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Focus on molecules: Cochlin

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1. Structure

Cochlin is the product of the coagulation factor C homology gene (*COCH*). Cochlin is highly conserved in human, mouse and chicken, with 94 and 79% amino acid identity of human to mouse and chicken sequences, respectively. Cochlin mRNA is expressed in the cochlea and in very low levels in the cerebellum, eye, spleen, lung, brain and thymus. Cochlin comprises a short predicted signal peptide (SP), an N-terminal factor C homology (FCH or LCCL) domain and two von Willebrand factor A-like domains (vWFA1 and vWFA2). Domain organization of cochlin is presented schematically in Fig. 1. Wild-type cochlin contains two potential N-linked glycosylation sites at positions 100 and 221 of its primary amino acid sequence. Three cochlin protein isoforms (~40, ~44 and ~60 KDa) have been detected in the cochlea and eye. The size heterogeneity of cochlin may be due to alternative mRNA splicing, exon skipping or posttranslational modification. Smaller forms lack the FCH domain. The N-terminal FCH domain is also referred as the LCCL domain, named after the horseshoe crab (*Limulus*) factor C, cochlin, and the late gestation lung protein (Lgl1). This domain has also been found in other proteins, for example, CocoaCrisp proteins of chicken (accession number AAK16497), mouse (NP_113579), and human (NP_113649); eye protein vitrin; and cub1 domain of human bone morphogenetic protein. Amino acid sequence comparisons and circular dichroism and NMR analysis have identified LCCL as an autonomously folding domain. Cochlin's two vWFA domains are encoded by exons 8–10 and 11–12, respectively. vWFA domains are present in a variety of extracellular matrix (ECM) components, including cartilage matrix protein and collagen types VI, VII, XII and XIV. Cochlin comprises the major non-collagen component of the ECM of the inner ear (reviewed in Robertson et al., 2003).

2. Function

The function of cochlin remains to be elucidated fully. Wild-type or other naturally existing mutant cochlins expressed in HeLa cells accumulate in extracellular deposits that closely parallel the ECM component fibronectin. Cochlin deposits in HeLa cells are not present in focal adhesion sites, and do not co-localize with known focal adhesion markers such as actinin, vinculin or paxilin. Full-length cochlin expressed in COS-7 or HeLa cells as HA-tagged or myc-tagged proteins, respectively, fail to show aggregation or degradation (Robertson et al., 2003). However, exogenous addition of cochlin leads to aggregation of primary trabecular meshwork (TM) cells in vitro. The aggregation of TM cells appears specific for cochlin and may be blocked by prior incubation of cochlin with anti-cochlin antibody (Bhattacharya et al., 2005a).

The function of the FCH domains remains to be elucidated. Evidence suggests that FCH domains are involved in antibody-independent host defense. Lgl1, present in fetal lungs and

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Limulus factor C are thought to be protective against pathogens. Another important cochlin domain, vWFA is known for shear-induced self-aggregation and adherence to platelets, macrophages or leukocytes. Formation of multimeric aggregates in vivo with physiological relevance has been known for several proteins such as apoptin, frataxin and basic fibroblast growth factor. Aggregation may be induced with or without shear and often the presence of ions or oxidative conditions are sufficient for induction. Cochlin undergoes aggregate formation when subjected to shear stress (Bhattacharya et al., 2005a). Whether multimeric aggregates of wild-type full-length cochlin are biologically active and elicit cell aggregation needs to be investigated. SDS-PAGE analysis reveals reduction of the molecular size of cochlin upon PNGase treatment (Robertson et al., 2003) indicating presence of glycosylation. The functional consequence of glycosylation in cochlin in the FCH and vWFA domains and interaction with protein partners remain unknown. Multiple protein–protein interactions mediated by FCH and/or vWFA domains may underlie cochlin localization and in vivo function.

3. Disease involvement

Several cochlin mutants in the FCH domain were found in patients with the autosomal dominant deafness and vestibular disorder, DFNA9, which is late-onset and progressive in nature (Robertson et al., 2003). In human DFNA9, cochlin has been found associated with microfibrillar deposits containing mucopolysaccharide (MPS) in the cochlea. Although the mechanism of pathogenesis in DFNA9 is still under investigation, aggregation of mutated cochlin has been implicated in the formation of such deposits (Robertson et al., 2003). Some DFNA9 patients develop vascular diseases such as cerebral ischemia and acute myocardial infarction, and the vWFA domain has been implicated in increased shear-induced platelet aggregation (SIPA).

Cochlin and MPS-cochlin deposits were found associated with glaucomatous but not normal human TM (Bhattacharya et al., 2005a). Early cochlin protein expression was found in the TM of the glaucomatous DBA/2J mouse model as early as 3 weeks (Bhattacharya et al., 2005a,b), much before appearance of signs of elevation in intraocular pressure (IOP) with the protein level reaching a plateau around 6–8 months of age. DBA/2J mice exhibit increased intraocular pressure at 6–8 months of age with progressive damage to the optic nerve and hearing loss. Presence of cochlin likely alters the ECM protein interactions. Increased presence of cochlin is concomitant with a decrease in collagen type II in glaucomatous TM tissue (Bhattacharya et al., 2005a,b). Many features of DFNA9 such as its late onset and progressive nature and neural damage and atrophy parallel the clinical characteristics of glaucoma. Glaucoma patients display enhanced SIPA as well. Protein and cell aggregation is a common theme in the pathogenesis of diverse diseases, including atherosclerosis, Parkinson and Alzheimer disease, as well as in glaucoma. vWFA domains of cochlin may have roles in response to different fluid flow regimes, local changes in ions or oxidative conditions in TM. Cochlin may undergo overexpression and multimeric aggregation in response to such changes.

4. Focus of future studies

Association of cochlin with glaucomatous TM tissue and early expression in DBA/2J mice occurs prior to elevation in IOP. Notably, the level of cochlin reaches a plateau around 6–8 months of age when elevation in IOP is observed, suggesting a role of cochlin in the observed increase in IOP. Future studies are designed to determine the association of cochlin with the elevation of IOP using different approaches. Studies are in progress to overexpress cochlin in the TM of control mice to determine whether cochlin expression leads to elevation in IOP and glaucomatous optic nerve damage. In addition, short-term viral vector-mediated cochlin expression experiments in cultured mammalian anterior segment have also been designed to

examine whether cochlin MPS deposits present in glaucomatous TM contribute to POAG pathology. Whether reduction of cochlin in DBA/2J mice will protect against IOP elevation is also under investigation using two approaches. One consists of breeding cochlin knockout mice (Rodriguez et al., 2004) with DBA/2J mice to assess whether this will abolish the phenotype of increased IOP. The second approach involves the use of shRNA for the coding region of cochlin. In human as well in DBA/2J mouse TM, concomitant decrease in collagen type II has been found with an increase in cochlin in the ECM. Whether this decrease in collagen is due to degradation or reduced, biosynthesis can be explored. It will also be interesting to assess whether other changes are present in other ECM proteins in glaucomatous TM. Future studies are designed to address such questions.

References

Bhattacharya SK, Rockwood EJ, Smith SD, Bonilha VL, Crabb JS, Kuchtey RW, Robertson NG, Peachey NS, Morton CC, Crabb JW. Proteomics reveals cochlin deposits associated with glaucomatous trabecular meshwork. J Biol Chem 2005;280:6080–6084. [PubMed: 15579465]

Bhattacharya SK, Annangudi SP, Salomon RG, Kuchtey RW, Peachey NS, Crabb JW. Cochlin deposits in the trabecular meshwork of the glaucomatous DBA/2J mouse. Exp Eye Res 2005;80:741–744. [PubMed: 15862180]

Robertson NG, Hamaker SA, Patriub V, Aster JC, Morton CC. Subcellular localisation, secretion, and post-translational processing of normal cochlin, and of mutants causing the sensorineural deafness and vestibular disorder, DFNA9. J Med Genet 2003;40:479–486. [PubMed: 12843317]

Rodriguez CI, Cheng JG, Liu L, Stewart CL. Cochlin, a secreted von willebrand factor type a domain-containing factor, is regulated by leukemia inhibitory factor in the uterus at the time of embryo implantation. Endocrinology 2004;145:1410–1418. [PubMed: 14657014]

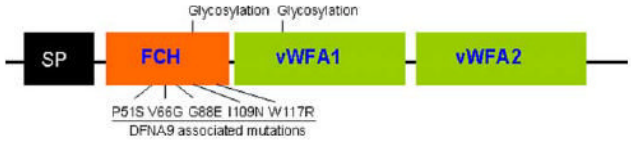

Fig. 1.

Schematic representation of cochlin protein. SP, signal peptide; FCH, factor C homology domain; vWFA1 and vWFA2, von Willebrand factor A homology domains 1 and 2; the reported mutations in FCH domain associated with DFNA9 are indicated. Glycosylation represents N-linked glycosylation sites.