was detected by reverse transcriptase-polymerase chain reaction. CK2 antibodies primarily reacted with retinal astrocytes, and staining was increased around new intraretinal vessels in mouse OIR and rat retinopathy of prematurity, whereas preretinal vessels were negative. Cultured astrocytes showed increased perinuclear CK2 staining compared to endothelial cells. In the OIR model, CK2 mRNA expression increased modestly on P13 but not on P17. Octreotide combined with emodin or 4,5,6,7-tetrabromobenzotriazole blocked mouse retinal neovascularization more efficiently than either compound alone. Based on its retinal localization, CK2 may be considered a new immunohistochemical astrocytic marker, and combination of CK2 inhibitors and octreotide may be a promising future treatment for proliferative retinopathies. ***(Am J Pathol 2006, 168:1722–1736; DOI: 10.2353/ajpath.2006.050533)***

Neovascularization, an important physiological process during retinal development, is a combination of vasculogenesis and angiogenesis.[1,2] The superficial retinal vessels grow radially from the optic nerve by vasculogenesis, where endothelial progenitor cells and hemangioblasts differentiate to form blood vessels. Deep vessels evolve by angiogenesis in which sprouting from superficial retinal vessels occurs, causing penetration of new blood vessels into the retina. In both processes, astrocytes migrating from the optic nerve to the retina play a guiding role for sprouting capillaries.[3]

Retinal neovascularization is usually considered to be the result of proliferation of endothelial cells from the existing blood vessels by angiogenesis. However, circulating adult hematopoietic stem cells may also contribute to this process by differentiating into endothelial cells.[4] It is still unclear what fraction of endothelial cells derives from hematopoietic stem cells during retinal angiogenesis. The identity of growth factors and cytokines that

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recruit bone marrow-derived precursors into circulation for participating in angiogenesis is not fully determined although some factors have been recently identified.[5] Also, adult mouse nonhematopoietic stem cells when injected into vitreous of neonatal eyes co-localize with retinal astrocytes that serve as a network for retinal angiogenesis.[6]

The initial stimulus for retinal neovascularization is thought to be hypoxia or ischemia that causes increases in the expression of growth factors, integrins, and proteinases resulting in the formation of new vessels. The first reaction to hypoxia is stimulation of hypoxia-inducible factor-1, which up-regulates angiogenic factors such as vascular endothelial growth factor (VEGF-A), fibroblast growth factor-2, insulin-like growth factor-I, hepatocyte growth factor, and platelet-derived growth factor.[7–9] The induction of angiogenesis depends on equilibrium between angiogenic factors and angiogenesis inhibitors including angiostatin, pigment epithelium-derived factor (PEDF), and thrombospondin-1.[9–11] Such a balance may become distorted in certain conditions leading to pathological retinal neovascularization, as seen in proliferative diabetic retinopathy (DR).

DR is the most severe ocular complication of diabetes and a major cause of blindness worldwide.[12,13] Hyperglycemia-induced advanced glycation end products[14] contribute to retinal hypoxia in diabetes, leading to early pericyte dropout and capillary closure. As the disease progresses, the resulting ischemia elicits compensatory retinal neovascularization triggered by angiogenic growth factors, notably VEGF, produced by retinal neuroglial cells, astrocytes, and Müller cells.[14,15] Animal oxygen-induced proliferative retinopathy (OIR), although developing on a nondiabetic background, proved to be a useful model in exploring roles of specific growth factors in angiogenesis[16,17] and the significance of altered cell interactions in DR pathogenesis.

Astrocytes and endothelial cells are believed to be interdependent cell populations,[2] but cellular mechanisms involved in the recruitment and differentiation of astrocyte precursor cells (spindle cells) in angiogenesis remain poorly understood. A captivating possibility is a cross talk between astrocytes and endothelial cells. The ability of astrocytes to secrete VEGF and promote retinal angiogenesis is well established.[18–20] Conversely, endothelial cell-derived leukemia inhibitory factor[21] and platelet-derived growth factor[22] can induce retinal astrocyte differentiation *in vitro*.

Studies of protein kinases in astrocyte-endothelial cell interactions may lead to establishing signaling pathways involved in retinal angiogenesis and to developing approaches for anti-angiogenic therapy. Protein kinase CK2 (formerly casein kinase 2) is a ubiquitous serine/threonine protein kinase that phosphorylates more than 300 substrates and is involved in a wide variety of biological processes, including cell proliferation, differentiation, apoptosis, and tumor development.[23–26] Our previous data have shown that CK2 inhibitors potently blocked adhesion, migration, and capillary-like tube formation by cultured retinal endothelial cells, as well as retinal neovascularization in a mouse model of OIR.[27] These data implicate CK2 in retinal angiogenesis and pathogenesis of diabetic and other proliferative retinal microangiopathies. Thus, it was of interest to examine the expression of CK2 in retinal cells. To this end, we studied CK2 localization in human retinal cells by immunohistochemistry using specific antibodies to CK2 subunits. These antibodies reacted with astrocytes of the human and rodent retinas. We also determined CK2 expression in mouse OIR and in rat retinopathy of prematurity (ROP). Finally, a combined action of CK2 inhibitors with somatostatin analogs that may inhibit different angiogenic signaling pathways was studied in the OIR model.

Materials and Methods

Immunohistochemistry

Postmortem human eyes were obtained from National Disease Research Interchange (Philadelphia, PA) within 24 hours after death. Isolated human retinal samples as well as whole human, mouse, and rat eyes were embedded in O.C.T. compound (Sakura Finetek U.S.A., Inc., Torrance, CA). Frozen sections (6 μm) were cut on a Leica CM1850 cryostat (McBain Instruments, Chatsworth, CA). Before immunofluorescent staining, sections were fixed for 5 minutes at room temperature in 3% *p*-formaldehyde in phosphate-buffered saline (PBS). Stained sections were mounted in Immuno-Fluoro mounting medium (ICN Immunobiologicals, Lisle, IL). The pictures were taken with MagnaFire digital camera (Optronics, Goleta, CA) attached to a BX40 Olympus microscope (Olympus USA, Melville, NY) and were combined using MagnaFire 2.1 software.

Specific antibodies were applied for 1 to 4 hours incubation at 20°C at the following dilutions: 1:100 mouse anti-human CK2α/α' (clone 1AD9; Santa Cruz Biotechnology, Santa Cruz, CA), 1:50 mouse anti-human CK2α/α' (clone D8E),[28] 1:50 rabbit anti-human CK2α (H-286, Santa Cruz Biotechnology), 1:200 rabbit anti-human CK2α, anti-CK2α', and anti-CK2β antibodies.[29] All tested anti-CK2 antibodies are listed in Table 1. Other antibodies included goat anti-mouse vimentin (ICN Biomedicals, Aurora, OH), goat anti-human glial fibrillary acidic protein (GFAP, Santa Cruz Biotechnology), mouse anti-pig GFAP (clone GA5; Sigma Chemical Co., St. Louis, MO), rabbit anti-human GFAP (Sigma), mouse anti-human/rat laminin γ1 chain (clone 2E8;[30] a gift from Dr. E. Engvall, the Burnham Institute, La Jolla, CA), rat anti-human/mouse perlecan (clone C11L1),[31] mouse anti-α-smooth muscle actin (clone 1A4, Sigma), rabbit anti-chicken desmin with broad interspecies cross-reactivity (Sigma), and affinity-purified rabbit anti-mouse/rat laminin (A.V. Ljubimov, unpublished). The latter three antibodies were used to visualize blood vessels in mouse and rat retinas. Immunofluorescence staining was performed as described,[31] with cross-species adsorbed secondary antibodies from Chemicon International (Temecula, CA).

In some experiments, the activity of anti-human CK2α/α' 1AD9 monoclonal antibody (mAb) was neutral-