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Mini Review

Collectrin, a homologue of ACE2, its transcriptional control and functional perspectives

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Abstract

Collectrin is a type I membrane protein and shares significant homology with C-terminal domain of angiotensin-converting enzyme-2 (ACE2). However, collectrin lacks catalytic domain and it suggests the presence of uncharacterized physiological functions of collectrin. Collectrin is transcriptionally regulated by hepatocyte nuclear factor-α and -β and is highly expressed on renal proximal tubules and collecting ducts as well as pancreatic β-cells. Recent in vitro and in vivo studies demonstrated interesting physiological roles of collectrin related to insulin secretion, formation of primary cilia, renal cyst formation and amino acid transport. The common underlying molecular mechanism may be suggested by the evidence that collectrin binds to SNARE complex by interacting with snapin. Collectrin is involved in the process of vesicle transport and membrane fusion and thus it delivers insulin for exocytosis or various membrane proteins to apical plasmalemma and primary cilia. Collectrin may be the new therapeutic target for various pathological processes such as diabetes, polycystic kidney disease, hypertension and aminoaciduria.

Keywords: Collectrin; Amino acid transport; Insulin secretion; Primary cilia

Collectrin was originally isolated by its up-regulation in 5/6 ablated kidneys, and identified as a homologue of angiotensin-converting enzyme-2 (ACE2). ACE2 has one catalytic domain and functions as carboxypeptidase [1,2] and it cleaves the C-terminal amino acid of angiotensin I to angiotensin 1–9 [3] or directly converts angiotensin II to angiotensin 1–7 [3,4]. Collectrin shares 47.8% identity with C-terminal end of ACE2, however, unlike ACE2, collectrin lacks active dipeptidyl carboxypeptidase catalytic domains [5].

We initially reported that collectrin localizes in the cytoplasm and apical membrane of renal collecting duct cells, and later its localization in pancreatic β-cells was also found. The studies with in vitro *cell* culture and transgenic mice revealed that collectrin plays an important role in insulin secretion and pancreatic β-cell proliferation [6,7]. In renal collecting duct cells, collectrin is demonstrated to be expressed in primary cilia and it plays critical roles in the maintenance of primary cilia and cell polarity [8]. Based on these evidences, one can speculate gene disruption of collectrin in mice may cause the phenotypes of diabetes or abnormality in water and salt handling, such as hypertension. Surprisingly, the generation of collectrin knockout mice by two independent groups resulted in a severe and general aminoaciduria [9,10]. In this mini review, we would like to discuss about the functional role of collectrin in diabetes and renal physiology.

Structure of collectrin

The deduced amino acid sequences of mouse (GenBank Accession No. AF178085), rat (AF178086) and human collectrin (AF229179) reveal striking homology and it is conserved during the evolution [5]. Collectrin protein has two hydrophobic domains: the N-terminal hydrophobic

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domain (amino acids 1–14) is a signal sequence; the second hydrophobic domain is a transmembrane domain stretching between residues 142 and 164; and a predicted cleavage site locates between amino acid residues 14 and 15. Thus, collectrin seems to be a type I membrane protein (Fig. 1). The extracellular domain included 127 amino acid residues, and it contains two N-linked and one O-linked glycosylation sites, which was confirmed by the reduction in molecular weight after deglycosylation treatment with *N*- and *O*-glycosidase [8].

The angiotensin-converting enzyme-2 (ACE2) (AF291820) mRNA encodes 805 amino acids and human collectrin shares 47.8% identity with the C-terminal end of ACE2 (amino acids 614–805) [11]. The ACE2 protein contains a single catalytic domain (amino acids 147–555) and shares 41.8% identity with the human angiotensin-converting enzyme (ACE). Collectrin lacks carboxypeptidase domains and does not share the segments or domains of ACE. The multiple sequence alignment of the three proteins, ACE, ACE2 and collectrin is shown in Fig. 2. Interestingly, human collectrin cDNA localizes to chromosome Xp22, 26 kb from the ACE2 gene and they may evolve from a common ancestral gene.

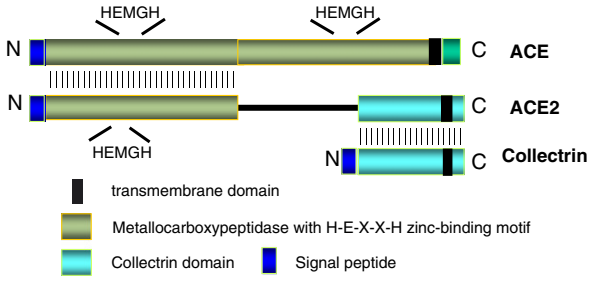

Fig. 2. A schematic drawing of the homologous domains of ACE, ACE2 and collectrin. Human collectrin shared 47.8% identity with the C-terminal end of ACE2 (amino acids 614–805). The ACE2 protein contains a single catalytic domain (amino acids 147–555) and shares 41.8% identity with both catalytic domains of the human ACE. Collectrin lacks carboxypeptidase active site domains and thus it does not share the segments or domains of ACE.

Tissue distribution and transcriptional regulation of collectrin

Collectrin was originally reported to express in the cytoplasm and apical membrane of collecting duct cells [5]. Later, extrarenal expression has been reported in several tissues and cell lines, including pancreatic β-cells, heart, liver, corpus callosum, hippocampus and MIN6 cells, a mouse insulinoma cell line [6,7]. Most recently, collectrin was also found to localize at apical brush border of proximal tubules in mouse kidney [9,10]. Collectrin was identified as hepatocyte nuclear factor-1α (HNF-1α) targeted gene and targeted disruption of HNF-1α resulted in diabetes and a renal phenotype with Fanconi syndrome manifested with glucosuria, phosphaturia, calciuria and aminoaciduria. Localization of collectrin and HNF-1α in proximal tubules and similar phenotype in collectrin and HNF-1α knockout mice suggested the expression of collectrin in proximal tubules is transcriptionally regulated by

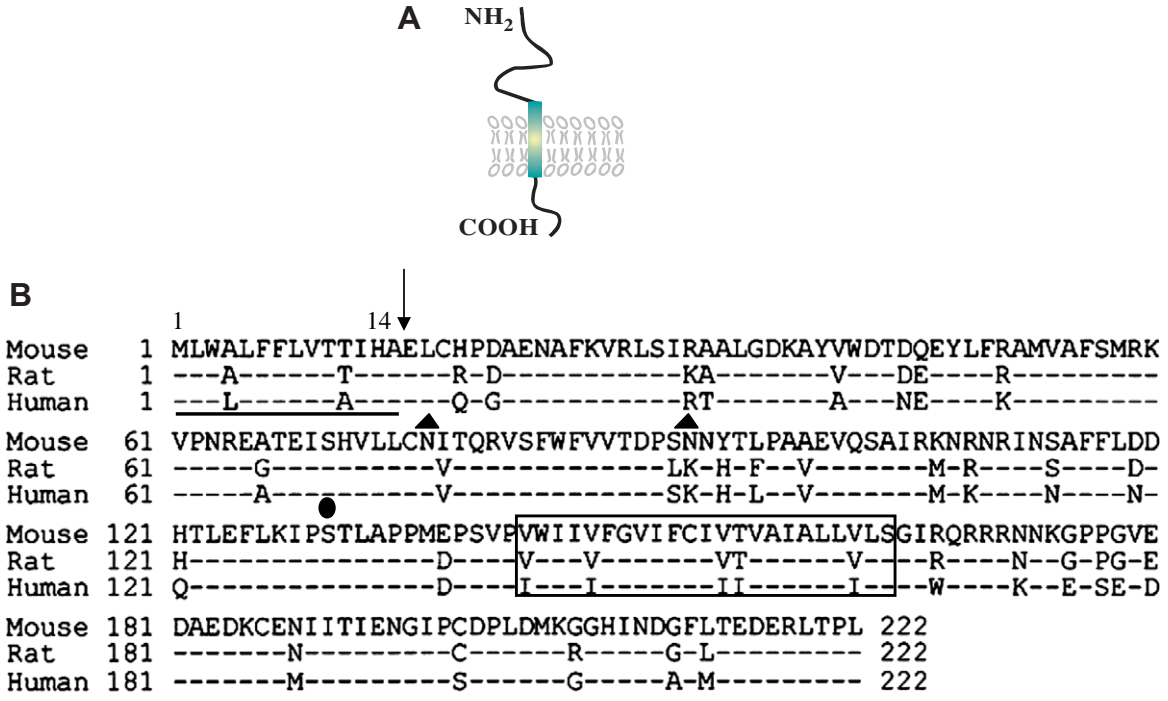

Fig. 1. The structure and amino acid sequence of collectrin protein. (A) The topology of collectrin protein. (B) Multiple sequence alignment of mouse, rat and human collectrin. The putative signal sequence is observed at the N-terminal (underlined) with the cleavage site (arrow). The putative transmembrane domain is boxed. The potential N-linked and O-linked glycosylation sites are indicated by filled triangles and filled circles, respectively.

HNF-1α. In contrast, mutations of HNF-1β gene in human MODY5 patients reveal type 2 diabetes and renal phenotype of cyst formation. HNF-1β and collectrin are co-localized in renal collecting duct cells and collectrin is also regulated by HNF-1β, thus gene disruption of collectrin may resulted in the diabetes, hypertension and renal cyst formation; however such phenotype was not observed in collectrin knockout mice and it may be due to the compensation of other genes such as ACE2.

Collectrin and cilia formation

Primary cilia are rod-like organelles and appear on most of cells throughout the body [12]. Their basic structure consists of nine doublets of microtubules and a ciliary membrane that is continuous with plasmalemma. One to two primary cilia are present on the surface of most vertebrate cells and also on the apical surface of tubular epithelia of mammalian kidney [13]. Primary cilia are frequently used for sensation, e.g. odorant receptors congregate on the cilia of olfactory epithelial cells and light-sensitive photoreceptors on the retina's rod and cone cells [14]. Although ciliary functions in renal tubules are incompletely understood, this organelle most likely functions as a flow-sensitive mechanosensor to luminal fluid flow, modulates flow-sensitive ion transport and possibly regulates cell proliferation and differentiation [15].

In mouse inner medullar collecting duct (mIMCD3) cells, the inhibiting of collectrin expression by siRNA resulted in absent or stunted primary cilia and they were associated with various abnormalities, such as shorter in length, disorganization of 9+0 microtubular structure, bulging basal bodies and stunted shaft projection [8]. Collectrin binds to γ-actin–myosin II-A, soluble *N*-ethylmaleimide-sensitive-factor attachment protein receptor (SNARE), and polycystin-2–polaris complexes, and all of these are involved in intracellular and ciliary movement of vesicles and membrane proteins. Polaris is the gene product of *tg737* and mutation in *tg737* gene causes recessive form of polycystic kidney disease (PKD) in orpk mice, and turned out to be part of intraflagellar transport particles (IFT), that carries cargo up and down the cilia, and the inhibition of IFT system shortens the primary cilia [16,17]. Thus collectrin may mediate specific vesicle transport, upon docking deliver the integral membrane proteins to ciliary plasmalemma, and maintain the primary cilia and cell polarity in collecting duct cells.

Collectrin and insulin secretion

The expression of collectrin in pancreatic β-cells was reported and collectrin is identified as a target of hepatocyte nuclear factor-α (HNF-1α) [6,7]. Genetic mutations in HNF-1α and HNF-1β, two structurally and functionally related homeodomain containing transcriptional factors, cause maturity-onset diabetes of the young (MODY), MODY3 and MODY5 [18,19], which is a monogenic form of type 2 diabetes characterized by impaired insulin secretion from pancreatic β-cells [20]. HNF-1 plays an important role in the multi-factorial steps for the proper insulin secretion including glucose transport, glycolysis and mitochondrial oxidation [21–26]. Insulin is secreted by exocytosis from secretory granules in pancreatic β-cells [27,28]. Overexpression of collectrin in INS-1 cells resulted in significant increase of insulin secretion, suggesting that collectrin can facilitate insulin secretion procedure [6]. The mechanism of how collectrin involved in insulin secretion was derived from the discovery of the binding of collectrin to SNARE complex *via* interaction with snapin, a SNAP-25 binding protein, which plays important roles in the exocytosis of insulin [6]. Furthermore, transgenic mice with increased expression of collectrin in pancreatic β-cells exhibit increased β-cell mass, suggesting a physiological role of collectrin in pancreatic β-cell proliferation [7]. Additional studies are needed to investigate the mechanism of collectrin in β-cell proliferation. It is intriguing to speculate that collectrin stimulates proliferation through cilium, an organelle closely related to cell proliferation and differentiation [15].

Loss of collectrin impairs renal amino acid transport

Two independent studies of targeted disruption of *collectrin* in mice resulted in a similar result: a severe aminoaciduria [9,10]. The aminoaciduria is generalized by loss of nearly every amino acid, especially tyrosine [9,10], phenylalanine [10] and glutamine [9]. Le et al.'s described reduced expression in amino acid transporters, such as neutral amino acid transporter *Slc6a19* (B^0AT1), *Slc7a9* ($B^{0,+}AT$) and *Slc3a1* (rBAT); and an altered cellular distribution of apical transporter responsible for glutamate/aspartate reabsorption EAAC1 was observed. Collectrin-deficient mice generalized by Danilczyk et al. showed markedly downregulated plasma membrane populations of amino acid transporter subtypes including *Slc6a19* (B^0AT1), XT3s1/SIT1, XT2 and XT3, and the EAAC1.

Amino acid transporters are located in the membrane of the proximal tubules, a microenvironment involved in amino acid reabsorption [29,30]. Collectrin expresses at the luminal side of brush border membranes in proximal tubules, directly binds to B^0AT1, XT2 and XT3, and co-localizes with B^0AT1 in the early proximal S1 tubule [9]. Co-expression of B^0AT1 with collectrin in *Xenopus laevis* oocytes and polarized MDCK cells resulted in an enhanced surface expression of B^0AT1 and a remarkable increase in L-isoleucine uptake. It was reported that *N*-ethylmaleimide-sensitive fusion attachment receptor (SNARE) complex proteins are involved in EAAC1 trafficking [31]. SNARE complexes are abundantly found in mammalian cells and are extensively involved in vesicular transport [32]. Collectrin can directly interact with snapin, a SNAP-25 binding protein, suggesting the possible involvement of collectrin in increasing the

functional amino acid transporters by trafficking of them to the proximal tubular apical membrane brush borders [6,9].

Concluding remarks and future perspectives

Since the identification of collectrin in 1999, certain knowledge has accumulated regarding the structure, expression and physiological functions of collectrin. In vitro study revealed collectrin is involved in the insulin secretion and primary cilia formation. In vivo collectrin gene disruption studies in mice highlighted the role of collectrin in the amino acid transport in the kidney. However, many questions still remain to be elucidated. What is the common molecular mechanism for the insulin secretion, primary cilia formation and amino acid transports? One insight into the molecular mechanism is that collectrin binds to SNARE complex protein such as snapin and the collectrin-mediated processes of vesicle transport and fusion to the plasma membrane in a polarized manner is crucial for delivering insulin and amino acid transporters. Impairment of collectrin function would link to various disease processes such as diabetes, hypertension, renal cyst formation and aminoaciduria. The elucidation of collectrin may be new target molecule for treatment of these pathological conditions.

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