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SCIENCE DIRECT

Brain Research 1054 (2005) 103 – 115

BRAIN RESEARCH

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Research Report

Long-term activation of c-Fos and c-Jun in optic nerve head astrocytes in experimental ocular hypertension in monkeys and after exposure to elevated pressure in vitro

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Accepted 14 June 2005
Available online 2 August 2005

Abstract

This study investigates whether the immediate early gene (IEG) products c-Fos and c-Jun are activated in vivo in monkeys with experimental glaucoma, and in vitro in cultured human ONH astrocytes exposed to hydrostatic pressure (HP). Three Rhesus monkeys with mild glaucomatous damage (mean intraocular pressure (IOP) 27 ± 1.3 mm Hg ~42 weeks) and three with moderate glaucomatous damage (mean IOP 44 ± 6.7% mm Hg ~11 weeks) were used for this study; the contralateral eye served as normal control (mean IOP 18.6 ± 1.7 mm Hg). ONH tissues were stained with GFAP, DAPI, and c-Jun or c-Fos, and transcription factor positive and negative nuclei were counted to determine nuclear localization. Cultured human normal and glaucomatous ONH astrocytes exposed to elevated HP served as the in vitro model of elevated pressure. Activation and nuclear localization of c-Fos and c-Jun increased significantly in the monkeys with elevated IOP. These data correlated with axonal loss, reactive astrocytes, and remodeling of the optic disc. Cultured human ONH astrocytes showed increased nuclear localization of c-Fos and c-Jun under exposure to HP. Immunohistochemistry demonstrated that the upstream regulators of c-Fos and c-Jun, ERK–MAPK and MAPKp38 localized to the nuclei of ONH astrocytes in monkeys with experimental glaucoma. Taken together, these results demonstrate c-Fos and c-Jun activation in ONH astrocytes in vivo and in vitro, and that activation of both transcription factors is associated with ERK and MAPKp38 activation in experimental glaucoma, suggesting that activation of transcription factors may participate in the induction and maintenance of the reactive astrocyte phenotype in glaucomatous optic neuropathy.

Theme: Disorders of the nervous system
Topic: Degenerative disease: other

Keywords: c-Fos; c-Jun; Astrocytes; Ocular hypertension; Hydrostatic pressure

Abbreviations: ALTS, argon laser scarification of the trabecular meshwork; DAPI, 4′,6-diamidino-2-phenylindole; ERK, extracellular signal-regulated kinase; ExpG, experimental glaucoma; FBS, fetal bovine serum; GFAP, glial fibrillary acidic protein; HP, hydrostatic pressure; IOP, intraocular pressure; JNK, c-Jun N-terminal kinase; MAPK, mitogen activated protein kinase; ON, optic nerve; ONH, optic nerve head; POAG, primary open angle glaucoma; PBS, phosphate buffered saline; TTBS, tris buffered saline + Tween

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doi:10.1016/j.brainres.2005.06.050

1. Introduction

Astrocytes are the most abundant cell type in the adult CNS. They are the major glial cell type in the non-myelinated optic nerve head (ONH) and provide cellular support functions to the axons, forming the interface between connective tissue surfaces and surrounding blood vessels. Astrocytes of the ONH have many of the same functions as astrocytes in the white matter in that they supply energy substrate to axons in the optic nerve, and maintain extracellular pH and ion homeostasis in the periaxonal space. In the lamina cribrosa, astrocytes form lamellae oriented perpendicular to the axons, and they maintain the extracellular matrix (ECM) which consists of collagens, elastic fibers, and glycoproteins such as laminin and proteoglycans [11]. In the normal ONH, astrocytes express a wide variety of growth factors and receptors, many of which serve as trophic and survival factors [20,21,30,46].

Primary open angle glaucoma (POAG) is a sight-threatening optic neuropathy characterized by loss of the axons of the retinal ganglion cells (RGC) and cupping of the optic disc in response to abnormally elevated intraocular pressure (IOP) in many patients [17]. In the glaucomatous ONH, differentiated, quiescent astrocytes become "reactive" and are thought to impede axonal survival or regrowth [29,34,36]. Reactive astrocytes have enlarged astrocyte cell bodies with a thick network of processes and increased expression of glial fibrillary acidic protein (GFAP) and vimentin [11,44]. In the glaucomatous ONH, reactive astrocytes increase expression of various cell surface molecules that play important roles in cell–cell recognition and in cell adhesion to substrates, as well as various growth factors, cytokines, and receptors. Reactive ONH astrocytes express many new ECM proteins such as laminin, tenascin C, and proteoglycans [11,12]. The expression of transforming growth factors, TGF-β1 and TGF-β2, ciliary neurotrophic factor (CNTF), fibroblast growth factor 2 (FGF-2), platelet-derived growth factor (PDGF), and their receptors have been reported to induce the transition of quiescent astrocytes into the reactive phenotype or to modulate the function of reactive astrocytes [6,22,47].

Expression of most genes associated with the transition and maintenance of the reactive astrocyte phenotype in neural degeneration is regulated at the transcription level. Very little is known about the transcriptional regulation of gene expression in optic nerve astrocytes in human and in experimental optic nerve diseases. The human proto-oncogene JUN is a transcription factor that interacts directly with specific target DNA sequences to regulate gene expression. The JUN gene product has also been shown to be structurally and functionally identical to the enhancer binding protein, AP-1; thus, AP-1 may be encoded by JUN [4]. The gene product of FOS is a leucine zipper protein that forms dimers with proteins of the Jun family in the nucleus, thereby forming the transcription factor complex activating protein 1 (AP-1) [16]. As such, the FOS proteins have been implicated as regulators of cell proliferation, differentiation, transformation, and apoptosis. Its product, the c-Fos protein, is a major component of the activator protein 1 (AP-1) complex, which controls the basal and inducible expression of several genes, including those encoding for basic fibroblast growth factor (bFGF) and intercellular adhesion molecule-1 (ICAM-1) among others [28,42,48].

It has been reported that c-Jun is expressed in RGCs after optic nerve lesions suggesting that c-Jun may promote RGC regeneration after injury [14,19]. c-Fos and c-Jun expression increased in neurons of the lateral geniculate neurons in response to RGC loss in glaucomatous rats [45]. Previous in vitro data provided by our laboratory have shown that c-Fos and c-Jun are positively affected in human ONH astrocytes exposed to elevated hydrostatic pressure (HP) [50]. In this study, we examined the activation and expression of c-Jun and c-Fos in vivo in monkeys with experimental glaucoma. Activation of these transcription factors in the monkey model correlated in our results with RGC axonal loss, reactive astrocytes, and remodeling of the optic disc. In addition, we investigated the role of c-Fos and c-Jun in human normal and glaucomatous ONH astrocytes exposed to elevated hydrostatic pressure (HP) and found evidence of increased activation and nuclear localization of the transcription factors under HP. Finally, it is known that members of the mitogen-activated protein kinase (MAPK) family are upstream regulators of the activation of c-Fos and c-Jun. Immunohistochemistry data here provide evidence that the ERK–MAPK and MAPKp38 are activated in the nuclei of ONH astrocytes in monkeys with experimental glaucoma (ExpG). These results demonstrate that c-Fos and c-Jun are expressed in ONH astrocytes both in vivo and in vitro and that activation of both transcription factors is associated with ERK and MAPKp38 activation in ExpG, suggesting that both transcription factors may be involved in the pathophysiology of glaucomatous optic neuropathy.

2. Materials and methods

2.1. Animal model

Six Rhesus monkeys with laser-induced unilateral elevated IOP were prepared in Dr. Paul Kaufman's laboratory (Department of Ophthalmology, University of Wisconsin Medical School at Madison). The clinical characteristics of the monkey eyes used in this study were reported earlier in detail [2,32]. The studies were performed following the guidelines of the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research.