Book Chapter

The Secreted Glycoprotein PAMR1: A New Potential Tumor Suppressor in Cancer

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Published **March 15, 2023**

This Book Chapter is partly adapted from a pre-published paper by Layla Hamour et al. at BioRXive (Haymour, L., Chaunavel, A., Diab-Assaf, M., Maftah, A. and Legardinier, S. PAMR1 negatively impacts cell proliferation and migration of Human Colon Cancer HT29 Cell Line. BioRXive, posted September 8, 2022. https://doi.org/10.1101/2022.09.07.506931)

How to cite this book chapter: Layla Haymour, Abderrahman Maftah, Sébastien Legardinier. The Secreted Glycoprotein PAMR1: A New Potential Tumor Suppressor in Cancer. In: Hussein Fayyad Kazan, editor. Immunology and Cancer Biology. Hyderabad, India: Vide Leaf. 2023.

Competing Interests: The authors declare that they have no competing interests.

Acknowledgements: The authors gratefully acknowledge the financial support of the “Ligue contre le Cancer”, France.

Introduction

This chapter focuses on PAMR1 (Peptidase domain containing Associated with Muscle Regeneration 1), a secreted multi-domain protein, which was first considered as a putative tumor suppressor in breast cancer in 2015 [1]. It was formerly named RAMP (Regenerative Associated Muscle Protease), because of its C-terminal trypsin-like domain and increased expression during muscle regeneration of injured muscles in mice [2]. After a structural description of PAMR1 (protein structure, isoforms and glycosylation), we will focus on its expression, role and potential mechanisms of action in cancer. We also will consider point mutations found in cancer and affecting all potential glycosylation sites of human PAMR1.

PAMR1: A Multidomain Protein

PAMR1 is a secreted multi-domain glycoprotein formed of five domains: an N-terminal Cubilin domain referred to as CUB (Complement C1r/C1s, Uegf, Bmp1), a unique EGF-like domain (Epidermal growth factor-like domains or ELD), two Sushi domains (Sushi 1 and 2) and C-terminal Peptidase S1 domain (Figure 1).

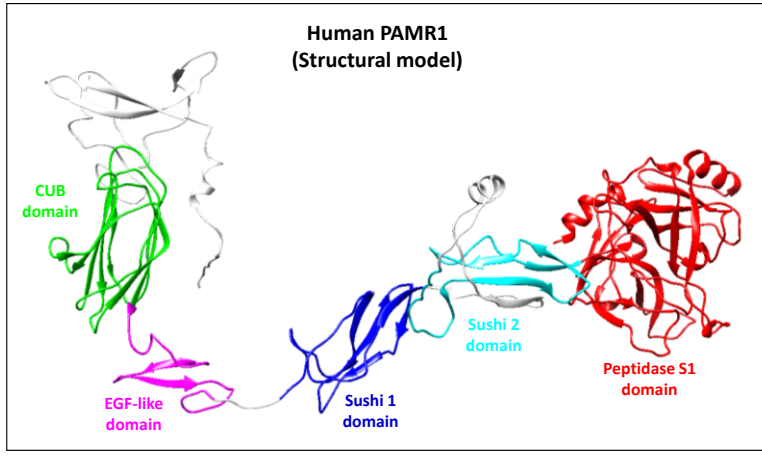

Figure 1: Predicted structural model of human PAMR1 canonical isoform 1 (AF-Q6UX9-F1-MODEL_V4.PDB) using AlphaFold (https://alphafold.ebi.ac.uk/). Boundaries of each colored protein domain were defined according information found in Uniprot database (https://www.uniprot.org/).

The CUB domain is a 110 residue-long extracellular domain, which is found in plasma-membrane and extracellular proteins. CUB-containing-proteins are involved in various functions (complement activation, tissue repair, cell signaling...) and also in tumor suppression [3,4], such as SCUBE2 (Signal peptide, CUB, and EGF-like domain-containing protein 2) [5]. In addition to a CUB domain, PAMR1 and SCUBE2 contain one and nine EGF-like domains respectively. EGF-like domains, which usually comprise about 30 to 40 amino-acid residues and are stabilized by three disulfide bonds, are often involved in protein-protein interactions [6], such as for those between NOTCH receptors and their ligands [7]. Indeed, CUB and EGF-like domains were shown to be involved in tumor suppressor activity of SCUBE2, through protein interactions with Bone Morphogenetic Proteins (BMPs) and E-cadherin respectively [8].

Sushi domains exist in a wide variety of complement and adhesion proteins but are also found in potential tumor suppressors such as SUSD4 (Sushi domain-containing protein 4) [9] and CSMD1 (CUB and Sushi Multiple Domains 1) [10].

The peptidase S1 domain is predominately found in serine proteases. Using Swiss-model server (https://swissmodel.expasy.org/), we found that this domain within human PAMR1 isoform 1 shared 26.53% identity with thrombin heavy chain, which is a serine protease composed of a “catalytic triad” in its active site. The structural model built with thrombin as a template for the peptide S1 domain of human PAMR1 shows a potential catalytic triad (Figure 2) with three key residues very close in space, namely a histidine at position 504, an aspartic acid at position 560 and a threonine (instead of serine as found in thrombin at this position) at position 665.

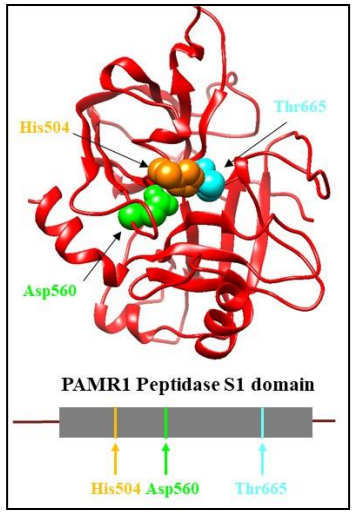

Figure 2: Predicted structure of human PAMR1 trypsin-like domain. The peptidase S1 domain of human PAMR1 (445-720) was built using Swiss-model Server with the X-ray structure of human thrombin (PDB 7SR9) used as a template. The three residues (H^{504}, D^{560}, T^{665}) are spatially close and at the same locations as residues composing the catalytic triad (H^{57}, D^{102}, S^{195}) of thrombin.

Although related to peptidase S1 family, PAMR1 might have no protease activity due to the presence of a threonine residue at position 665 instead of the conserved serine residue. However, some proteins like TSP50, which is homologous to serine proteases, have also a threonine in their catalytic triad [11]. This threonine catalytic site was shown to be essential for TSP protease activity [11] and its function in cell proliferation [12]. Although the threonine residue is known to be substantially less nucleophilic than a serine residue, it might be interesting to determine whether or not PAMR1 might have a protease activity. No evidence in the literature supports this hypothesis.

PAMR1 and Isoforms

There are several human PAMR1 transcripts coding for different isoforms (ENSEMBL database), some of which are well described, and others are predicted. By referring to the UniProt database, three human PAMR1 isoforms (named Iso 1-3) were described and obtained by alternative splicing (Figure 3).

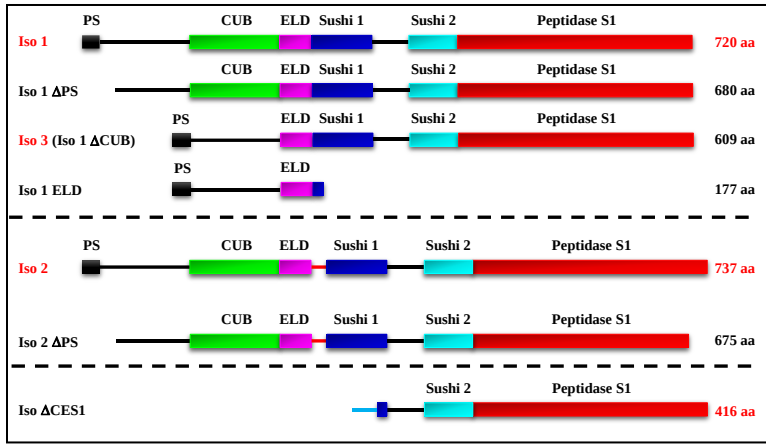

Figure 3: Representation at scale of human PAMR1 isoforms, according to data available in the Uniprot (www.uniprot.org/) and Ensembl (www.ensembl.org/) databases. Three main protein isoforms are described: Iso 1 (canonical isoform), Iso 2 and Iso 3 (named Iso1 ΔCUB). Iso 2 is distinguished from Iso 1 by the presence of 17 additional residues (red line) between the EGF-like domain (ELD) and Sushi 1 domains. The 4 other potential isoforms are: Iso 1 ΔPS (without signal peptide), Iso1 ELD (only ELD is present), Iso 2 ΔPS (without signal peptide) and Iso ΔCES1 (without the CUB, ELD and Sushi1 domains).

Isoform 1 (Iso 1) is considered as the canonical form with a non-mature 720 amino acid (aa) sequence. After cleavage of its signal peptide (21 aa), Iso 1 does not have more than 699 amino acid residues and its theoretical molecular weight (MW) is 77849.95 Da. Isoform 2 (Iso 2) differs from Iso 1 by the presence of 17 additional aa at position 274 (737 aa) following the EGF-like domain (ELD). Iso 2 is therefore composed of 716 aa after cleavage of its signal peptide and its theoretical MW is 79593.93 Da. Isoform 3 (Iso 3) differs from Iso 1 by lacking the CUB domain (Iso1 ΔCUB), it is of 609 aa and 67541 Da of MW. Other described or potential isoforms exist, Iso 1 without signal peptide (Iso 1 ΔSP) or with only the EGF-like domain called ELD (Iso 1 ELD). For Iso 2, there would also be an isoform without signal peptide (Iso2 ΔSP). Finally, there would be an isoform with only the Sushi 2 and peptidase S1 domains (Iso ΔCES1). If the Iso 1 ΔSP and Iso 2 ΔSP isoforms are indeed expressed by cells, it can be expected that they would encode non-secreted isoforms of PAMR1 due to the absence of a signal

peptide. We can wonder what would be the exact location and role of these intracellular isoforms.

PAMR1 is a Glycoprotein with *N*- and *O*-glycans

Human PAMR1 possesses several potential *N*- and *O*-glycosylation sites, as predicted by NetNGlyc, NetOGlyc databases and the presence of consensus sequences of *O*-glycosylation within its EGF-like domain (Figure 4).

Although possessing five *N*-glycosylation consensus sequences of the type Asn-Xaa-(Ser/Thr), only four sites are predicted by the NetNGlyc server [13] to be occupied by *N*-glycans on asparagine residues at positions 96, 279, 451 and 614. Except Asn 96, the three others are located within domains of PAMR1, namely Sushi and Peptidase S1 domain. In humans, *N*-glycans are mainly sialylated and fucosylated complex-type *N*-glycans as shown in Figure 4, with two, three or four antennae. Although PNGase F treatment showed the presence of *N*-glycans on PAMR1, we have not determined yet the number nor the structure of these *N*-glycans. The presence of these *N*-glycans could be required for PAMR1 folding and/or secretion.

Different types of *O*-glycosylation are predicted for human PAMR1. Concerning secreted glycoproteins like PAMR1, the most common *O*-glycosylation is mucin-type *O*-GalNAc glycosylation, for which there is no defined consensus sequence yet but which can be predicted using NetOGlyc [13]. For human PAMR1, 18 sites are predicted with most of them within Sushi 2 and inter-Sushi region (Figure 4) but the occupation of these glyco-sites must be experimentally demonstrated. However, the most relevant predicted *O*-GalNAc glyco-sites are those between the two Sushi domains because mucin-type *O*-glycosylation is often found in not structured proline-rich protein regions. The simplest form of mucin *O*-glycans is formed of attached GalNAc to the serine or threonine. This *O*-GalNAc can be extended by various sugar moieties (galactose, N-acetylglucosamine and sialic acid), forming different “core” structures that are counted to be 8 cores [14]. An example: Galβ1-3GalNAc- is the most common *O*-GalNAc and is referred to as core 1.

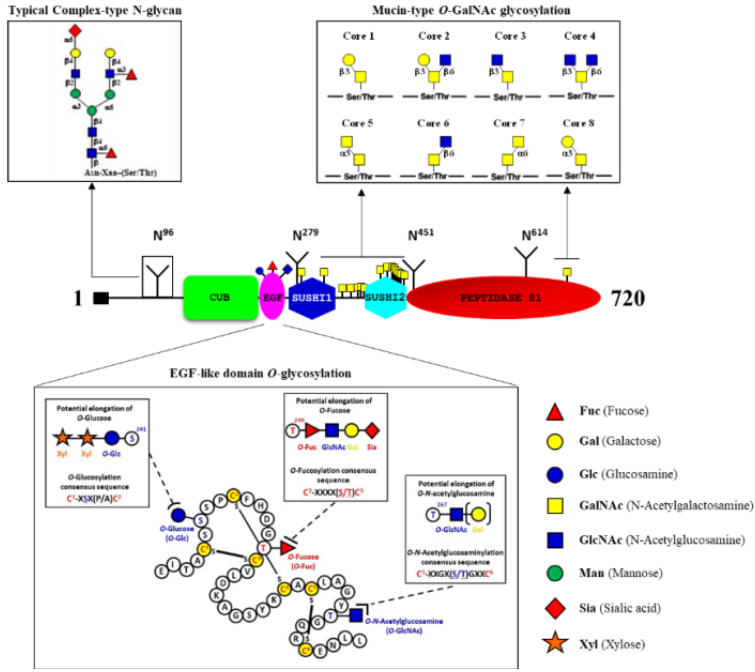

Figure 4: Representation at scale of multi-domain human PAMR1 and its potential *N*- and *O*-glycosylation sites, predicted according to NetNGlyc-1.0 and NetOGlyc-4.0 databases or linked to presence of consensus sequences. An example of sialylated and fucosylated complex-type *N*-glycan with two antennae found in human glycoproteins is shown. Among the eight structures known for *O*-GalNAc mucin-type glycosylation, Core 1-4 are the most abundant ones and can be elongated and terminated with fucoses and/or sialic acids. The EGF-like domain of human PAMR1 exhibits three consensus sequences, for specific addition of *O*-Glucose, *O*-Fucose and *O*-GlcNAc. As shown in inserts, these *O*-linked monosaccharides can be elongated or not by other monosaccharides.

Despite a prediction of one *O*-GalNAc site in the EGF-like domain (see Table I), it is unlikely that a residue would actually be attached as this has never been shown for Notch receptors having 29-36 EGF-like repeats [15]. However, other rare *O*-glycosylations with well-defined consensus sequences can be found in EGF-like domains such as *O*-Glucose, *O*-Fucose and *O*-GlcNAc type glycosylations. Human PAMR1 exhibits the three consensus sequences within its EGF-like domain.

O-Glucosylation is less familiar than most *O*-linked glycosylation, it refers to the attachment of *O*-Glucose to Epidermal growth factors repeats (EGF-like) of proteins, especially NOTCH receptors. It is mediated by *O*-Glucosyltransferases, including POGLUT1 known to add *O*-Glucose on serine of consensus sequence C^1-X-S-X-(P/A)-C^2 of EGF-like domain. *O*-Glucosylation is crucial for the regulation of NOTCH trafficking [16]. Human PAMR1 exhibits a potential *O*-Glucosylation site within its EGF-like domain at Serine 241 between C1 and C2 (Figure 4) but doesn't possess the consensus sequence for specific addition of Glc between C3 and C4 of the EGF-like domain, mediated by ER (endoplasmic reticulum)-resident POGLUT2/3 [17]. In some cases, *O*-Glucose can be elongated by xylose residues [18].

O-Fucose can be added on consensus sequences of EGF-like repeats and Thrombospondin Type 1 Repeats (TSRs) in properly folded proteins, following specific action of ER-resident Protein *O*-Fucosyltransferase 1 (POFUT1 or FUT12) [19,20] and Protein *O*-Fucosyltransferase 2 (POFUT2 or FUT13) [21,22] respectively [23]. POFUT1-mediated *O*-Fucosylation occurs on serine and threonine of the following consensus sequence C^2-XXXX-(S/T)-C^3 of an EGF-like domain, as it is the case for human PAMR1 which possesses a potential *O*-Fucosylation site at threonine 249 (Figure 4). This *O*-Fucose can be elongated, in some cases, by successive additions of GlcNAc, Gal and sialic acid to form a sialylated tetrasaccharide *O*-Fucosylglycan [24]. It is well known that *O*-Fucose added by POFUT1 car modulate protein-protein interactions. Indeed, the *O*-Fucosylation of EGF-like repeats within the extracellular domains of NOTCH receptors modulates the receptor–ligand interactions, which are essential for activation of NOTCH signaling [25].

Another *O*-linked glycosylation found in EGF like domains of glycoproteins is *O*-N-Acetylglucosamine (*O*-GlcNAc) added by ER-resident EGF-domain specific *O*-GlcNAc transferase (EOGT). The *O*-GlcNAc added on NOTCH1 by EOGT was shown to promote Notch signaling by enhancing its interaction with mammalian DLL ligands [26]. EOGT mediated *O*-GlcNAc addition occurs on serine or threonine of the consensus sequence

$C^5XXGX(S/T)GXXC^6$ [27] of an EGF-like domain. As shown in Figure 4, the threonine 267 of human PAMR1 exhibiting the sequence $C^5LAGYTGQRC^6$ is thus a potential *O*-GlcNAcylation site. Interestingly, the occupation of this threonine 267 by an *O*-GlcNAc was experimentally demonstrated by mass spectrometry [28].

By Copper-catalyzed Azide-Alkyne Cycloaddition referred to as click chemistry and target mass spectrometry, we demonstrated that mouse PAMR1 (homolog to human PAMR1 isoform 1) was, as expected, composed of a unique triple-modified EGF-like domain with *O*-Glucose, *O*-Fucose and *O*-GlcNAc [29].

The role of all these glycosylations is not known but they could be important for the folding and the secretion of PAMR1 but also for its interactions with other protein partners. It is therefore interesting to know whether these different potential or proven glycosylation sites could be affected in cancer.

Alteration of the Glycosylation Sites of Human PAMR1 in Cancer

BioMuta database (BioMuta v4.0), which is a single-nucleotide variation and disease association database, contains non-synonymous single-nucleotide variations associated with cancer. All alterations of the glycosylation sites of human PAMR1 occurring in cancer, either by change of keys residues (Asn, Ser, Thr) or change of keys residues in consensus sequences, are presented in the following table.

Table I: Point mutations found in cancer, affecting *N*- and *O*-glycosylation sites found in human PAMR1 or key residues in consensus sequences of glycosylation.

Type of glycosylation	Predicted Occupation	Location	Ref. residue	Position	Alt. residue	Variation	Cancer type	Functional predictions
N-glycan	Yes ($\underline{N}^{96}$GS)		Asn	96		none		
O-Glc	Yes (CS$\underline{S}^{241}$SPC)	EGF-like	Ser	241		none		
O-Fuc	Yes (CFHDG$\underline{T}^{249}$C)	EGF-like	Thr	249	Lys	**T249K**	Liver cancer	probably damaging
O-Fuc	Yes (CFHDG$\underline{T}^{249}$C)	EGF-like	Thr	249	Met	**T249M**	Melanoma	probably damaging
O-GalNAc	Never detected in EGF-like	EGF-like	Ser	257		none		
O-GlcNAc	Yes (CXXG$\underline{X}^{267}$GXXC)*	EGF-like	Thr	267		none		
	No	EGF-like	Gly	265	Cys	**G265C**	Uterine cancer	probably damaging
N-glycan	Yes ($\underline{N}^{279}$CS)	Sushi 1	Asn	279		none		
O-GalNAc	Yes (T^{294}, NetOGlyc)	Sushi 1	Thr	294		none		
N-glycan	**No** (N^{316}NS)	Sushi 1	Asn	316		none		
O-GalNAc	Yes (T^{328}, NetOGlyc)	Sushi 1	Thr	328		none		
O-GalNAc	Yes (S^{352}, NetOGlyc)		Ser	352	Leu	**S352L**	Melanoma	probably damaging
O-GalNAc	Yes (S^{366}, NetOGlyc)		Ser	366		none		
O-GalNAc	Yes (S^{376}, NetOGlyc)		Ser	376		none		
O-GalNAc	Yes (S^{380}, NetOGlyc)		Ser	380		none		
O-GalNAc	Yes (S^{386}, NetOGlyc)		Ser	386		none		
O-GalNAc	Yes (T^{389}, NetOGlyc)	Sushi 2	Thr	389		none		
O-GalNAc	Yes (T^{408}, NetOGlyc)	Sushi 2	Thr	408	Ala	**T408A**	Kidney cancer	probably damaging
O-GalNAc	Yes (S^{416}, NetOGlyc)	Sushi 2	Ser	416		none		
O-GalNAc	Yes (S^{424}, NetOGlyc)	Sushi 2	Ser	424		none		
O-GalNAc	Yes (S^{425}, NetOGlyc)	Sushi 2	Ser	425	Arg	**S425R**	Sarcoma	probably damaging
O-GalNAc	Yes (T^{428}, NetOGlyc)	Sushi 2	Thr	428		none		
O-GalNAc	Yes (T^{432}, NetOGlyc)	Sushi 2	Thr	432		none		
O-GalNAc	Yes (S^{436}, NetOGlyc)	Sushi 2	Ser	436		none		
O-GalNAc	Yes (S^{441}, NetOGlyc)	Sushi 2	Ser	441		none		
N-glycan	Yes ($\underline{N}^{451}$IT)	Peptidase S1	Asn	451		none		
	No	Peptidase S1	Thr	453	Ala	**T453A**	Uterine cancer	benign**
N-glycan	Yes ($\underline{N}^{614}$DT)	Peptidase S1	Asn	614		none		
O-GalNAc	Yes (S^{672}, NetOGlyc)	Peptidase S1	Ser	672		none		
*** demonstrated experimentally (Alfaro et al., 2012)**								
**** functional prediction according the change of Thr into Ala**								

There is no variation listed in the Biomuta database concerning the asparagine residues potentially bearing *N*-glycans, namely N^{96}, N^{279}, N^{451} and N^{614}. On the other hand, the T453A mutation causes the loss of the *N*-glycosylation site $\underline{N}^{451}$IT in uterine cancer. This variation is predicted to be benign by BioMuta. However, if we consider that this asparagine $\underline{N}^{451}$, located in the peptidase S1 domain, is really occupied, it is possible that the loss of the *N*-glycan at this position impacts the folding and/or the secretion of the protein or even its activity.

Concerning mucin-type *O*-GalNAc glycosylation, only the sites with scores higher than 0.5, predicted as glycosylated by NetOGlyc, are listed in the table. Occupancy of these *O*-glycosylation sites can change *in vivo*, depending on the cells expressing the protein. In addition, some local regions in a protein are more likely to carry *O*-GalNAc than others. By example, the presence of *O*-GalNAc site is predicted within the EGF-like domain of human PAMR1 at S^{257}. Regarding NOTCH

receptor, which contains a large number of EGF-like repeats (29-36), mucin-type *O*-glycosylation can be present but always outside the EGF-like repeats [15].

The regions most likely to carry this type of modification are destructured and prolin-rich regions such as that between the two Sushi domains (region 345-386) of human PAMR1. This region matches the sequence below and contains 5 serine residues with each of them predicted as an *O*-GalNAc site, 3 proline residues and unpredicted threonine T^{369}.

ACRE**P**KIS^{352}DLVRRRVL**P**MQVQS^{366}RET**P**LHQLYS^{376}AAF S^{380}KQKLQS^{386}

The S352L mutation found in melanoma, probably damaging, could thus lead to the elimination of *O*-GalNAc or extended *O*-GalNAc glycan important for the secretion and/or activity of human PAMR1.

If taking account the nine predicted *O*-GalNAc sites in the Sushi 2 domain and predicted structure of human PAMR1 by Alphafold (Figure 1), seven of them are relevant since located in unstructured regions, without periodic secondary structures including T^{408} and S^{425}, for which there is variation in cancer (T408A in kidney cancer and S425R in sarcoma).

Human PAMR1 also contains an EGF-like domain, which is a protein domain often involved in protein-protein interactions and which potentially bears three *O*-linked monosaccharides (*O*-Glucose, *O*-Fucose and *O*-GlcNAc) like mouse PAMR1 [29] or corresponding extended *O*-glycosylglycans capable of modulating these interactions. The T249K and T249M mutations found in liver cancer and melanoma, respectively, target the potential *O*-Fucosylation site, while the G265C mutation found in uterine cancer affects the consensus sequence for the addition of an *O*-GlcNAc. Besides the amino acid change, the loss of *O*-Fucose or *O*-GlcNAc on the EGF-like domain is probably damaging for PAMR1 function.

Expression of PAMR1 in Normal and Cancer Tissues

Little is known about the expression of PAMR1 in normal and malignant/cancerous tissues, as well as its role in different tissues/cell lines. PAMR1 is a low-tissue-specific protein. It is known to be overexpressed in cervical and endocervical tissues at its mRNA level. It is recorded to be highly present in the serum and gallbladder at its protein level (Gene card database). The analysis of Lo et al, to 27 normal tissues, showed that the highest expression of PAMR1 transcriptome is found in brain, bladder, aorta, and colon tissues, with lower expression in breast and skeletal tissues of both isoforms 1 and 2 [1]. On the other hand, no data are available concerning PAMR1 expression in embryonic and stem cells.

RNA Seq data concerning PAMR1 expression in tumoral tissues from patients *versus* normal ones can be found in the Firebrowse database (Figure 5).

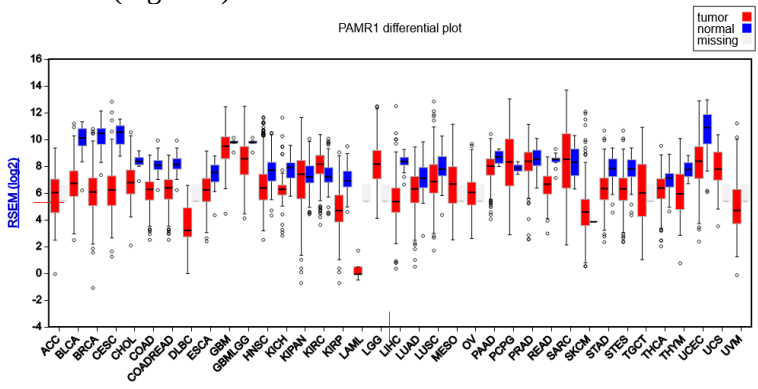

Figure 5: Expression of PAMR1 in normal and tumoral cancer samples from TCGA Firebrowse database (http://firebrowse.org/). PAMR1's expression is downregulated in many cancers such as breast invasive carcinoma (BRCA), Cervical and Endocervical Cancers (CESC), Colon Adenocarcinoma (COAD), Colorectal Adenocarcinoma (COADREAD) and Rectal Adenocarcinoma (READ).

According to data from Firebrowse, PAMR1 is downregulated in tumoral tissues of several cancers including BRCA (Breast invasive carcinoma), CESC (Cervical and Endocervical

carcinoma), LIHC (Liver hepatocellular carcinoma) confirming earlier research on breast [1], cervical [30], hepatocellular [31] cancers, as well as for cutaneous squamous cell carcinoma [32]. Recently, we also confirmed PAMR1 downexpression in colorectal cancer [33], consistent with public data from Firebrowse concerning COAD (Colon Adenocarcinoma), READ (Rectal Adenocarcinoma) and COADREAD (Colorectal Adenocarcinoma).

PAMR1 could be, as well, overexpressed in some/exceptional tumoral tissues such as the case of KIRC (Kidney renal clear cell carcinoma), PCPG (Pheochromocytoma, and Paraganglioma), and SKCM (Skin Cutaneous Melanoma), and also meningioma. On the other hand, data is missing for PAMR1's expression in the following cancers: ACC (Adrenocortical Carcinoma), DLBC (Lymphoid Neoplasm Diffuse Large B-cell Lymphoma), LAML (Acute Myeloid Leukemia), LGG (Brain Lower Grade Glioma), MESO (Mesothelioma), OV (Ovarian Serous Cystadenocarcinoma), TGCT (Testicular Germ cell Tumors), UCS (Uterine Carcinosarcoma), and UVM (Uveal Melanoma). All these differences of PAMR1 expression according to the considered cancer type could be correlated to potentially different roles of this glycoprotein in normal different cell types before their malign transformation.

PAMR1 as a Potential Tumor Suppressor

Recent studies showed a reduced expression of PAMR1 in breast [1] and cervical [30] cancers. Different mechanisms could explain this very low expression of PAMR1 in cancer such as epigenetic silencing. Indeed, PAMR1 was shown to be down-expressed by promoter hypermethylation in breast cancer, such as other tumor-suppressor genes including APC, BRCA1, p16, P21 and TIMP3 [1].

Since a long time, DNA methyltransferase inhibitors such as 5-aza-2' deoxycytidine (also named decitabine [34]) are used for DNA demethylation inducing reactivation of epigenetically silenced tumor suppressor genes. In addition, the use of decitabine was shown to lead to cell cycle arrest (G2/M phase)

and apoptosis in human cancer cells [35]. Due to its anti-cancer properties, decitabine was thus used in cancer chemotherapy in different cancers such as lung cancer [36], leukemia [37] and gastric cancer [38]; sometimes in combination of inhibitors of deacetylation [39]. In gastric cancer, decitabine led to inhibition of tumor cell proliferation and up-regulation of E-cadherin [38].

Due to its recovered expression by decitabine and its suppressor role of cancer cell growth, PAMR1 was regarded as a potential tumor suppressor gene in breast cancer [1]. PAMR1 was also shown to be downregulated in cervical cancer and correlated with favorable prognosis [30]. In this latter study, cervical cancer cell proliferation, migration, and invasion were shown to be increased after PAMR1 knockdown by siRNA in HeLa and Me180 cells and on the contrary decreased following its overexpression. These data, in addition to more recent data on colorectal cancer [33], reinforce the idea that PAMR1 is indeed a tumor suppressor gene.

PAMR1 Inhibits Myc and mTORC1 Signaling Pathways

Cancer's molecular alterations are intricate and induce alteration in numerous signaling pathways. Recently, it was revealed by Yang et al. that PAMR1 could be involved in the suppression of MYC and mTOR signaling pathways [30]. MYC is a proto-oncogene activated among others by the MAPK pathway, which plays a role in favoring proliferation, migration, apoptotic resistance, and angiogenesis. MYC has many properties: it can regulate the transcription of other genes and stabilize mRNA and proteins [40]. When it is positively deregulated, it will activate the transcription of target genes which will have a favorable effect on tumorigenesis and tumor progression [41]. mTOR, a serine/threonine kinase protein, is activated by the PI3K/AKT pathway involved in the same biological processes as the MAPK pathway [30]. mTOR is known also to activate the metastatic cascade in cancers. SIN1 and MLST8 are two subunits of mTORC1 and mTORC2 that promote cell migration and invasion. The regulation of ULK1 by PAMR1, a mTORC1

negative regulator, as well as SIN1 and MLST8, could suppress cell migration and invasion in cervical cancer.

Conclusion

PAMR1 is a secreted multi-domain *N*- and *O*-linked glycoprotein, which was initially discovered as a potential actor in muscle regeneration [2] and later as a potential tumor suppressor in breast cancer, where its expression was epigenetically silenced by promoter hypermethylation [1]. In addition of an anti-proliferative role in breast [1] and colorectal [33] cancer, PAMR1 exhibited a negative effect on migration and invasion of cervical cancer cell lines [30]. Although PAMR1 was able to inhibit Myc and mTORC1 signaling pathways, the precise molecular mechanism by which PAMR1 exerts its action is still unclear. Among its potential roles, PAMR1 might have an anti-protease activity due to the presence a threonine (less nucleophile than serine) at position 665 in its C-terminal trypsin-like domain, instead of serine residue usually found in the catalytic triad at this position. If PAMR1 indeed possesses even weak proteolytic activity, one can wonder about its protein targets.

To exert its specific action on proliferation and other cell properties, PAMR1 might be involved in protein-protein interactions with extracellular and/or membrane protein partners via its CUB and EGF-like domains. By analogy with the SCUBE2 protein, which contains CUB and EGF-like domains and also exhibits anti-tumor activity toward breast cancer cells [42], PAMR1 could interact with a surface membrane protein such as E-cadherin [5] and affect signaling pathways as mentioned above. However, the protein partners of PAMR1 are not known and remain to be discovered depending on the tumor context.

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