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Identification and characterization of a novel tight junction-associated family of proteins that interacts with a WW domain of MAGI-1

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Abstract

The membrane-associated guanylate kinase protein, MAGI-1, has been shown to be a component of epithelial tight junctions in both Madin–Darby canine kidney cells and in intestinal epithelium. Because we have previously observed MAGI-1 expression in glomerular visceral epithelial cells (podocytes) of the kidney, we screened a glomerular cDNA library to identify the potential binding partners of MAGI-1 and isolated a partial cDNA encoding a novel protein. The partial cDNA exhibited a high degree of identity to an uncharacterized human cDNA clone, KIAA0989, which encodes a protein of 780 amino acids and contains a predicted coiled-coil domain in the middle of the protein. In vitro binding assays using the partial cDNA as a GST fusion protein confirm the binding to full-length MAGI-1 expressed in HEK293 cells, as well as endogenous MAGI-1, and also identified the first WW domain of MAGI-1 as the domain responsible for binding to this novel protein. Although a conventional PP*x*Y binding motif for WW domains was not present in the partial cDNA clone, a variant WW binding motif was identified, LP*x*Y, and found to be necessary for interacting with MAGI-1. When expressed in Madin–Darby canine kidney cells, the full-length novel protein was found to colocalize with MAGI-1 at the tight junction of these cells and the coiled-coil domain was found to be necessary for this localization. Because of its interaction with MAGI-1 and its localization to cell–cell junctions, this novel protein has been given the name MAGI-1-associated coiled-coil tight junction protein (MASCOT).

Keywords: MAGI-1; Tight junction; WW domain; Coiled-coil; MAGI-1-associated coiled-coil tight junction protein; AMOTL2

1. Introduction

The epithelia and endothelia of organisms play a fundamental role in establishing a physical barrier between two extracellular compartments of different composition within tissues. The free diffusion of solutes between the compartments is regulated by tight junctions that act as molecular seals. In addition to this "barrier" function, tight junctions are also responsible for maintaining the unique apical and basolateral membrane distribution of proteins and lipids that characterizes polarized cells. A specialized epithelial cell that is gaining interest because of its importance in normal kidney function is the glomerular visceral epithelial cell, or podocyte. Its highly complex and unique morphology manifests itself in the interdigitating nature of its minor, or foot, processes as they wrap around capillaries within the glomerulus. A specialized cell–cell junction called the slit diaphragm serves to laterally join the foot processes and potentially adds to the isoporous filtering nature of the kidney. In addition, the slit diaphragm serves to delineate distinct apical and basal membrane domains in the foot processes, thereby imparting a degree of polarization in their plasma membranes [1]. The nature of the slit diaphragm membrane domain is believed to be that of a modified epithelial adherens junction although it does

Abbreviations: JAM, junctional adhesion molecule; MAGI-1, membrane-associated guanylate kinase inverted-1; MAGUK, membrane-associated guanylate kinase; SH3, Src homology 3; PTB, phosphotyrosine binding; PDZ, PSD-95/Dlg/ZO-1; YAP, Yes-associated protein; GuK, guanylate kinase; DRPLA, dentatorubral and pallidoluysian atrophy; AIP, atrophin-1 interacting protein; GST, glutathione-*S*-transferase; HEK293, human embryonic kidney 293; MDCK, Madin–Darby canine kidney; JEAP, junctional-enriched and -associated protein; LCCP, Leman coiled-coil protein; AMOTL1, angiomotin-like 1; AMOTL2, angiomotin-like 2

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contain molecular components found in tight junctions as well [2]. Therefore, the exact nature of the slit diaphragm is best viewed at this point as a specialized cell–cell junction structure.

At the molecular level, epithelial tight junctions consist of a growing number of transmembrane and submembranous proteins whose functions are only now being realized in the context of this plasma membrane microdomain (for a detailed review see [3,4]). Transmembrane proteins of tight junctions consist mainly of the tetraspan proteins claudin and occludin and a few members of the JAM family of immunoglobulin (Ig) domain-containing adhesion molecules. Tight junction-associated submembranous proteins are more numerous and diverse than the transmembrane proteins and can be divided into two discreet groups based on the presence or absence of a protein–protein interaction domain referred to as the PSD-95/dlg/ZO-1 (PDZ) domain.

Many of the PDZ containing proteins of the tight junction belong to a large family of molecules referred to as the membrane-associated guanylate kinases (MAGUKs) that include ZO-1 [5], ZO-2 [6] and ZO-3 [7], as well as Pals1 [8], MAGI-1 [9] and MAGI-3 [10]. The MAGI proteins consist of three members that together make up a MAGUK subfamily. We and others have found that MAGI-1 is a component of tight junctions in MDCK cells [9,11] and it is found localized in a podocyte-like fashion in kidney glomeruli [12]. MAGUK proteins share a common structural organization and are proposed to function as molecular scaffolds within cells [3,13]. The unique organization of protein–protein interaction domains seen in conventional MAGUK proteins is inverted in the MAGI family of proteins. In addition, two WW domains in the MAGI proteins replace the SH3 domain observed in the conventional MAGUKs. Many of these MAGUK proteins are found at special subcellular regions such as post-synaptic densities within neurons as well as the tight and adherens junctions of epithelial cells and are believed to play a role in the structure and function of these specialized complexes.

Although the binding of a number of proteins to the PDZ domains and GuK domain of MAGI-1, MAGI-2 and MAGI-3 has been reported, there is little data on proteins that interact with the WW domains of MAGI proteins. In a screen to identify interacting partners for the DRPLA gene product atrophin-1, partial cDNAs containing the WW domains of MAGI-1 (AIP-3) and MAGI-2 (AIP-1) were isolated [14], but no further characterization on these interactions has yet been reported. This lack of identified binding proteins for the MAGI WW domains and our finding that MAGI-1 is expressed in a glomerular podocyte-like fashion prompted us to screen a mouse glomerular cDNA library using the two WW domains of MAGI-1 as a probe. We have previously reported on the initial results of this screen that identified a region of the actin-bundling protein synaptopodin (containing its two PP*x*Y motifs) as an interacting partner for MAGI-1 [11]. A more thorough characterization of this interaction showed that synaptopodin preferentially recognized the second WW domain of MAGI-1 and demonstrated the first complete characterization of a MAGI family member binding to another protein via a WW domain interaction.

From the same screen that identified synaptopodin as a MAGI-1 binding partner, an additional binding partner for the WW domains of MAGI-1 has been identified and is reported here. The isolated protein belongs to a novel family of poorly characterized molecules that share the distinction of having a predicted coiled-coil domain in the middle of the protein with no other known protein domains. In addition to the protein identified here, one other member of this protein family is capable of binding specifically to the first WW domain of MAGI-1. Finally, the two members of this protein family can self-associate through their coiled-coil domain and are observed to localize to the tight junction of epithelial cells.

2. Experimental procedures

2.1. Expression cloning

Details on the screening of the glomerular cDNA library with the GST-MAGI-1 WW12 probe (amino acids 219–416 of mouse MAGI-1) has been described elsewhere [11]. Four of the 11 total independent clones isolated in the screen comprise the same region of mouse MASCOT/AMOTL2 and are represented in this study by clone 18.1.

2.2. Plasmid constructs

Clone 18.1 was cloned into pGEX-4T and myc-RK5 [15] to make GST-clone 18.1 and myc-clone 18.1, respectively. The proline within the LP*x*Y motif of myc-clone 18.1 was mutated to an alanine (myc-clone 18.1 P105A) with the QuickChange™ kit (Stratagene, La Jolla, CA) using the manufacturer's instructions.

Full-length human and mouse MASCOT/AMOTL2 as well as human JEAP/AMOTL1 expressed sequence tag (EST) cDNAs were obtained from commercial sources (ATCC, Manassas, VA; ResGen™, Invitrogen Corp., Carlsbad, CA) (human MASCOT/AMOTL2, clone CS0DI067YC01; mouse MASCOT/AMOTL2, IMAGE ID# 4191248; human JEAP/AMOTL1, IMAGE ID# 3533422). All cDNAs were sequenced in their entirety. The entire coding sequences for human MASCOT/AMOTL2 and human JEAP/AMOTL1 were isolated by PCR and cloned into Flag-pcDNA3.1(−) and 3× HA-pcDNA3.1(−) to make Flag-MASCOT/AMOTL2, Flag-JEAP/AMOTL1, HA-MASCOT/AMOTL2 and HA-JEAP/AMOTL1. The following deletion constructs of human MASCOT/AMOTL2 were generated by PCR using primers flanking the region to be isolated: MASCOT/AMOTL2 1–307, MASCOT/AMOTL2 1–578, MASCOT/AMOTL2 308–780, and MASCOT/AMOTL2 579–780. The deletion