Membrane-associated guanylate kinase with an inverted arrangement of protein-protein interaction domains (MAGI2) also called synaptic scaffolding molecule (S-SCAM), atrophin-1-interacting protein 1, activin receptor-interacting protein 1 (also called synaptic scaffolding protein 1) is a scaffold protein that binds a wide variety of receptors, cell adhesion molecules and signalling molecules. It also interacts with other scaffold proteins and adaptors, and forms a protein network that supports cell junctions. As it is highly expressed in brain, the study on its roles in synaptic organization initially preceded. However, mounting evidence indicates that MAGI2/S-SCAM functions as a tumour suppressor and plays essential roles to maintain the integrity of cell structures in non-neuronal tissues. We review the articles regarding MAGI2/S-SCAM outside brain and discuss future perspectives for the research of MAGI family proteins.

Keywords: cancer/intestine/kidney/scaffold protein/synapse.
The Molecular Mechanism Underlying the Tumour Suppressive Role of MAGI2/S-SCAM

In most reports, the tumour suppressive role of MAGI2/S-SCAM is explained by the stabilization of PTEN. However, MAGI2/S-SCAM is involved in versatile signal pathways. MAGI2/S-SCAM interacts with regulators of small guanosine triphosphate (GTP)-binding proteins, signalling molecules of the Wnt pathway, transforming growth factor (TGF)β pathway and the Notch pathway, and regulates the endocytosis of membrane proteins (Table I). Therefore, the tumour suppressive role of MAGI2/S-SCAM may be mediated not only by PTEN.

MAGI3 is reported to compete with Na+/H+ exchanger regulatory factor 2 (NHERF-2) for the binding with Lysophosphatidic acid receptor 2 (LPA2) in colon cancers (18). When LPA2 binds to NHERF-2, phospholipase C is activated to generate via Gαq diacylglycerol and inositol 1,4,5-triphosphate, eventually resulting in the activation of nuclear factor κB and c-Jun N-terminal kinase. In contrast, when LPA2 interacts with MAGI3, RhoA is activated via Gα12. Thus MAGI3 suppresses LPA-induced migration and invasion of colon cancer cells. The interaction between MAGI2/S-SCAM and LPA2 has not yet been reported, but it is possible that MAGI2/S-SCAM plays a similar role.

The findings regarding to MAGI1 also provide an insight into a potential mechanism underlying the tumour suppressive role of MAGI2/S-SCAM. In colon cancer cells, MAGI1 enhances the phosphorylation of focal adhesion kinase, extracellular signal-regulated kinase 1/2 and Akt to promote integrin cell-mediated cell adhesion, and inhibits migration, invasion and adhesion-independent growth (19). MAGI1 also inhibits β-catenin-dependent Wnt signalling. As MAGI2/S-SCAM interacts with β-catenin and axin, and forms a complex with adenomatous polyposis coli, MAGI2/S-SCAM might exert its tumour suppressive role through the regulation of the Wnt signalling (20–23).

The genetic screen using siRNA oligonucleotides for 710 kinase genes revealed that MAGI1 depletion upregulates a transcriptional co-activator, yes-associated protein 1 (YAP1), which is negatively regulated by the tumour suppressive Hippo pathway (24). That is, MAGI1 suppresses YAP1 activity. It is possible that MAGI2/S-SCAM also inhibits YAP1.

MAGI Proteins, RhoA and the Hippo Pathway

However, the negative regulation of YAP1 by MAGI1 is twisting. MAGI1 like MAGI3 activates RhoA through Gα12 and PDZ-Rho guanine nucleotide exchange factor downstream of BAI1 (25). Thereby, MAGI1 and MAGI3 are thought to be a scaffold that activates RhoA signalling. Knockout mice of MAGI2/S-SCAM exhibit the impairment of RhoA activation under the stimulation of NMDA receptors in neurons, suggesting that MAGI2/S-SCAM also...
Fig. 2 Protein interactions mediated by MAGI2/S-SCAM. MAGI2/S-SCAM assembles cell adhesion molecules, receptors and signalling molecules and link them to the cytoskeleton. (A) MAGI2/S-SCAM interacts with other scaffold proteins and adaptors in synapses. Both of PSD-95 and S-SCAM interact with neuroligin, NMDA receptor and SAPAP/GKAP. (B) PSD-95 and S-SCAM directly and indirectly interact with each other. TARP is the auxiliary subunit that regulates the surface expression of the a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR). β-Catenin is an adaptor that binds to cadherin. This figure illustrates the role of MAGI2/S-SCAM at the excitatory synapses. MAGI2/S-SCAM plays a similar role in the inhibitory synapses and at the slit diaphragm. Thus MAGI2/S-SCAM forms a large protein complex that supports the architecture of cell junctions. (C) The interactome conserved in synaptic dendritic spines and foot processes of kidney podocytes. Nephrin is expressed not only in kidney but also in brain. Dendrin, Kibra and synaptopodin are abundantly expressed in kidney and brain. Dendrin binds to the WW domains of MAGI2/S-SCAM and Kibra. Dendrin binds to CIN85. Synaptopodin regulates RhoA signalling and the actin cytoskeleton. Kibra is a component of the tumour suppressive Hippo pathway. CIN85 regulates the endocytosis of receptors. The functional significance of these protein interactions remains to be clarified.
activates RhoA (26). However, as RhoA activates YAP1 (27), the property of MAGI proteins as the RhoA signalling scaffold is not consistent with that of the negative regulator of YAP1. To be more complicated, MAGI2/S-SCAM interacts with Gzs-coupled receptors such as β1-adrenergic receptor (β1AR) and vasoactive intestinal polypeptide Type 1 receptor (VPAC1) (28, 29). Cyclic AMP is known to inhibit YAP1. MAGI2/S-SCAM does not alter cyclic AMP production in response to β1AR-agonist, but inhibits VPAC1-induced cyclic AMP production. Here again, MAGI2/S-SCAM seems to down-regulate cyclic AMP and eventually activate YAP1. These findings are incongruous and our knowledge is still too fragmentary. If MAGI2/S-SCAM negatively regulates YAP1, the underlying mechanism might be independent of RhoA. In this point of view, the interaction of MAGI2/S-SCAM with Kibra is intriguing. Kibra, which is a well-known component of the Hippo pathway, interacts with MAGI2/S-SCAM via dendrin (30–32). Further studies will be necessary to understand how the suppression of MAGI2/S-SCAM leads to oncogenesis.

MAGI2/S-SCAM in Intestine

MAGI2/S-SCAM binds to VPAC1 in intestine (29). VPAC1 is a Gz-c, protein coupled receptor that regulates fluid and electrolyte secretion in intestine. MAGI2/S-SCAM inhibits VPAC1 and blocks its agonist-induced internalization. This report illustrates a physiological role of MAGI2/S-SCAM in intestine. Coeliac disease (gluten-sensitive enteropathy) and inflammatory bowel disease show enhanced intestinal epithelial permeability. Based on this finding, the researchers tested using Dutch and British cohorts whether genes encoding tight junction proteins are relevant to these diseases (33). They found weak association of MAGI2 with coeliac disease and ulcerative colitis. The independent study also revealed the association of MAGI2 variations with Crohn’s disease and ulcerative colitis (34). It is proposed that the dysfunction of MAGI2/S-SCAM at tight junctions impairs the barrier against bacteria in the gut lumen and leads to inflammatory bowel diseases.

MAGI2/S-SCAM in Kidney

A cell adhesion molecule, nephrin, is an essential component of the slit diaphragm. Glutathione S-transferase (GST) pull-down assay was performed using kidney glomerular lysates to search for nephrin-interacting proteins, and MAGI2/S-SCAM was identified by mass spectrometry (35). Venus knock-in mice corroborated the expression of MAGI2/S-SCAM in kidney (36). MAGI2/S-SCAM binds Vangl2, a core protein of the planar cell polarity pathway in kidney (37), MAGI2/S-SCAM interacts with dendrin and forms a complex with CIN85 (31). Knockout mice have been generated by three groups including ours. There are three alternative splicing variants of MAGI2/S-SCAM (α, β and γ) (38). We knocked out only the longest variant, MAGI2/S-SCAMα (26). Although the remaining two variants, MAGI2/S-SCAMβ and γ, were expressed, the mice died within 24 hr after birth. We focused on brain in the analysis of mutant mice and found the dysregulation of RhoA signalling in dendritic spines. Balbas et al. (39) targeted exon 4 and knocked out all variants. In MAGI2-null mice, nephrin expression was decreased, while CIN85 was up-regulated. The mice exhibited proteinuria and podocytes loss. Ihara et al. (40) replaced exon 6 with Venus fluorescent protein and knocked out all the three variants. Their mice died within 3 weeks and showed anuria and abnormal podocytes. Nephrin and dendrin protein levels were reduced. These reports clearly indicate the essential role of MAGI2/S-SCAM in podocytes. MAGI1 is reported to be involved in focal segmental glomerulosclerosis (FSGS) (41). A cell adhesion molecule, sidekick-1, is up-regulated in podocytes in FSGS, and causes the dysfunction of podocytes. However, without MAGI1, sidekick-1 does not exhibit deleterious effects, suggesting that MAGI1 is implicated in the pathogenesis in FSGS. By analogy, it is speculated that MAGI2/S-SCAM is involved in glomerular diseases. Expression of dominant-active or dominant-negative RhoA in podocytes

Table I. Molecules that interact with MAGI2/S-SCAM.

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<thead>
<tr>
<th>Receptors</th>
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<tr>
<td>Nr3c1-GABA receptor (2)</td>
<td>G2 Glutamate receptor (32, 33)</td>
</tr>
<tr>
<td>β1AR (28)</td>
<td>VPAC1 (29)</td>
</tr>
<tr>
<td>Brain-specific angiogenesis inhibitor-1 (4)</td>
<td>Activin Type II receptors (6)</td>
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<tr>
<th>Cell adhesion molecules</th>
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<tr>
<td>Neutropilisin (2)</td>
<td>β-Dystroglycan (54)</td>
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<tr>
<td>Dendrite arborization and synapse maturation 1 (Dasml1/IgSF9b) (55, 36)</td>
<td>Nectin (37)</td>
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<tr>
<td>Neprhin (35)</td>
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<th>Other transmembrane proteins</th>
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<tr>
<td>Dacll (58, 59)</td>
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<tr>
<th>Scaffold proteins, adaptor proteins and cytoskeleton-associated proteins</th>
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<tr>
<td>SAPAP/GKAP (2)</td>
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<tr>
<td>MAGUK-interacting protein (MAGUI)/connector enhancer of ksr2 (Cnk2) (60)</td>
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<tr>
<td>Postsynaptic density-95 (PSD-95)/synapse-associated protein 90 (SAP90) (38)</td>
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<tr>
<td>Tamalin/general receptor for phosphoinositides 1-associated scaffold protein (GRASP) (61)</td>
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<tr>
<td>Stargazin/transmembrane AMPAR regulatory proteins (TARPs) (62, 63)</td>
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<tr>
<td>SANS (45)</td>
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<tr>
<td>Dendrin (31)</td>
<td></td>
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<tr>
<td>Atoxin-1 (5)</td>
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<tr>
<td>β-Catenin (20, 21)</td>
<td>6-Catenin (64)</td>
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Signalling molecules

| PTEN (10)                                                                |                     |
| RAPGEF2/PDZ-GEF/nRapGEP (65)                                             |                     |
| SynArGF1 (66)                                                            |                     |
| SMAD2/3 (6)                                                              |                     |
| Axin (22)                                                                |                     |

JAM4, Carom and RASSF6 were identified as MAGI1-interacting proteins and could also interact with MAGI2/S-SCAM (67–69).
disrupts the integrity of the slit diaphragm and causes proteinuria (42). As MAGI proteins regulate RhoA signalling, it is not surprising if MAGI proteins are implicated in nephrotic syndrome. Furthermore, MAGI1 is cleaved by caspase and the products of cleavage mediate apoptosis in Madin-Darby canine kidney cells (43). As MAGI2/S-SCAM is also cleaved by caspase, MAGI2/S-SCAM might induce apoptosis in a similar manner (44). MAGI2/S-SCAM binds to a scaffold protein, scaffold protein containing ankyrin repeats and SAM domain (SANS), which is encoded by USH1G, one of human Usher syndrome (USH)-causing genes (45). USH syndrome belongs to the ciliopathies and is characterized by inner ear defect and retinitis pigmentosa. As MAGI2 knockdown impairs ciliogenesis in mouse inner medullary collecting duct IMCD3 cells, MAGI2/S-SCAM might be implicated in other ciliopathies with kidney disorders.

Conclusions and Perspectives

The importance of MAGI2/S-SCAM outside brain is now obvious. We are wondering why MAGI2/S-SCAM is so important in the tissues with high expressions of MAGI1 and MAGI3. Three MAGI proteins are highly homologous to each other. Researchers do not always use the antibody specific for each MAGI protein in their experiments. Therefore, we must be careful about which MAGI protein was detected and evaluated in each experiment. Even so, the expression of MAGI2/S-SCAM in prostate, liver and lung, is confirmed at mRNA level and it is indisputable that MAGI2/S-SCAM functions as a tumour suppressor, and its depletion leads to oncogenesis in these tissues. Why does not MAGI1 compensate for the down-regulated MAGI2/S-SCAM? MAGI1 is highly expressed in kidney, but MAGI2/S-SCAM deletion gives severe damage to the slit diaphragm. Do not podocytes express MAGI1? Although most molecular interactions are shared by MAGI1 and MAGI2/S-SCAM, do they still have distinct roles? The finding that MAGI2/S-SCAM knockdown impairs ciliogenesis in IMCD3 cells also suggests that the specific role of MAGI2/S-SCAM in inner medullary collecting ducts. On the other hand, the neural expression of MAGI1 was confirmed with the specific antibody (46). The surface expression of glutamate transporter-1 is regulated by MAGI1 in astrocytes (47). MAGI1 copy number variations were proposed to be associated with bipolar affective disorder and schizophrenia (9). These findings suggest that MAGI1 is also important for brain function. It is essential to characterize three MAGI proteins specifically and clarify their specific roles in each tissue.

The first and second exons are specific for the longest variant, MAGI2/S-SCAMz, and are far away from the remaining exons that are common for three variants. We targeted the first exon and specifically knocked out MAGI2/S-SCAMz (26). The knockout mice died within 24 hr. Other groups targeted exon 4 and exon 6 and knocked out all variants (39, 40). Their MAGI2/S-SCAM null mice died by 3 months and within 3 weeks, respectively. It sounds that the phenotype of MAGI2/S-SCAMz-specific knockout mice is more severe. As we used BDF1 mice and Balbas et al. used 129 mice, it might be due to the different genetic backgrounds. However, we also need to consider the possibility that the remaining shorter variants play a certain dominant and detrimental role. It will be intriguing to investigate into the specific role of the longest variant.

Finally, we are attracted by the similarity in the protein networks that support synaptic dendrites and foot processes of podocytes (Fig. 2C). MAGI2/S-SCAM, dendrin, and CIN85 form a complex in both tissues. Nephrin is also expressed in brain (48). Synaptopodin and Kibra are enriched in brain and kidney (30, 49). Synaptopodin, which regulates RhoA and the actin cytoskeleton, interacts with MAGI1, and is highly likely to bind to MAGI2/S-SCAM (50, 51). Kibra is known to bind dendrin (30). MAGI2/S-SCAM, dendrin, CIN85, synaptopodin and Kibra form a common protein interactome in brain and kidney. What does this mean? Is it related to the morphological similarity between dendritic spines and foot processes of podocytes? Does it mean some common functional requirement shared by the synapse and the slit diaphragm? There are many questions to be answered.

The study focused on particular molecules seems to be out of date. Nevertheless, if we study MAGI proteins carefully, we might make unexpected discoveries and find therapeutic targets for cancer, inflammatory bowel diseases and kidney diseases.

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Conflict of Interest
None declared.

References


