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Inositol 1,4,5-triphosphate-associated cGMP kinase substrate:

Basis Sequence: Mouse

Sarabeth M Graham¹, Sandra Longoria², Pabak Sarkar³, and Peter Koulen⁴

¹Integrative Physiology, UNTHSC, TX 76107, US.

²Pharmacology and Neuroscience, University of North Texas Health Science Center, TX 76116, US.

³Molecular Biology and Immunology, University Of North Texas Health Science Center, TX 76107, US.

⁴Departments of Ophthalmology and Basic Medical Sciences, University of Missouri - Kansas City, School of Medicine, MO 64108, US.

Abstract

Inositol 1, 4, 5-trisphosphate (IP₃) receptor associated cyclic GMP (cGMP) kinase substrate (IRAG, also known as Mrv1) is a type-2 integral membrane endoplasmic reticulum (ER) protein, which interacts with IP₃ Receptor type 1 (IP₃R1), cGMP kinase I- β (cGKI β) and other associated proteins. It plays a key role in NO, cGMP, and cGKI β mediated inhibition of IP₃R1 activity and thus relaxes smooth muscle tone and inhibits platelet aggregation. As a scaffolding protein Mrv1 maintains the conformation of a heteroprotein complex containing cGKI β , IP₃R1 and other proteins and enables efficient activity of cGKI β within the complex. Increased expression of Mrv1 or IRAG in the absence of tumor related transcription factor in pancreatic cancer cells suggest that it might be involved in tumorigenesis. Downregulation of Mrv1 during megakaryocyte maturation indicates that it is involved in cell growth and differentiation.

Protein Function

Mrv1 or inositol 1, 4, 5-trisphosphate (IP₃) receptor associated cyclic GMP (cGMP) kinase substrate (IRAG) is an approximately 125kDa protein with approximately 900 amino acids encoded by the Mrv1 gene located in the distal region of mouse chromosome 7 (homologous with the human 11p15 region). Two splice variants are reported in both mouse (Mrv1a and Mrv1b) and human (MRV1A and MRV1B). Both of these gene products contain an alpha-helical coiled coil domain, a C-terminal hydrophobic domain, a stretch of basic amino acid at N-terminal forming leucine zipper motif, an ATP binding site and multiple N-linked glycosylation and phosphorylation sites. IRAGa (or Mrv1a) contains an extra stretch of 84 amino acids, which is thought to form a hydrophobic domain at the N-terminus.

IRAG is a type-2 integral membrane endoplasmic reticulum (ER) protein, which coimmunoprecipitates with IP₃ Receptor type 1 (IP₃R1), cGMP kinase I- β (cGKI β) and

other associated proteins. It interacts with cGKI β through a Leucine Zipper motif at its N-terminus and cGKI β phosphorylates IRAG at Ser⁶⁹⁶ the N- terminus of its coiled-coil motif. Deletion of IRAG's N-terminal coiled coil region or mutation of Ser⁶⁹⁶ leads to a loss of its interaction with IP3R1.

Mrv1 plays a key role in NO, cGMP, and cGKI β mediated inhibition of IP3R1 activity and thus relaxes smooth muscle tone and inhibits platelet aggregation during plaque formation. Some studies suggest that IRAG acts as a scaffolding protein that maintains the conformation of a heteroprotein complex containing cGKI β , IP3R1 and other proteins like phospholamban. As part of this complex, it enables efficient activity of cGKI β within the complex. Increased expression of Mrv1 or IRAG in the absence of tumor related transcription factor in pancreatic cancer cells suggest that it might be involved in tumorigenesis. Downregulation of Mrv1 during megakaryocyte maturation indicates that it is involved in cell growth and differentiation.

PM ID	Author	Title	Journal	Pub Date
10321731	Shaughnessy JD Jr <i>et al.</i>	Mrv1, a common MRV integration site in BXH2 myeloid leukemias, encodes a protein with homology to a lymphoid-restricted membrane protein Jaw1.	Oncogene. 18, 12	25 Mar 1999
10724174	Schlossmann J <i>et al.</i>	Regulation of intracellular calcium by a signalling complex of IRAG, IP3 receptor and cGMP kinase Ibeta	Nature. 404, 6774	9 Mar 2000
12480535	Koller A <i>et al.</i>	Association of phospholamban with a cGMP kinase signaling complex.	Biochem Biophys Res Commun. 300, 1	3 Jan 2003
11309393	Ammendola A <i>et al.</i>	Molecular determinants of the interaction between the inositol 1,4,5-trisphosphate receptor-associated cGMP kinase substrate (IRAG) and cGMP kinase Ibeta.	J Biol Chem 276, 26	29 Jun 2001
16166082	Casteel D. E. <i>et al.</i>	Identification of the interface between cGMP-dependent protein kinase Ibeta and its interaction partners TFII-I and IRAG reveals a common interaction motif.	J Biol Chem. 280, 46	18 Nov 2005
15483626	Geiselhoringer A <i>et al.</i>	IRAG is essential for relaxation of receptor-triggered smooth muscle contraction by cGMP kinase.	EMBO J. 23, 21	27 Oct 2004
14729908	Fritsch R.M. <i>et al.</i>	InsP3R-associated cGMP kinase substrate (IRAG) is essential for nitric oxide-induced inhibition of calcium signaling in human colonic smooth muscle.	J Biol Chem. 279, 13	26 Mar 2004
16990611	Antl M <i>et al.</i>	IRAG mediates NO/cGMP-dependent inhibition of platelet aggregation and thrombus formation.	Blood. 109, 2	15 Jan 2007
17312387	Kusumawidjaja G <i>et al.</i>	Basic transcription factor 3 (BTF3) regulates transcription of tumor-associated genes in pancreatic cancer cells. Cancer	Cancer Biol Ther 6, 3	6 Mar 2007

Regulation of Activity

In pancreatic cancer cells Mrv1 expression is regulated by basic transcription factor 3 (BTF3). Mrv1 expression has shown to be downregulated in macrophage differentiation of BXH2 myelomocytic leukemias when increased mRNA degradation is observed. The highly hydrophobic carboxyl terminal with an encoded PEST sequence also suggests that Mrv1 may be regulated by protein degradation.

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Interactions with Ligands and Other Proteins

The Mrv1 gene interacts with cyclic GMP-dependent protein kinase I (cGKI) and inositol-1,4,5-trisphosphate (IP3) receptor (IP3R) type I (IP3R1) in a macrocomplex assembly. Phosphorylation by cGKI at Ser644 and Ser677 causes the Mrv1 gene product to

bind to IP3R1 with its coiled-coil domain. Mrv1 contains an α -helix structure with several basic amino acids on the face of the helix creating an electropositive region which can uniquely bind to the N-terminal leucine zipper domains of cyclic GMP-dependent protein kinase I β (PKG1 β). PKG1 β can then phosphorylate Mrv1 resulting in the inhibition of IP3R dependent calcium release. The protein also contains an 84 amino acid carboxyl terminal hydrophobic region domain, which may serve as an anchoring domain for endoplasmic reticulum localization. Near this carboxyl terminal is also a PEST sequence providing access to enzymes involved in protein degradation. Mrv1 also contains an ATP-binding site, however its function significance is unknown.

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Regulation of Concentration

The transcription factor BTF3 controls the downregulation of Mrv1 expression in several cell lines.

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Subcellular Localization

Mrv1 is associated with endoplasmic reticulum (ER) membranes as evidenced by perinuclear staining patterns characteristic of the ER and predicted by the presence of an 84 amino acid carboxyl terminal hydrophobic region domain potentially anchoring the protein in the endoplasmic reticulum.

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Major Sites of Expression

Mrv1 expression was detected in bone marrow, fetal liver, spleen, Paneth cells in the base of the crypts of Lieberkuhn in the small intestine, heart, trachea, uterus, placenta, skeletal muscle, smooth muscle, megakaryocytes, platelets, lymph nodes, appendix, lung, colon, kidney, and testis. Mrv1 is expressed in many brain regions including the nucleus-motorius, nucleus-trigemini, and the hippocampus.

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Phenotypes

A 75% reduction in Mrv1 was seen in mice with a mutation in the IRAG-IP3R1 interaction domain. Pylorus stenosis and a severe intestinal movement disorder were observed in these animals.

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Splice Variants

Mrv1a and Mrv1b are splice variants of Mrv1. Two splice variants are reported in both mouse (Mrv1a and Mrv1b) and human (MRV1A and MRV1B). Both of these gene products contain an alpha-helical coiled coil domain, a C-terminal hydrophobic domain, a stretch of basic amino acid at N-terminal forming leucine zipper motif, an ATP binding site and multiple N-linked glycosylation and phosphorylation sites. IRAGa (or Mrv1a) contains an extra stretch of 84 amino acids, which is thought to form a hydrophobic domain at the N-terminus.

Antibodies

Rabbit anti-bovine IP3R-associated cGMP kinase substrate (IRAG) antibody recognizes mouse Mrv1 protein and is commercially available from ABR–Affinity BioReagents, 4620 Technology Drive, Suite 600, Golden, CO 80403, USA (catalog no. PA3-851).