

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

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July, 2009

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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

July, 2009

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 |
| <i>g</i> | relative centrifugal force |
| <i>g</i> | gauge |
| hr | hour(s) |
| HA | 9 aa tag from influenza haemagglutinin (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 |
| TNF α | tumour necrosis factor α |
| Ub | ubiquitin |
| v/v | volume per volume |
| w/v | weight per volume |

Acknowledgements

Firstly, I would like to thank my supervisors Graham Mayrhofer and Richard Ivell. I'd especially like to thank Graham for 'adopting' me when things got complicated and taking over the role as my primary supervisor. Thank you very much for 'getting excited', editing my thesis, and for putting up with my 'crap'! A big thank you to Nick Eyre, for being my mentor and helping me so much, even after you'd left our lab. I would also like to thank both Steven Wood and Dan Peet for their support and intellectual input into this project, and Lyn Waterhouse from Adelaide Microscopy for answering all of my annoying questions and fixing the confocal microscope when it was playing silly buggers. Thanks to Peter Hoffmann, Chris Bagley, Megan Penno and Sandra Hack from the Adelaide Proteomics Centre who also answered my annoying questions with great patience. Michael Roberts, Grigory Rychov, Greg Barritt, Roland Gregory and Joel Castro were great to help me with the calcium experiments, thank you. Next I need to thank almost everyone in the building, but in particular the members of the McColl, Beard, Morona, Dent and Paton labs, for letting me 'steal' reagents, use equipment, help with protocols, providing me with animal carcasses to scavenge from, or just listening to my babble... I never actually stole anything, but I did get pretty good at obtaining stuff for free (thanks for the lessons Nick!). Seriously, I could not have done many of my experiments without your help. Thankyou Connor for giving me this thesis template, it made a horrible task that little bit easier. Thanks also to Chris C, Ros, John, Shirley, Adrianna, Garry and Serge for making stuff, selling stuff and lending stuff to me. Thanks to Martin and Lester for being great 'fix it' men. I'd like to say to Nick E, Sarah, Gorjana, Michelle, Minky, Nicole, Julie, Mei Mei, Brock, Fran, Damien, Tom, Damon, Matt, Mark, Iain, Wendel, Steven, Paul, Melissa, Oli, Jane, Adriana, Erin, Geroget, Maggie, Thomas, Ann, Peter, Carolina, Shelly, Soki, Chris W, Jamie, and everyone else that I have probably missed out (I'm sorry, it's not you, it's my terrible memory!), thank you so much for your friendship, making my time in this building (almost) enjoyable. Finally I'd like to thank my fantastic partner Mark, and my beautiful family and friends, for giving me great friendship and support (both mental and financial!) over the last too many years. Thank you!!!