Distribution and Functions of the Novel Membrane-Spanning Four-Domains, Subfamily A Member HCA112

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Abstract

Members of the membrane-spanning four-domains, subfamily A (MS4A) family are small polypeptides that share the structural features of four-transmembrane domains and unevenly sized extracellular loops. The family includes CD20, FcεRIβ and HtM4, plus a number of relatively uncharacterised proteins / predicted proteins. MS4A proteins are discussed in relation to other protein families, such as the tetraspanins, that are also characterised by four-transmembrane domains. The aim of this study was to identify the cell and tissue distribution, subcellular localisation, and function of a newly discovered member of the MS4A family, hepatocellular carcinoma-associated antigen 112 (HCA112).

At a subcellular level, HCA112 was found on the plasma membrane of transfected COS-7 cells, and also within the Golgi complex, trans-Golgi-network, and early endosomes. The molecule is orientated such that the large loop is extracellular and the N- and C-terminal domains are cytoplasmic. The presence of HCA112 associated with components of the endocytic pathway raised the question of whether some originated from the surface membrane. Antibody was used to label a HA epitope tag engineered into the large extracellular loop of HCA112, and the bound antibody was tracked through early endosomes to the recycling compartment. Here it co-localised with internalised transferrin, indicating strongly that HCA112 is internalised via clathrin-dependent mechanisms. Several endocytic sorting motifs within the intracellular domains of HCA112 were investigated for their ability to direct internalisation of HCA112. Deletion of a di-leucine motif was found to slow but not prevent endocytosis, suggesting that it is involved in endocytosis of HCA112, although not essential for the process. When HCA112 expression constructs featuring N- and C-terminal domain truncations were examined, it was found that the N-terminal tail does not affect the subcellular localisation or trafficking of HCA112, while deletion of the C-terminal intracellular domain resulted in retention of the mutant protein in the ER.

HCA112 has a wide tissue distribution and is highly expressed in the lining/covering and parenchymal epithelium of some tissues, proximal renal tubules, ductal epithelium in a number of organs, endothelial cells, some steroidogenic endocrine cells, adipocytes, smooth muscle cells, follicular dendritic cells and macrophages. The expression of

HCA112 by a wide range of cell types suggests that its function(s) has general importance and is not limited to any specific cell type(s). After reflection on the functions of the HCA112-expressing cells, a common theme that emerged was one of endocytic activity. This lead to speculate that one function of HCA112 might be related to uptake of macromolecules, for instance, in antigen processing and presentation. This might be a general function, such as facilitating uptake of other cell membrane proteins, or directing the traffic of endocytic vesicles. It was noted that HCA112 has a similar cell and tissue distribution to the scavenger receptor and fatty acid translocase FAT/CD36 (Zhang *et al.*, 2003). Furthermore, in cells co-transfected with HCA112 and FAT/CD36, the two molecules co-localise in early endosomes and co-immunoprecipitate, suggesting that the molecules physically and spatially associate. Thus, HCA112 could be involved with (or complement) FAT/CD36 in its functions as a long chain fatty acid transporter and scavenger receptor.

A proteomics study of proteins that co-immunoprecipitated with HCA112 detected putative interactions with a number of proteins. These included LR8, transferrin receptor, interferon induced transmembrane proteins 2 and 3, Calpain-6, stomatin, PDGF α receptor, and heat shock 70 kDa protein 8 (HSPA8, formerly known as clathrin un-coating ATPase). Of these, LR8 and the transferrin receptor were investigated in more detail. The results provide strong evidence that HCA112 forms a novel complex with LR8, and that this may be involved in macromolecule internalisation or trafficking of membrane proteins, such as FAT/CD36 or the transferrin receptor. In the case of the transferrin receptor, this traffic appears to involve the clathrin-dependent pathway, but it is possible that when HCA112 is associated with FAT/CD36, it functions within lipid raft domains.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Wendy Parker

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Abbreviations

°C	degrees Celsius
µg	microgram(s)
µL	microlitre(s)
aa	amino acid(s)
APC	allophycocyanin
APC	antigen presenting cell
az	azide
bp	base pairs
BMDC	bone marrow-derived DC
CD	cluster of differentiation
cDNA	complementary deoxyribonucleic acid
CTB	cholera toxin B subunit
Cy3	cyanine
DAPI	4',6-diamidino-2-phenylindole
DC	dendritic cell
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EDTA	ethylene-diamine-tetra-acetic-acid disodium salt
EGFP	enhanced green fluorescent protein
FCS	feotal calf serum
FDC	follicular dendritic cell
FITC	fluorescein isothiocyanate
GFP	green fluorescent protein
GKO	gene knock-out
GTS	glysine, tris, SDS buffer for PAGE
g	relative centrifugal force
g	gauge
hr	hour(s)
HA	9 aa tag from influenza haemaglutanin (YPYDVPDYA)
HCA112	hepatocellular carcinoma-associated antigen 112

HCl	hydrochloric acid
IPTG	isopropyl-β-D-thio-galactopyranoside
IL	interleukin
kb	kilobase/s
kDa	kilodalton/s
KEG2	kidney expressed gene product 2
kg	kilogram/s
l	litre/s
LB	Luria Bertani broth
LPS	lipopolysaccharide
M	molar
mAb	monoclonal antibody
mg	milligram(s)
min	minute(s)
mL	milliliter(s)
mM	millimolar
nm	nanometers
NMS	normal mouse serum
OD	optical density
OD_{260}	optical density at 260 nm
OD_{280}	optical density at 280 nm
O/N	overnight
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PE	Phycoerythrin
PMSF	phenylmethylsulphonyl fluoride
PNS	post-nuclear supernatant
RNA	ribonucleic acid
RO	reverse osmosis distilled water

RT	room temperature
RT-PCR	reverse transcription PCR
SDS	sodium dodecyl sulphate
SOC	super optimal broth with catabolite repression
SOC	store operated calcium channel
s	second(s)
TAE	tris-acetate EDTA buffer
TBS	tris-buffered saline
TBST	tris-buffered saline plus 0.1% Tween 20
TCA	trichloroacetic acid
TNFα	tumour necrosis factor α
Ub	ubiquitin
v/v	volume per volume
w/v	weight per volume

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