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VMP1

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Gene symbols: Vmp1, TMEM49

1. General Function

VMP1 (Vacuole Membrane Protein 1), also now as TMEM49, is a transmembrane protein (UniProtKB/Swiss-Prot Q96GC9) of 406 amino-acids length with 7 putative transmembrane domains and with no known homologues in yeast. Vector expression of EGFP-tagged VMP1 fusion protein in several mammalian cell lines, induces formation of cytoplasmic vesicles and VMP1 is located in the membrane of these structures (1) (Figure). Rat VMP1 mRNA presents three alternative splicing variants. Two of those variants (1.9 and 2.7 kb) are highly expressed in intestine, kidney, ovary and placenta, moderately expressed in liver, lung, stomach, thymus, brain and testis, and slightly expressed in thyroid and retina. The third splicing form (3.5 kb) is observed in intestine, liver, lung, kidney, stomach, thymus, ovary and placenta (1).

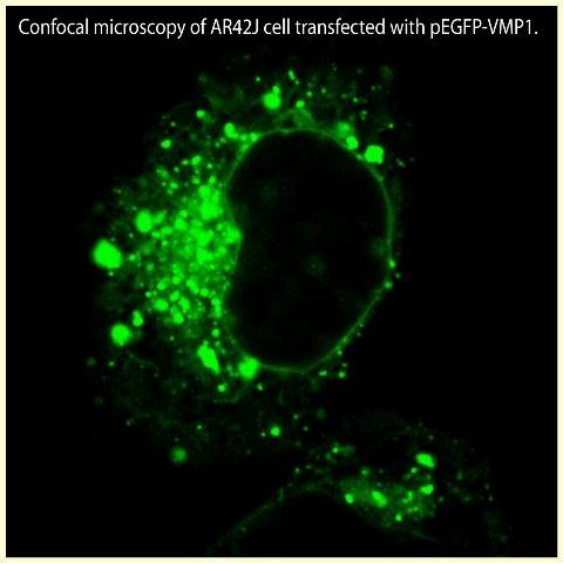

Figure 1. AR42J rat acinar cell transfected with the pEGFP-VMP1 expression plasmid. VMP1 expression induces formation of cytoplasmic vesicles and VMP1-EGFP fusion protein is located in these structures. Confocal microscope image, Nikon Eclipse C1, SALES Foundation, Buenos Aires, Argentina.

A significant reduction of VMP1 mRNA has been observed in kidney cancer metastases versus primary tumors as well as in breast cancer cell lines such as HCC1954 and MDA-MB-231 compared to non-invasive cell lines MCF-12A, T-47D and MCF-7, suggesting a relevant roll of VMP1 protein in

cancer progression. Moreover, VMP1 has been described as an essential plasma membrane protein for cell adherence, and therefore loss of VMP1 could contribute to increase the invasive capacity of cancer cells (5).
Recently a significant advance in the molecular insight underlying VMP1 function has been made. The VMP1 protein has been implicated in induction of autophagosome formation (6). It was demonstrated that VMP1 expression is sufficient to trigger the autophagy process in mammalian cells. Indeed, pcDNA4-VMP1 expression induces cytoplasmic autophagosomes formation and LC3-I to LC3-II conversion. Moreover, VMP1 co-localizes with LC3, a marker of the autophagosomes. VMP1 is induced by starvation and rapamycin treatments. Notably, its expression is necessary for autophagy, because VMP1 small interfering RNA inhibits autophagosome formation under both autophagic stimuli.

Mechanistically, VMP1 interacts with the autophagy protein Beclin1 through VMP1-carboxyterminal domain *ATG*, which is essential for autophagosome formation. $VMP1^{\Delta ATG}$, a mutated VMP1 protein where the ATG domain is deleted, is unable to induce autophagy suggesting that VMP1-Beclin1 interaction is a key event in autophagosome formation and the autophagy pathway (6, 7).

Recently, it has been reported that the scaffold protein TP53INP2 interacts with VMP1, mediating the recruitment of LC3 to the autophagosome membrane and supporting a key role of VMP1 in the autophagic pathway (9).

The importance of VMP1 function is demonstrated by its conservation among eukaryotes cells. The social amoeba *Dictyostelium discoideum* has a *vmp1*-related gene that it is necessary for the integrity of endoplasmic reticulum and it is implicated in membrane traffic-dependent processes such as organelle biogenesis and structure, endocytosis, and protein trafficking (8). Indeed, VMP1 is required for autophagy in *Dictyostelium discoideum* and its absence causes defects in autophagic cell death and the accumulation of ubiquitin-positive protein aggregates. Moreover, the expression of a VMP1 mammalian homologue in the *Dictyostelium* mutant is able to complement this last phenotype suggesting the functional conservation across species (13).

2. VMP1 in Pancreas

VMP1 was cloning from analysis and characterization of a large number of transcripts from rat pancreas with acute pancreatitis. VMP1 expression is part of the acinar cell molecular response to pancreatitis (1, 4). During cerulein-induced experimental acute pancreatitis, VMP1 mRNA is detected in rat pancreas as early as 30 min after cerulein first injection with a maximal after 1 h treatment (1). Moreover, high VMP1 mRNA levels are observed in the fetal pancreas on day 19 and remains elevated in the newborn until day 11. Then, it suddenly decreases and remains low until day 23, after which it is no longer detectable. *In situ* hybridization studies confirmed that VMP1 transcript is highly and specifically expressed in pancreas acinar cells during experimental acute pancreatitis (1). Moreover, early VMP1 expression was correlated with acinar cell cytoplasmic vacuolization in arginine-induced acute pancreatitis (2).

In the WBN/Kob rat, VMP1 expression, evaluated by RT-PCR and in situ hybridization, is strongly induced in acinar cells at 12 week, when spontaneous chronic pancreatitis begins and it is correlated with prominent vacuolar formation (3).
VMP1-triggered autophagy is induced *in vivo* during experimental acute pancreatitis, where it is associated with Beclin1 and LC3 in the membrane of autophagosomes. Indeed a ElaI-VMP1 transgenic mouse has been recently developed,

where VMP1-EGFP expression is under the control of elastase 1 promoter inducing a constitutive and pancreas specific expression of VMP1. The ElaI-VMP1 mouse was born and grew up without any noticeable abnormality. Histological studies of this transgenic animal show normal tissue architecture, acinar cells have zymogen granules with normal morphology and polarization. However in acinar cell cytoplasm there is an increased number of autophagosomes where VMP1, Beclin1 and LC3 co-localize (6).

VMP1 expression triggers autophagy and precedes apoptotic cell death in pancreas beta cells as a direct response to streptozotocin-induced experimental diabetes (10).

Recently, VMP1 has been implicated in pancreatic cancer cells. Gemcitabine, the standard chemotherapy for pancreatic cancer, induces early expression of VMP1 in PANC-1 and MIAPaCa-2 pancreatic cancer cell lines. VMP1 induction by gemcitabine promotes the autophagy pathway and apoptotic cell death mediating gemcitabine-induced citotoxicity in pancreatic cancer cells (14, 15).

3. Tools for study of VMP1

a. cDNA clones

Two human VMP1 clones are available from Invitrogen (Cat. #392737 and #IOH22902). Also OriGene offer a GFP-tagged clone (Cat #RG202623), a Myc-DDK-tagged clone (Cat. #RC202623), and an untagged clone in CMV expression vector. (Cat. #SC109258). None of these have been checked by us. We have developed a pEGFP-VMP1 (Figure).

b. Antibodies

Rabbit antibody raised against the C-terminal 20 amino-acids of rat VMP1 (Met386 to Lys406) have been used to identify VMP1 by western blotting, IP, immunofluorescence and IHC (6). This antibody recognizes VMP1 from mouse, rat and human origin. An antibody against this region is sold by Novus Biologicals as a rabbit polyclonal IgG (NBP1-19041). We used this antibody for western blot and inmunofluorescence at a dilution of 1:100-1:500.

c. Viral Vectors

None.

d. Mouse Models

ElaI-VMP1 transgenic mice have been recently developed (6). In this animal, transgenic expression of VMP1-EGFP fusion protein is driven by pancreatic elastase I promoter and therefore there is a constitutive and pancreas specific transgen expression.

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