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Review article

Myocilin and optineurin: Differential characteristics and functional consequences

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ABSTRACT

Myocilin and optineurin are two genes linked to glaucoma, a major blinding disease characterized by progressive loss of retinal ganglion cells and their axons. This review describes the characteristics of myocilin and optineurin protein products and summarizes the consequences of ectopically expressed wild-type and mutant myocilin and optineurin in trabecular meshwork and/or neuronal cells. Myocilin and optineurin exhibit differential characteristics and have divergent functional consequences. They contribute to the development of glaucoma probably via distinct mechanisms.

1. Introduction

Glaucoma is one of the leading causes of irreversible blindness worldwide. This disease is characterized by progressive loss of retinal ganglion cells (RGCs) and accompanying axons. Primary open-angle glaucoma (POAG), the most common form of glaucoma, is frequently associated with increased intraocular pressure (IOP). The IOP is controlled by a balance between the production and outflow of the aqueous humor contained in the anterior chamber. The trabecular meshwork (TM), a specialized tissue, is the major site for regulation of the aqueous humor outflow.[1,2]

Studies over the past decades have revealed that POAG is genetically heterogeneous, caused by several susceptibility genes and environmental factors.[3,4] To date, a total of 15 chromosomal loci have been mapped and designated as GLC1A to GLC1O.[3–7] Four candidate genes have been identified that include myocilin as the GLC1A,[6] optineurin as the GLC1E,[7] WDR36 as the GLC1G,[3,4] and neurotrophin-4 as the GLC1O[3,4] gene.

Myocilin is the first identified gene for both juvenile- and adult-onset POAG.[6] More than 70 myocilin mutations have been found in several families.[3,5] Glaucoma patients with myocilin mutation tend to have high IOP.[5] Among the myocilin mutations, the Gln368Stop (Q368X) mutation is the most frequent[8] and the Pro370Leu (P370L) mutation is responsible for one of the most severe glaucoma phenotypes.

Optineurin is a gene that links principally to normal tension glaucoma (NTG), a subtype of POAG.[7] Optineurin mutations are noted to vary with ethnic background.[9] The Glu50Lys (E50K) mutation, found in Caucasian and Hispanic populations,[9] seems to be associated with a more progressive and severe disease in NTG patients.[10]

2. Gene structure and expression

The myocilin gene contains three exons and two introns that span 17 kb pairs.[11] The protein product encoded by the human gene contains 504 amino acids. There is a hydrophobic signal peptide sequence (amino acids or aa 1–32) and non-muscle myosin-like domain near the amino (N)-terminus and an olfactomedin-like domain (aa 326–501) at the carboxyl (C)-terminus. The N-terminus has two initiation sites. Within the myosin-like domain, there are leucine zipper motifs within two coiled-coil regions (aa 74–110 and aa 118–186).[11,12] The leucine zipper and the olfactomedin domains are well conserved across species. At the secondary level, the N-terminal region is primarily α-helical and the C-terminal consists mostly of β-strands.[13] In glaucoma patients, most of the myocilin mutations are mapped to the third exon of the gene within the C-terminal olfactomedin domain.

The expression of myocilin is mainly in the eye, but it has also been observed in several other tissues in the body including the skeletal muscle and brain. In the eye, myocilin is seen in many tissues such as the sclera, ciliary body and the optic nerve head,[14] although its expression is the highest in the TM.

The human optineurin gene has a total of 16 exons; the first 3 are non-coding and the remaining 13 exons code for a 577-amino acid protein that contains multiple coiled-coil domains and a C-terminal zinc finger.[15,16] The optineurin protein from different species has

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high amino acid homology.[16] The amino acid 50 glutamic acid residue is conserved in mouse, rat, chicken and cow. Optineurin is also ubiquitously expressed in non-ocular tissues such as the heart and brain, and in ocular tissues including the retina, trabecular meshwork and non-pigmented ciliary epithelium. In the retina, RGCs are immunolabeled with high intensity.[15,16]

3. Protein characteristics

Myocilin was initially identified as a protein secreted into the media of TM cultures after induction with glucocorticoids such as dexamethasone (DEX).[12] One unique feature is that myocilin expression is highly upregulated by DEX in TM cells but not in other cell types such as corneal fibroblasts.[11,17] Containing a signal peptide sequence at the N-terminus, myocilin is, at least in part, secreted via the traditional secretory pathway. Myocilin has additionally been shown to associate with exosome-like vesicles and may also use this alternative mechanism to be released from the cell.[11]

Myocilin has been shown to be a glycoprotein, N-glycosylated at amino acid residues 57–59 (Asn-Glu-Ser).[11] On Western blots, it is seen as 53–57 kDa doublet bands although some antibodies also yield a 66-kDa protein band. When subjected to membrane protein extraction, myocilin in TM cell lysates distributed largely in the hydrophobic fraction in association with membranes (Fig. 1). The myocilin protein has also been reported to be cleaved into a 20-kDa N-terminal fragment and a 35-kDa C-terminal fragment both *in vitro* and *in vivo*. The proteolytic processing of myocilin is suggested to have a role in regulating its molecular interactions.[18]

Myocilin interacts with itself at sites of the leucine zipper/coiled-coil domain to form dimers and possibly multimers.[11,19,20] It also interacts with several proteins including flotillin-1 (a structural protein of lipid rafts), optimedin, extracellular proteins such as fibronectin and fibrillin-1, as well as matricellular proteins hevin and SPARC.[11,18]

Optineurin, by contrast, is not secreted.[21] It is neither N- nor O-glycosylated but is serine- and tyrosine-phosphorylated.[22] Optineurin is shown to be, exclusively, a hydrophilic, cytosolic protein.[22] It possesses a ubiquitin-binding domain, is ubiquitinated and is processed through the ubiquitin–proteasome pathway.[22,23] By native blue gel electrophoresis, optineurin is found to be capable of forming 420 kDa homo-oligomers, which, based on the 67-kDa monomer size, is estimated to be hexamers.[22] Furthermore, optineurin interacts and associates with Rab8, myosin VI and transferrin receptor,[22,24] either singly or in combination, to form supermolecular complexes with sizes larger than 400 kDa.[22]

Optineurin has also been shown to interact with transcription factor IIIA,[25] huntingtin,[26] metabotropic glutamate receptor[27] and TANK-binding kinase 1 (TBK1).[28] It shares 53% amino acid homology with NEMO (NF-κB essential modulator) and is identified as a NEMO-related protein.[29] Optineurin expression is upregulated by tumor necrosis factor-α (TNFα)[15] and interferon and its phosphorylation is induced upon phorbol 12-myristate 13-acetate stimulation.[29]

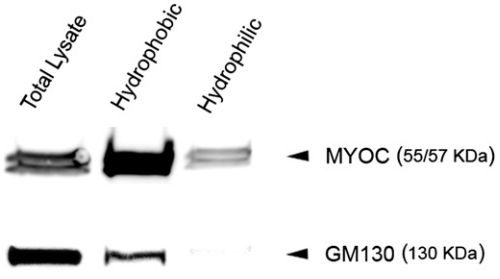

Fig. 1. Western blot analysis for myocilin in hydrophilic and hydrophobic fractions of human TM cell lysates. Total proteins in TM lysates were subjected to membrane protein extraction followed by Western blotting. The hydrophobic fraction contained membrane proteins and the hydrophilic fraction contained predominantly non-membrane cytosolic proteins. Myocilin (MYOC) in total cell lysates was detected largely in the hydrophobic fraction, although a small portion was also seen in the cytosolic, hydrophilic fraction. GM130, a Golgi marker used as a membrane protein positive control, was detected exclusively in the hydrophobic fraction.

4. Localization

Myocilin protein is localized to both intracellular and extracellular sites in TM cells. Immunofluorescence has shown that the intracellular form of myocilin is diffusely distributed in the cytoplasm including perinuclear regions.[11,17] Subcellular fractionation and/or immunoelectron microscopy indicated that intracellular myocilin in TM cells is associated not only with endoplasmic reticulum, Golgi apparatus, vesicles but also with mitochondria.[11,30] Immunogold labeling documented the extracellular localization of myocilin.[11,31,32] It is intriguing that, in TM tissues, myocilin is mainly associated with microfibrillar architecture in sheath-derived plaques where pathologic changes have been reported to occur in the eyes of POAG patients.[32]

Optineurin also has a diffuse, cytoplasmic distribution but a proportion of the protein is associated with the Golgi apparatus.[21] As optineurin is a cytosolic protein not associated with membranes, the optineurin–Golgi association is probably indirect via interactions of other Golgi-associated proteins such as Rab8.[22]

5. Consequences of overexpression and mutation and/or possible functional roles

5.1. Deadhesive activity, Rho inactivation, mitochondrial association and inhibition on neurite outgrowth: Myocilin phenotype

The extracellular myocilin was demonstrated to be a very poor substrate for TM cells.[33] It blocks the TM attachment to fibronectin, retards migration, causes a dramatic reduction in actin stress fibers and focal adhesions,[33,34] and triggers TM cells to assume a stellate morphology with microspikes.[34] Myocilin has been shown to interact with fibronectin via the heparin (Hep) II domain.[35,36] This domain, along with the Arg-Gly-Asp site, is where fibronectin is linked to the actin cytoskeleton, transducing signals from the exterior to the interior of the cells. The fibronectin–TM cell interaction is possibly mediated via binding of myocilin to the Hep II domain.[36] Purified recombinant myocilin protein has also been more recently noted to be a modulator of Wnt signaling.[37]

Overexpressing wild-type or full-length myocilin intracellularly[38] by transfection or by protein transduction[39] in TM cells results in an alteration in actin architecture, similar to that seen with the extracellular form of myocilin. Key findings include a loss of actin stress fibers and vinculin-positive focal adhesions, and a reduction in cell adhesion to fibronectin, vitronectin and collagen types I and IV.[40] The fibronectin deposition is diminished and the trypsinization time needed to round up or suspend TM cells from plates is significantly shorter for myocilin transfectants than that for mock controls, signifying compromised cell–matrix adhesiveness.[40] Myocilin therefore appears to possess a deadhesive activity, capable of changing TM cells in culture from a state of strong adherence, containing robust focal adhesions and actin-containing stress fibers, to a state of compromised adhesiveness. The deadhesion process, on a long-term chronic basis, may render TM cells vulnerable. Myocilin-overexpressing cells have been shown to display an increased susceptibility to apoptotic challenge.[38] It is speculated that cell vulnerability, in conjunction with additional