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Functional links between gelatinase B/matrix metalloproteinase-9 and prominin-1/CD133 in diabetic retinal vasculopathy and neuropathy

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ABSTRACT

Retinopathy, a common cause of blindness, is a hallmark of diabetes and depends on two pathological mechanisms: vasculopathy and neuropathy. Whereas vasculopathy is well understood and has been associated with changes in gelatinase B/matrix metalloproteinase-9 (MMP-9) and other vasculotropic factors, specific markers for diabetes-induced retinal neuropathy are not yet described. Neuropathy may result from damages to the blood-retinal barrier (BRB) and from loss of neuroprotective factors. We studied diabetes-induced changes in vascular, inflammatory and regenerative markers and demonstrated that MMP-9 was increased, whereas prominin-1/CD133 was decreased in retinal extracts. *In vitro*, MMP-9 specifically destroyed prominin-1/CD133. Streptozotocin-induced diabetes resulted in BRB breakdown as a sign of vasculopathy and in prominin-1/CD133 destruction in photoreceptors as an *in situ* parameter of diabetic neuropathy. Both *in vivo* phenotypes were completely reversed in single MMP-9 gene knockout mice, demonstrating that MMP-9 mediates both diabetes-induced retinal vasculopathy and neuropathy, with prominin-1/CD133 being a critical and specific substrate of MMP-9. This functional link between gelatinase B/MMP-9 and prominin-1/CD133 explains mechanistically both the vasculopathy and neuropathy of diabetic retinopathy and suggests that specific MMP-9 inhibition is an interesting therapeutic avenue to investigate.

1. Introduction to diabetic retinal vasculopathy and neuropathy

Diabetes is worldwide the most common autoimmune disease and is reaching epidemic scales. It is characterized by a number of organ-specific pathologies, known as diabetic nephropathy, neuropathy and retinopathy. These and other hallmarks of diabetes have common pathogenic mechanisms in which vascular changes, leading to insufficiency, predominate. Diabetic retinopathy (DR) remains one of the leading causes of blindness worldwide and is characterized by progressive retinal vasculopathy with endothelial cell dysfunction, breakdown of the blood-retinal barrier (BRB), ischemia-induced retinal neovascularization, expansion of extracellular matrix (ECM) and outgrowth of fibrovascular membranes at the vitreoretinal interface. Formation of fibrovascular tissue results in severe complications such as vitreous hemorrhage and traction retinal detachment. Chronic low-grade inflammation is implicated in the pathogenesis of DR. Recently, this inflammation has been linked to activation of innate immune mechanisms by advanced glycation end products (AGE), resulting from chemical modifications of proteins by hyperglycemia. Low-grade inflammation is ignited through leukocyte receptors of AGE, named receptor for advanced glycation end products (RAGE). By the inflammatory reaction vascular leakage and edema formation may occur. Thickening of basement membranes and edema may result in tissue hypoperfusion and endothelial cell damage may be associated with leukocyte recruitment and adhesion to the retinal vasculature (Miyamoto et al., 1999; Joussen et al., 2004). Angiogenesis and vasculogenesis play a pronounced role in DR. Angiogenesis, the sprouting of new blood vessels from preexisting blood vessels,

Abbreviations: BRB, blood-retinal barrier; CBB, Coomassie brilliant blue; DR, diabetic retinopathy; ECM, extracellular matrix; EPC, endothelial progenitor cell; FITC, fluorescein isothiocyanate; ICAM-1, intercellular adhesion molecule-1; MMP, matrix metalloproteinase; PDR, proliferative diabetic retinopathy; SCF, stem cell factor; SDF-1, stromal cell-derived factor-1; SDS-PAGE, sodium docecyl sulfate polyacrylamide gel electrophoresis; VEGF, vascular endothelial growth factor; ZO, zonula occludens.

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requires the degradation of basement membranes and ECM, endothelial cell migration, endothelial cell proliferation, and capillary tube formation (Deryugina and Quigley, 2010; Carmeliet and Jain, 2011). Vasculogenesis, the *de novo* formation of blood vessels from circulating bone marrow (BM)–derived endothelial precursor cells, promotes neovascularization (Asahara et al., 1997). BM-derived endothelial progenitor cells (EPCs) and c-kit$^+$ cells contribute to new vessel formation in proliferative diabetic retinopathy (PDR) (Abu El-Asrar et al., 2010; Abu El-Asrar et al., 2011). DR has classically been regarded as a deficiency of the retinal microvasculature and a consequence of vascular cell damage, rather than resulting from changes in photoreceptor biology. However, recent studies proved that neurodegeneration, as evidenced by the presence of apoptotic cells in all retinal layers, and impaired visual function are initiated early after the onset of diabetes and progress independently of vascular lesions. These studies show indirect or correlative evidences of neurodegeneration, e.g. up-regulated expression of active caspase-3 in the diabetic retina (Abu-El-Asrar et al., 2004). However, direct molecular mechanisms underlying diabetes-induced retinal neurodegeneration and dysfunction are not documented.

1.1. Prominin-1/CD133

Prominin-1/CD133 was discovered in 1997 independently by two groups. Miraglia et al. (1997) identified CD133 as the target of AC133, a monoclonal antibody that binds a CD34$^+$ subset of human hematopoietic stem cells (HSC). Weigmann et al. (1997) identified prominin-1 as the target of their antibody raised against mouse neuroepithelial cells. Both groups described their antigen as a 115–120 kDa glycoprotein, incorporated into the plasma membrane with five-transmembrane domains (Miraglia et al., 1997; Weigmann et al., 1997). Since prominin shared no sequence homology or similar motifs with other proteins it made part of a completely new family of membrane proteins; the prominin family (Corbeil et al., 2001).

Prominin-2 is a membrane protein related to prominin-1 but originating from a different gene. Prominin-2 was discovered in humans and rodents (Zhang et al., 2002; Fargeas et al., 2003) and shares about 60% of its amino acids identity with prominin-1 (Fargeas et al., 2003). Analogous to prominin-1, prominin-2 is also found in plasma membrane protrusions (Fargeas et al., 2003). Prominin-1 and prominin-2 are also detected in tears (Jaszai et al., 2007b), as are MMP-2 and MMP-9 (Ollivier et al., 2007). The prominins are also present in small membrane particles (Marzesco et al., 2005).

Human prominin-1 has 60% amino acid identity to murine prominin (Corbeil et al., 1998; Miraglia et al., 1998). It is also produced as different splice variants or isoforms and, therefore, a specific nomenclature became necessary (Fargeas et al., 2007). The overall structure of human prominin-1 consists out of five transmembrane domains, connected by two short intracellular loops and two large extracellular loops of, respectively, 225 and 290 amino acids, quenched between an extracellular N-terminus and an intracellular C-terminus (Fig. 1). The protein contains a cysteine-rich region behind the first N-terminal extracellular tail and within the first intracellular loop (Miraglia et al., 1997; Weigmann et al., 1997; Corbeil et al., 2001) (Fig. 1). The short C-terminal cytoplasmic domain of prominin-1 holds five tyrosine residues, which are available for phosphorylation. *In vitro* and in cells, prominin-1 was found to be phosphorylated at tyrosine-828 and tyrosine-852 (Fig. 1) by the Src-family tyrosine kinases. The phosphorylated tyrosine-828 forms a potential interaction site for SH2-domain containing proteins (Boivin et al., 2009) (Fig. 1).

Prominins are associated with plasma membrane protrusions such as microvilli, microspikes, filopodia, lamellipodia and cilia (Marzesco et al., 2005; Dubreuil et al., 2007). Hence the name "prominin", derived from the latin word "*prominere*", to stand out or to be prominent (Weigmann et al., 1997; Corbeil et al., 2000, 2001). Indeed, prominins interact with the cholesterol present in the plasma membrane in the so-called cholesterol-based lipid rafts (Corbeil et al., 2001). These rafts are concentrations of many molecular entities at the cell surface, forming active signaling platforms, orchestrated by the cell cytoskeleton (Head et al., 2014). Remarkably, depletion of cholesterol from the cell surface, results in a completely different distribution of prominin molecules (Roper et al., 2000). Furthermore, prominin-1 interacts with protocadherin 21 (PCDH21), a cadherin specific for photoreceptor cells, and with actin filaments (Yang et al., 2008). Nevertheless, the exact physiological mechanisms of action of prominin remain unknown and may become better understood, once the three dimensional structure will be known.

Prominin-1 is expressed very early during retinal development (Zacchigna et al., 2009) and distortions in the prominin-1 protein structure have pronounced effects on vision (Gurudev et al., 2013). Prominin-1 is typically found in the photoreceptor layer of the retina of different species (Maw et al., 2000; Jaszai et al., 2007a, 2011; Han et al., 2012), more specifically, in the plasma membrane evaginations, present at the base of the outer segments of rod photoreceptors. Prominin-1 has been found associated with the membranes of newly generated disks and in the regions where new disks are being formed (Maw et al., 2000). Loss of prominin-1 causes retinal degeneration, possibly because of impaired generation of the evaginations and impaired conversion of the evaginations to disks (Maw et al., 2000). In mice, loss of prominin-1 results in progressive degeneration of photoreceptors and loss of vision due to defects in photoreceptor disk formation (Zacchigna et al., 2009). Mutations in the human prominin-1 gene were found to lie at the bases of several cases of family-related retinal degeneration. A frameshift mutation of prominin-1 (G614EfsX12, see Fig. 1) which results in premature termination of translation, caused human retinal degeneration in an Indian family. The predicted protein lacks half of the second extracellular loop, the final membrane spanning segment and the cytoplasmic C-terminal domain and does not reach the cell surface (Maw et al., 2000). A Pakistanian family, suffering from severe retinal degeneration accompanied by macular degeneration, was found to have a mutation at the *PROM1* gene locus on human chromosome 4p15. This mutation resulted in a premature stop codon at Gln576 (Q576X, see Fig. 1) (Zhang et al., 2007). A R373C (Fig. 1) missense mutation was associated with families with macular dystrophy and yellow fundus flecks (STGD4), retinal pigment epithelial atrophy (MCDR2) and autosomal dominant cone-rod dystrophy resulting in the degeneration of cone and rod photoreceptors (Yang et al., 2008). Transgenic mice, expressing the R373C mutant form of prominin-1 presented with subretinal deposits, photoreceptor atrophy and progressive photoreceptor degeneration. Microscopically, the prominin-1 mutants presented with overgrown and misoriented photoreceptor disk membranes (Yang et al., 2008). An Arab family presented with autosomal recessive cone-rod dystrophy (CORD) and high myopia, due to a frame-shift mutation in *PROM1*. A homozygous insertion in exon 12 was found, which induced a frame-shift starting from codon 452 and a stop codon 12 amino acids downstream of the translated proteins (Y452fsX12, see Fig. 1) (Pras et al., 2009). Finally, a Spanish family with autosomal recessive retinitis pigmentosa (arRP) and premature macular atrophy and myopia, was found to have a new homozygous *PROM1* mutation. This mutation also resulted in a frameshift, introducing a premature stop codon and yielding a shorter protein (S289EfsX1, see Fig. 1). This shorter prominin-1 form was found to be degraded by the nonsense-mediated decay pathway, resulting in absence of prominin-1 (Permanyer et al., 2010).