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HM1.24/BST-2 is constitutively poly-ubiquitinated at the N-terminal amino acid in the cytoplasmic domain

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ABSTRACT

HM1.24 (also known as BST-2, CD317, and Tetherin) is a type II single-pass transmembrane glycoprotein, which traverses membranes using an N-terminal transmembrane helix and is anchored in membrane lipid rafts via a C-terminal glycosylphosphatidylinositol (GPI). HM1.24 plays a role in diverse cellular functions, including cell signaling, immune modulation, and malignancy. In addition, it also functions as an interferon-induced cellular antiviral restriction factor that inhibits the replication and release of diverse enveloped viruses, and which is counteracted by Vpu, an HIV-1 accessory protein. Vpu induces down-regulation and ubiquitin conjugation to the cytoplasmic domain of HM1.24. However, evidence for ubiquitination site(s) of HM1.24 remains controversial. We demonstrated that HM1.24 is constitutively poly-ubiquitinated at the N-terminal cytoplasmic domain, and that the mutation of all potential ubiquitination sites, including serine, threonine, cysteine, and lysine in the cytoplasmic domain of HM1.24, does not affect the ubiquitination of HM1.24. We further demonstrated that although a GPI anchor is necessary and sufficient for HM1.24 antiviral activities and virion-trapping, the deleted mutant of GPI does not influence the ubiquitination of HM1.24. These results suggest that the lipid raft localization of HM1.24 is not a prerequisite for the ubiquitination. Collectively, our findings demonstrate that the ubiquitination of HM1.24 occurs at the N-terminal amino acid in the cytoplasmic domain and indicate that the constitutive ubiquitination machinery of HM1.24 may differ from the Vpu-induced machinery.

1. Introduction

HM1.24, also known as BST-2, CD317, and Tetherin, is a type II transmembrane protein that is highly expressed on myelocytes and tumor cells derived from B and T cell lymphocytes and is also present in activated lymphocytes [1–4]. In addition to myeloma cells, increased expression of HM1.24 has also been documented in a wide variety of invasive solid tumor cell lines [5], in pancreatic ductal adenocarcinomas [6], and in pancreatic endocrine tumors [7]. HM1.24 has also been also identified as an interferon-induced cellular restriction factor that inhibits the release of enveloped viruses from the cell surface. Since then, much of the research on HM1.24 has been directed towards exploration of its antiviral function.

HM1.24 is composed of an N-terminal cytoplasmic domain followed by a transmembrane domain, a large extracellular domain containing two possible N-glycosylation sites and a coiled-coil domain, and a glycosylphosphatidylinositol (GPI) attached to the C-terminus [8]. Thus, HM1.24 is anchored in lipid rafts at the cell surface via a C-terminal GPI, however, the transmembrane domain near the N-terminus lies outside the lipid rafts [8]. Such a highly unique dual-anchor topology of HM1.24 is critical for its antiviral activity [9].

We have previously shown that HM1.24 localizes to the cell surface and the *trans*-Golgi network and/or recycling endosomes, and is internalized from lipid rafts on the cell surface in a clathrin-dependent manner with a dual tyrosine motif (YxY; x represents any amino acid) [10]. Moreover, a humanized anti-HM1.24 monoclonal antibody (AHM) was rapidly internalized from the cell surface in a clathrin-dependent manner, and the internalized AHM was subsequently delivered to, and degraded in, late endosomes/lysosomes, indicating that part of HM1.24 is also transported to late endosomes/lysosomes, and degraded [11]. A

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previous study demonstrated that a significant fraction of HM1.24 in HeLa cells is constitutively degraded with relatively rapid turnover rates, and which is mediated via ESCRT (endosomal sorting complex required for transport)-dependent sorting steps [12]. The ESCRT machinery is involved in the sorting of ubiquitinated membrane proteins into the intralumenal vesicles of multivesicular endosomes and their lysosomal degradation [13]. Viral protein u (Vpu), a protein encoded by HIV-1, counteracts an antiviral activity of HM1.24, which leads to downregulation of HM1.24 from the cell surface and enhanced ESCRT-mediated lysosomal degradation of HM1.24 [14,15]. Vpu induces ubiquitination and downregulation of HM1.24 [16]. The N-terminal cytoplasmic domain of HM1.24 contains several potential ubiquitination sites, such as two lysines (at positions 18 and 21 from the N-terminus), two serines (positions 3 and 5 from the N-terminus), a threonine (position 4 from the N-terminus), and two cysteines (positions 9 and 20 from the N-terminus) (see Fig. 1). Tokarev et al. demonstrated that mutations of all these potential ubiquitination sites in the cytoplasmic domain of HM1.24 abrogates Vpu-mediated ubiquitination [17]. By contrast, it has been shown that all these potential ubiquitination sites are not involved in Vpu-dependent HM1.24 ubiquitination, suggesting the possibility that tyrosine and/or N-terminus amino acid are ubiquitinated [18]. Thus, the site of HM1.24 that undergoes ubiquitination is still a matter of debate. So far, much of the research on ubiquitination in HM1.24 has been conducted under Vpu expression. In addition to antiviral activity, however, HM1.24 has a wide range of biological activities including cell signaling, immune modulation, and malignancy [16]. Moreover, ubiquitination regulates diverse cellular functions, including protein degradation, cell division, differentiation, protein trafficking, and signal transduction [19]. Therefore, elucidation of the ubiquitination mechanism of HM1.24 in the steady state is expected to lead to a better understanding of the physiological functions of HM1.24. In this study, we aimed to identify the constitutive ubiquitination site of HM1.24.

2. Materials and methods

2.1. Cell culture

HeLa and NIH3T3 cells were cultured in Dulbecco's modified Eagle's medium (GIBCO Invitrogen) supplemented with 10% fetal bovine serum (GIBCO Invitrogen), 2 mM glutamine, and 1% penicillin-streptomycin in humidified 95% air and 5% CO_2 at 37 °C. In transfection experiments, the cells were plated onto 35 mm dishes the day before transfection.

2.2. Antibodies

Rabbit polyclonal antibodies to HM1.24 were raised against glutathione *S*-transferase (GST) fusion protein of the extracellular domain corresponding to amino acid residues 49–162 [10]. Mouse monoclonal antibodies to FLAG (M2) were purchased from Sigma-Aldrich.

2.3. Constructs and transfection

Human HM1.24 cDNA [10] in the mammalian expression vector pcDNA3.1 (Invitrogen, Groningen, the Netherlands) were used as template for the amplification of the DNA fragments by polymerase chain reaction (PCR). PCRs were performed using Pfu DNA polymerase (Toyobo, Osaka, Japan) or YieldAce DNA polymerase (Stratagene, La Jolla, CA, USA) according to manufacturer's instructions. The component parts of each chimeric DNA were joined by the splicing of overlap extension (SOE) method, as previously described [20]. All deletion mutants of HM1.24 were also constructed by SOE method, as previously

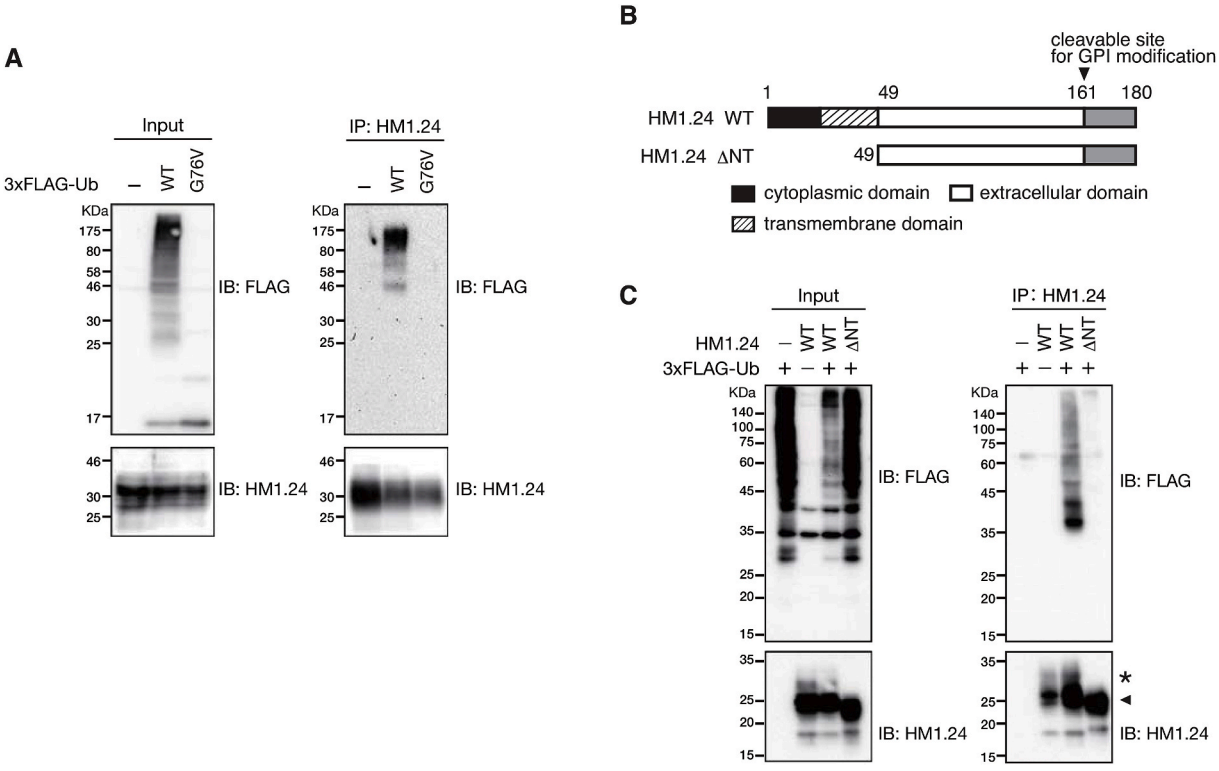

Fig. 1. Ubiquitination of HM1.24. (A) HeLa cells were transiently transfected with 3xFLAG-Ub or 3xFLAG-UbG76V expression vector. Cells were lysed 36h post-transfection, and HM1.24 was immunoprecipitated using anti-HM1.24 antibody. Ubiquitination of HM1.24 was assessed by Western blot analysis of the immunoprecipitates (IP) with anti-FLAG antibody. Western blot analysis of cell lysates (Input) with anti-HM1.24 or anti-FLAG antibody was performed to assess the levels of expressions of HM1.24 and ubiquitinated HM1.24. (B) Schematic representation of the N-terminal cytoplasmic and transmembrane domain-deletion mutant (HM1.24 ΔNT). (C) NIH3T3 cells were co-transfected with 3xFLAG expression vector and HM1.24 WT, HM1.24 ΔNT, or empty vector. Ubiquitination of HM1.24 mutants was determined as described in the legend to Fig. 1A. Asterisk and arrowhead represent the mature form of WT and ΔNT, respectively.

described [10]. Other mutants of HM1.24 were made using the QuikChange site-directed mutagenesis kit (Stratagene, Cedar Creek, TX). All HM1.24 constructs were ligated within BamHI and Xba I sites in the mammalian expression vector pcDNA3.1. Transfections were carried out with FuGENE6 (Roche Molecular Biochemicals) according to the manufacturer's instructions. After 36 h, the cells were used for immunoprecipitation or pull-down experiments.

2.4. Immunoprecipitations

NIH3T3 cells were co-transfected with wild-type (WT) or mutants HM1.24 and 3xFLAG-ubiquitin (Ub) or 3xFLAG-UbG76V. 36 h after transfection, cells were harvested with SDS solution [10 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 1% SDS] and boiled for 5 min. Cell extracts were the diluted 10-fold with lysis buffer [50 mM Tris-HCl, pH 7.5, containing 150 mM NaCl, 5 mM EDTA, and 1% (v/v) Triton X-100], supplemented with a protease inhibitors mixture containing E64d, leupeptin, and pepstatin A (Peptide Institute) and 20 mM N-ethylmaleimide (Calbiochem) to minimize deubiquitination during extract preparation. Cell lysates were centrifuged for 10 min at 10,000×*g*, and supernatants were mixed with an antibody to the HM1.24 bound to protein A-Sepharose (Amersham Biosciences). Immunoprecipitates were subjected to SDS-polyacrylamide gel electrophoresis and followed by immunoblotting.

2.5. western blotting

Cell lysates and immunoprecipitates were subjected to SDS-polyacrylamide gel electrophoresis under reducing conditions. Immunoblot analysis was performed as previously described [21].

3. Results

3.1. HM1.24 is ubiquitinated at a N-terminal cytoplasmic domain

HeLa cells that are constitutively expressing HM1.24 were transfected with a plasmid encoding 3xFLAG-Ub or 3xFLAG-Ub G76V, a mutant which lacks conjugation activity. HM1.24 was immunoprecipitated from the cell lysates with an anti-HM1.24 antibody and subjected to Western blotting with anti-FLAG antibody to detect ubiquitinated HM1.24. Lipid raft-associated proteins have the characteristic of resistance to solubilization by nonionic detergent, such as Triton X-100 [22]. We have previously demonstrated that effective solubilization of HM1.24 was achieved with 1% SDS (10). In this study, therefore, cells were lysed by boiling in 1% SDS and then HM1.24 was immunoprecipitated. This procedure enables not only effective solubilization of HM1.24 from the membrane, but also dissociation of other Ub-conjugated proteins noncovalently associated with HM1.24. Therefore, it is thought that most, if not all, of FLAG-tagged Ub-positive bands detected in immunoprecipitates of HM1.24 are derived from ubiquitinated HM1.24. As shown in Fig. 1A, although HM1.24 revealed a molecular mass of approximately 30–36 kDa (Fig. 1C), ubiquitinated forms of HM1.24 were observed as high-molecular-weight smears, ranging from 48 to 200 kDa, in the cells expressed 3xFLAG-Ub, but not 3xFLAG-Ub G76V, thereby indicating that HM1.24 is polyubiquitinated via the carboxy group of the C-terminal glycine residue of Ub, which is essential for generating a covalent isopeptide bond with substrate [23].

We next examined whether the N-terminal cytoplasmic domain of HM1.24 is ubiquitinated. To this end, both the N-terminal cytoplasmic tail and the subsequent transmembrane domain-deleted mutant (HM1.24ΔNT), illustrated in Fig. 1B, was transiently co-expressed with 3xFLAG-Ub in NIH3T3 cells and immunoprecipitated from extracts using the anti-HM1.24 antibody. We have previously demonstrated that HM1.24ΔNT mutant exclusively localizes as a membrane-associated form on the cell surface (10). HM1.24 WT expressed in NIH3T3 was observed as a mature form, with broad bands with molecular masses ranging from 26 to 30 kDa and a 23 kDa precursor form, which represent the mature and precursor forms, respectively [17]. The observed difference in molecular weight of HM1.24 expressing endogenously in HeLa cells and exogenously in NIH3T3 cells is considered to be due to differences in processing of asparagine-linked oligosaccharides [17], and also may reflect differences in the ubiquitination patterns of HM1.24 observed between HeLa cells (Fig. 1A) and NIH3T3 (Fig. 1C). As shown in Fig. 1C, a smear of bands migrating similar to the ubiquitinated forms of HM1.24 expressing in HeLa cells was observed when HM1.24 WT was transiently expressed in NIH3T3 cells. On the other hand, HM1.24ΔNT expressing in NIH3T3 cells was ~5 kDa smaller than WT by deletion of the cytoplasmic and transmembrane domains, and was not ubiquitinated (Fig. 1C). These results indicate that HM 1.24 is polyubiquitinated at the N-terminal cytoplasmic region and/or transmembrane region.

Since the transmembrane domain also contains a conventional Ub acceptor lysine residue (position 47), we constructed a mutant, HM1.24 (K47R), in which the lysine residue within the transmembrane region was replaced with an arginine residue (Fig. 2A). HM1.24 K47R was transiently co-expressed with 3xFLAG-Ub in NIH3T3 cells and immunoprecipitated with anti-HM1.24 antibody. Subsequent Western blot analysis using anti-FLAG antibody showed that the HM1.24 (K47R) mutant was also found to undergo ubiquitination (Fig. 2B). These results therefore suggest that HM1.24 is polyubiquitinated in the N-terminal cytoplasmic domain.

3.2. Ubiquitin is conjugated to the N-terminal amino acid of HM1.24

The site within the N-terminal cytoplasmic domain of HM1.24 that undergoes ubiquitination still remains controversial. We sought to determine the ubiquitination site within the N-terminal cytoplasmic region of HM1.24. In addition to conventional Ub acceptor, lysine, Ub can be also conjugated to cysteine and serine or threonine residues [24,25]. To examine involvement of these potential ubiquitination sites, we created two mutants; HM1.24ΔN deleted the N-terminus 12 amino acids including two serines, a threonine, and a cysteine, and HM1.24K18, 21R/C20A substituted two lysines (positions 18 and 21) and a cysteine (20) with arginines and an alanine, respectively (Fig. 2C). As shown in Fig. 2D, ubiquitination was detected in both HM1.24ΔN and HM1.24K18,21R/C20A as in WT. These results suggest that lysine, serine, threonine, and cysteine residues within the N-terminal cytoplasmic domain of HM1.24 do not participate in ubiquitin-conjugation to HM1.24.

3.3. GPI anchoring of HM1.24 is not a prerequisite for its ubiquitination

We have previously shown that the C-terminal GPI anchor of HM1.24 is the predominant domain that determines localization in lipid raft [10]. However, GPI-anchor-deleted mutant (HM1.24ΔGPI) had little influence on the ubiquitination (Fig. 3). Thus, these results indicate that localization of HM1.24 in lipid raft is dispensable for the ubiquitin-conjugation. Conversely, HM1.24ΔGPI was mainly detected as a 30 kDa mature form, thereby demonstrating that HM1.24ΔGPI is more effectively processed to mature form, compared to WT. Moreover, since HM1.24ΔGPI was also shown to undergo ubiquitination in a similar manner to WT (Fig. 1C), we further created mutants; deleted or substituted ubiquitination potential amino acids present in the cytoplasmic and transmembrane domains of HM1.24ΔGPI to analyze the ubiquitination site of HM1.24, as illustrated in Fig. 3A. The results indicate that all ubiquitination potential amino acids present in the N-terminal cytoplasmic and the transmembrane domains are not involved in the ubiquitination of HM1.24. Finally, we produced the mutant HM1.24ΔNΔGPI K18,21R/C20A/T45A/K47R, which combined all the deletions and mutations of potential ubiquitination sites (Fig. 4A) and evaluated its ubiquitination by comparison with HM1.24ΔGPI and HM1.24ΔNΔGPI. In cells co-expressed each mutant with 3xFLAG-Ub,

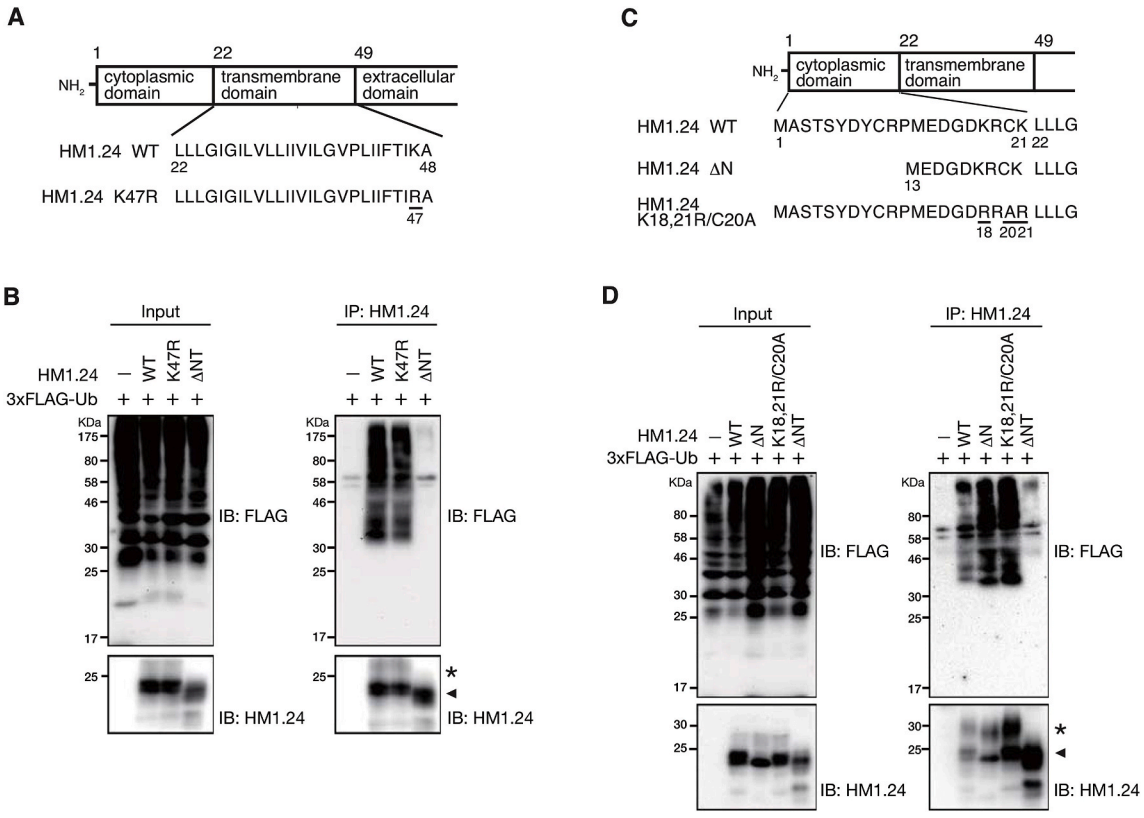

Fig. 2. Ubiquitination of HM1.24 occurs at the N-terminal amino acid. (A) Schematic representation of the N-terminal 12 amino acids-deleted mutant (HM1.24ΔN), and lysines-to-arginines and cysteine-to-alanine mutations introduced in the cytosolic domains of HM1.24. Amino acid number and sequence abbreviated in single letter code from start to end of each domain, and substituted amino acids are shown on bottom of each sequence. (B) NIH3T3 cells were co-transfected with 3xFLAG expression vector and HM1.24 WT, as well as each mutant, or empty vector. Ubiquitination of HM1.24 mutants was determined as described in the legend to Fig. 1A. Asterisk and arrowhead represent the mature form of WT and K47R and ΔNT, respectively. (C) Schematic representation of the lysine-to-arginine mutation introduced in the transmembrane domains of HM1.24 to generate the HM1.24 K47R construct. (D) Schematic depiction of the domain structure of LGP85 and LGP107 chimeras. Each domain of LGP85 and LGP107 is shown in red and white, respectively. The nomenclature indicates as follows: N, N-terminal signal sequence; L, luminal domain; T, transmembrane domain near to the C-terminus; C, C-terminal cytoplasmic tail. NIH3T3 cells were co-transfected with 3xFLAG expression vector and each HM1.24 WT, K47R mutant or empty vector. Ubiquitination of HM1.24 chimeras was determined as described in the legend to Fig. 1A. Asterisk and arrowhead represent the mature form of WT, DNT, and K18,21R/C20A and ΔNT, respectively.

ubiquitination was observed in all mutants (Fig. 4B). These results were further corroborated by reciprocal coimmunoprecipitation experiments, using antibodies against 3xFLAG (Fig. 4C). In particular, the result that the mutant of HM1.24ΔNΔGPI K18,21R/C20A/T45A/K47R was ubiquitinated in spite of the absence of all potential ubiquitination sites supports our conclusion that HM1.24 is ubiquitinated at the N-terminal amino acid of the cytoplasmic domain. It should be noted that in all cells expressing each mutant, the non-ubiquitinated form was also co-immunoprecipitated by the anti-FLAG antibody (see asterisks in Fig. 4C). As HM1.24 exists as a disulfide linked dimer, which is mediated by three cysteine residues in the extracellular domain [26], this result suggests that; at least part of non-ubiquitinated or deubiquitinated HM1.24 forms dimers with the ubiquitinated HM1.24, and that lipid raft localization by GPI-anchor addition is not essential for dimer formation of HM1.24.

4. Discussion

In the present study we show that HM1.24 undergoes constitutive ubiquitination at the N-terminal cytoplasmic domain. We further mapped the site within the cytoplasmic domain that is available for the ubiquitination of HM1.24 by substituting and/or deleting potential ubiquitination sites. Although HM1.24 has many potential ubiquitination sites, including two serines, one threonine, two cysteines, and two lysines, within the cytoplasmic domain, our results show that mutation or deletion of all of these sites individually or in combination had no effect on the ubiquitination of HM1.24. Therefore, we conclude that Ub conjugation to the cytoplasmic domain of HM1.24 occurs at only one restricted site, the N-terminus amino acid. We further demonstrated that although a GPI anchor is necessary and sufficient for HM1.24 antiviral activities and virion-trapping [9], the deleted mutant of GPI did not influence the ubiquitination of HM1.24. This suggests that the lipid raft localization of HM1.24 is not a prerequisite for the ubiquitination.

To date, most of the studies concerning the ubiquitination of HM1.24 have been performed using cells expressing Vpu. The ubiquitination sites of HM1.24 induced by Vpu indicate that serine and threonine residues [17] or tyrosines, cysteines, or the N-terminal methionine residue [18] could function as potential Ub acceptor sites in the cytoplasmic domain of HM1.24. On the other hand, like Vpu, Kaposi's sarcoma-associated herpesvirus (KSHV) also counteracts HM1.24 [27]. KSHV encodes two immunomodulatory MARCH E3 Ub ligases, K3 and K5 [20]. K5 specifically ubiquitinates a lysine residue at position 18 in the cytoplasmic domain of HM1.24 [27]. These differences in ubiquitination sites of HM1.24 might reflect the action of an alternative Ub conjugation mechanism induced by virus infection. Moreover, viruses that have evolved strategies to counteract the antivirus function may ubiquitinate HM1.24 via a different ubiquitination mechanism from that under constitutive conditions. Indeed, it has been demonstrated that Vpu of human HIV-1 does not hijack the constitutive ubiquitination machinery of HM1.24 [12].

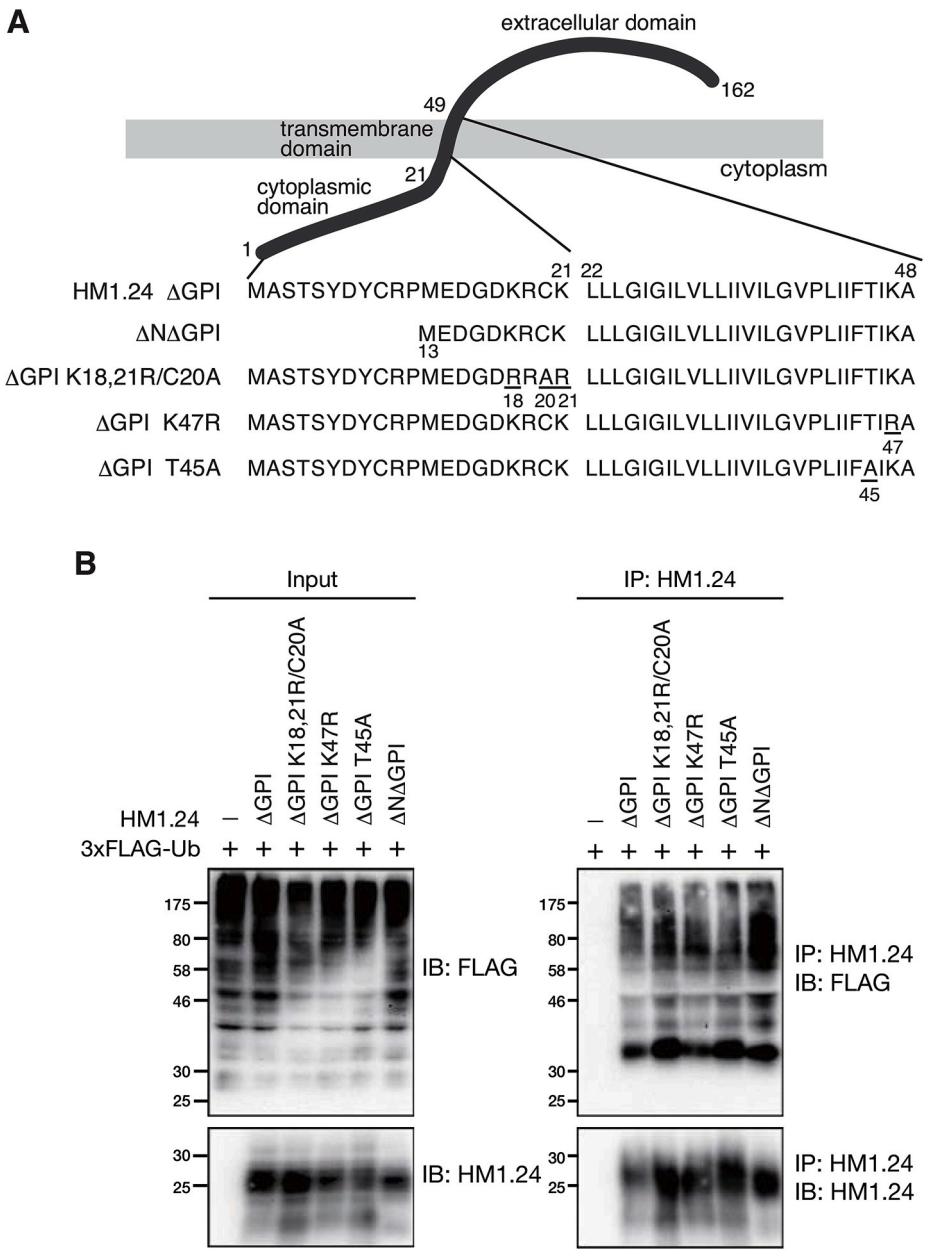

Fig. 3. Ubiquitination of the GPI anchor-deleted HM1.24. (A) Schematic of the mutations introduced in the cytosolic and transmembrane domains of HM1.24ΔGPI. (B) NIH3T3 cells were co-transfected with 3xFLAG expression vector and HM1.24ΔGPI, each mutant illustrated in (A), or empty vector. Ubiquitination of HM1.24 mutants was determined as described in the legend to Fig. 1A.

In addition to the potential ubiquitination sites mentioned above, the cytoplasmic domain of HM1.24 contains two methionine residues: one is an N-terminus and the other is positioned 13 from the N-terminus. Indeed, HM1.24 is expressed as a long or short isoform that lacks 12 amino acids from the first methionine [28]. Our data show that HM1.24ΔN, the short isoform, is ubiquitinated as well as the long isoform (WT). Moreover, mutation of all potential ubiquitination sites in the cytoplasmic domain of HM1.24ΔN did not affect its ubiquitination. Collectively, these results suggest that Ub is conjugated to the ε-NH_2 group of the N-terminal amino acid of HM1.24. Although the two isoforms have distinct sensitivities to vial antagonists and activation of NF-κB signaling [28], our results indicate that such different biological functions between two isoforms cannot be explained by the ubiquitination alone.

We cannot completely rule out the possibility that mutation of one or more potential Ub acceptor sites may enable Ub attachment to other sites, as suggested by previous studies [29]. Interestingly, using an monoclonal antibody against the C-terminal 13 amino acids of Ub left on ubiquitinated peptides after LysC digestion, Akimov et al. have recently identified approximately 63,000 unique ubiquitination sites mapped on 9200 distinct proteins [30]. It should be noted that among them 104 proteins underwent N-terminal ubiquitination, in which the ubiquitinated peptide was derived from the N-terminally ubiquitinated long isoform, but not the short, of HM1.24. In this case, the ubiquitin was attached to the first methionine of the long form. Altogether, our findings demonstrate that HM1.24 is constitutively polyubiquitinated at the N-terminal methionine residues.

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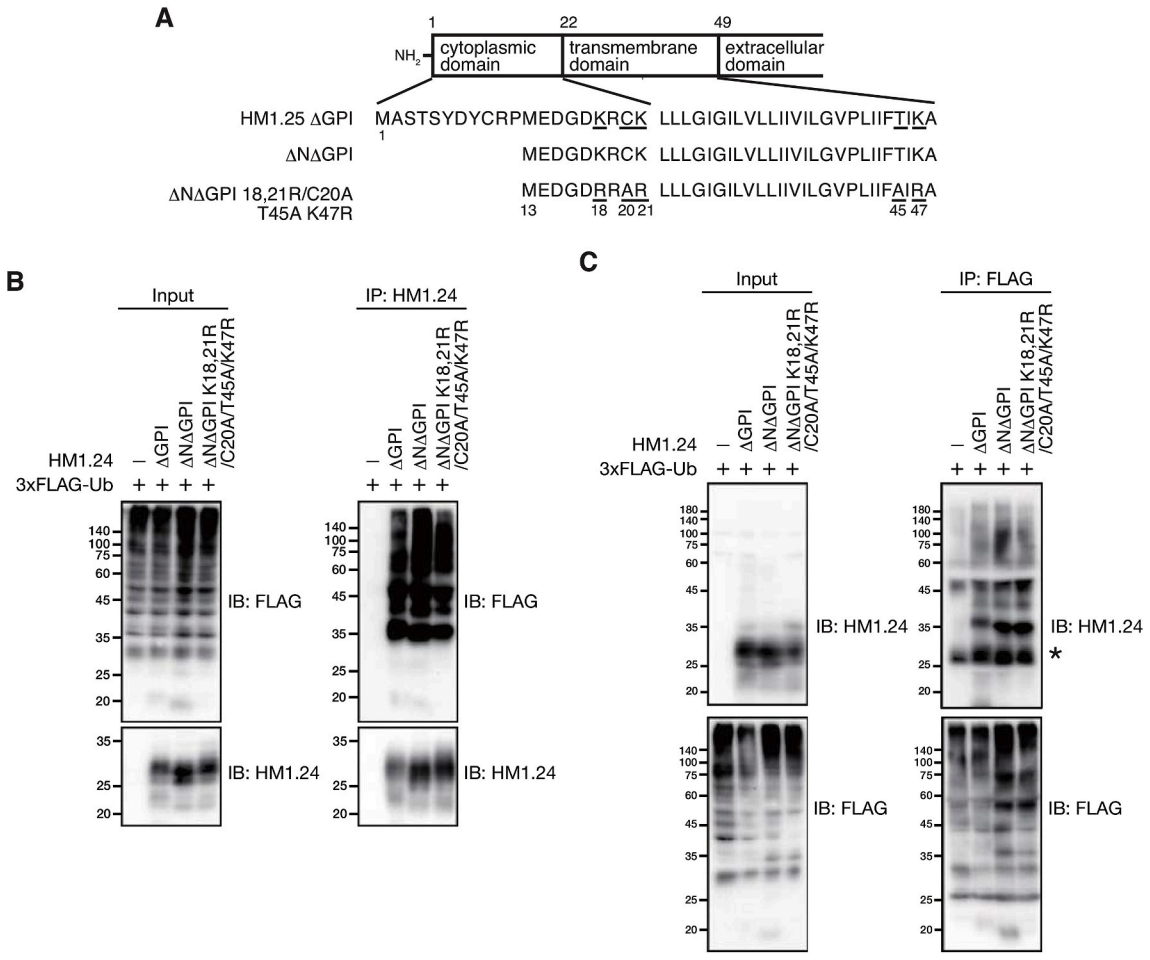

Fig. 4. N-terminus ubiquitination of GPI-deleted HM1.24 mutant. (A) Schematic representation of the mutant which combined all of deletions and mutations of potential ubiquitination sites in the cytoplasmic and transmembrane domains of HM1.24ΔGPI to generate the HM1.24. (B, C) NIH3T3 cells were co-transfected with 3xFLAG-Ub expression vector and HM1.24ΔGPI, HM1.24ΔNΔGPI, HM1.24ΔNΔGPI K18,21R/C20A/T45A/K47R, or empty vector. (B) Cells were lysed 36h post-transfection, and HM1.24 was immunoprecipitated using anti-HM1.24 antibody. Ubiquitination of HM1.24 was assessed by Western blot analysis of the immunoprecipitates (IP) with anti-FLAG antibody. (C) Cells were lysed 36h post-transfection, and ubiquitinated proteins were immunoprecipitated using the anti-FLAG antibody. Ubiquitination of HM1.24 was assessed by Western blot analysis of the immunoprecipitates (IP) with anti-HM1.24 antibody. Western blot analysis of cell lysates (Input) with anti-HM1.24 or anti-FLAG antibody was performed to assess the levels of expression of HM1.24 and ubiquitinated HM1.24. Asterisk represents non-ubiquitinated form of each HM1.24 mutant.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Keiko Fujimoto: Conceptualization, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Sanae Nakashima:** Investigation, Methodology. **Shotaro Uchida:** Investigation, Methodology. **Riham N.S. Amen:** Investigation, Methodology. **Yuji Ishii:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. **Yuko Hirota:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. **Yoshitaka Tanaka:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition.

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References

[1] T. Goto, S.J. Kennel, M. Abe, M. Takishita, M. Kosaka, A. Solomon, S. Saito, A novel membrane antigen selectively expressed on terminally differentiated human B cells, Blood 84 (1994) 1922–1930.

[2] S. Ozaki, M. Kosaka, S. Wakatsuki, M. Abe, Y. Koishihara, T. Matsumoto, Immunotherapy of multiple myeloma with a monoclonal antibody directed against a plasma cell-specific antigen, HM1 24 (1997) 3179–3186. Blood 90.

[3] J. Ishikawa, T. Kaisho, H. Tomizawa, B.O. Lee, Y. Kobune, J. Inazawa, K. Oritani, M. Itoh, T. Ochi, K. Ishihara, Molecular cloning and chromosomal mapping of a bone marrow stromal cell surface gene, BST2, that may be involved in pre-B-cell growth, Genomics 26 (1995) 527–534, https://doi.org/10.1016/0888-7543(95)80171-h.

[4] M. Vidal-Laliena, X. Romero, S. March, V. Requena, J. Petriz, P. Engel, Characterization of antibodies submitted to the B cell section of the 8th Human Leukocyte Differentiation Antigens Workshop by flow cytometry and immunohistochemistry, Cell. Immunol. 236 (2005) 6–16, https://doi.org/10.1016/j.cellimm.2005.08.002.

[5] J. Walter-Yohrling, X. Cao, M. Callahan, W. Weber, S. Morgenbesser, S.L. Madden, C. Wang, B.A. Teicher, Identification of genes expressed in malignant cells that promote invasion, Canc. Res. 63 (2003) 8939–8947.
[6] R. Grützmann, H. Boriss, O. Ammerpohl, J. Lüttges, H. Kalthoff, H.K. Schackert, G. Klöppel, H.D. Saeger, C. Pilarsky, Meta-analysis of microarray data on pancreatic cancer defines a set of commonly dysregulated genes, Oncogene 24 (2005) 5079–5088, https://doi.org/10.1038/sj.onc.1208696.
[7] G. Capurso, S. Lattimore, T. Crnogorac-Jurcevic, F. Panzuto, M. Milione, V. Bhakta, N. Campanini, S.M. Swift, C. Bordi, G. Delle Fave, N.R. Lemoine, Gene expression profiles of progressive pancreatic endocrine tumours and their liver metastases reveal potential novel markers and therapeutic targets, Endocr. Relat. Canc. 13 (2006) 541–558, https://doi.org/10.1677/erc.1.01153.
[8] S. Kupzig, V. Korolchuk, R. Rollason, A. Sugden, A. Wilde, G. Banting, Bst-2/HM1.24 is a raft-associated apical membrane protein with an unusual topology, Traffic 4 (2003) 694–709, https://doi.org/10.1034/j.1600-0854.2003.00129.x.
[9] D. Perez-Caballero, T. Zang, A. Ebrahimi, M.W. McNatt, D.A. Gregory, M. C. Johnson, P.D. Bieniasz, Tetherin inhibits HIV-1 release by directly tethering virions to cells, Cell 139 (2009) 499–511, https://doi.org/10.1016/j.cell.2009.08.039.
[10] N. Masuyama, T. Kuronita, R. Tanaka, T. Muto, Y. Hirota, A. Takigawa, H. Fujita, Y. Aso, J. Amano, Y. Tanaka, HM1.24 is internalized from lipid rafts by clathrin-mediated endocytosis through interaction with alpha-adaptin, J. Biol. Chem. 284 (2009) 15927–15941, https://doi.org/10.1074/jbc.M109.005124.
[11] J. Amano, N. Masuyama, Y. Hirota, Y. Tanaka, Y. Igawa, R. Shiokawa, T. Okutani, T. Miyayama, M. Nanami, M. Ishigai, Antigen-dependent internalization is related to rapid elimination from plasma of humanized anti-HM1.24 monoclonal antibody, Drug Metab. Dispos. 38 (2010) 2339–2346, https://doi.org/10.1124/dmd.110.035709.
[12] N. Roy, G. Pacini, C. Berlioz-Torrent, K. Janvier, Characterization of E3 ligases involved in lysosomal sorting of the HIV-1 restriction factor BST2, J. Cell Sci. 130 (2017) 1596–1611, https://doi.org/10.1242/jcs.195412.
[13] R.C. Piper, J.P. Luzio, Ubiquitin-dependent sorting of integral membrane proteins for degradation in lysosomes, Curr. Opin. Cell Biol. 19 (2007) 459–465, https://doi.org/10.1016/j.ceb.2007.07.002.
[14] K. Janvier, A. Pelchen-Matthews, J.B. Renaud, M. Caillet, M. Marsh, C. Berlioz-Torrent, The ESCRT-0 component HRS is required for HIV-1 Vpu-mediated BST-2/tetherin down-regulation, PLoS Pathog. 7 (2011), e1001265, https://doi.org/10.1371/journal.ppat.1001265.
[15] T. Kueck, S.J. Neil, A cytoplasmic tail determinant in HIV-1 Vpu mediates targeting of tetherin for endosomal degradation and counteracts interferon-induced restriction, PLoS Pathog. 8 (2012), e1002609, https://doi.org/10.1371/journal.ppat.1002609.
[16] A. Tokarev, M. Skasko, K. Fitzpatrick, J. Guatelli, Antiviral activity of the interferon-induced cellular protein BST-2/tetherin, AIDS Res. Hum. Retrovir. 25 (2009) 1197–1210, https://doi.org/10.1089/aid.2009.0253.
[17] A.A. Tokarev, J. Munguia, J.C. Guatelli, Serine-threonine ubiquitination mediates downregulation of BST-2/tetherin and relief of restricted virion release by HIV-1 Vpu, J. Virol. 85 (2011) 51–63, https://doi.org/10.1128/JVI.01795-10.
[18] J.K. Gustin, J.L. Douglas, Y. Bai, A.V. Moses, Ubiquitination of BST-2 protein by HIV-1 Vpu protein does not require lysine, serine, or threonine residues within the BST-2 cytoplasmic domain, J. Biol. Chem. 287 (2012) 14837–14850, https://doi.org/10.1074/jbc.M112.349928.
[19] C.M. Pickart, M.J. Eddins, Ubiquitin: structures, functions, mechanisms, Biochim. Biophys. Acta 1695 (2004) 55–72, https://doi.org/10.1016/j.bbamcr.2004.09.019.
[20] K. Fujimoto, S. Uchida, R.N.S. Amen, Y. Ishii, Y. Tanaka, Y. Hirota, Lysosomal integral membrane protein LGP85 (LIMP-2) is ubiquitinated at the N-terminal cytoplasmic domain, Biochem. Biophys. Res. Commun. 524 (2020) 424–430, https://doi.org/10.1016/j.bbrc.2020.01.095.
[21] K. Fujimoto, H. Ida, Y. Hirota, M. Ishigai, J. Amano, Y. Tanaka, Intracellular dynamics and fate of a humanized anti-interleukin-6 receptor monoclonal antibody, tocilizumab, Mol. Pharmacol. 88 (2015) 660–675, https://doi.org/10.1124/mol.115.099184.
[22] K. Simons, E. Ikonen, Functional rafts in cell membranes, Nature 387 (1997) 569–572, https://doi.org/10.1038/42408.
[23] C. Grabbe, K. Husnjak, I. Dikic, The spatial and temporal organization of ubiquitin networks, Nat. Rev. Mol. Cell Biol. 12 (2011) 295–307, https://doi.org/10.1038/nrm3099.
[24] K. Cadwell, L. Coscoy, Ubiquitination on nonlysine residues by a viral E3 ubiquitin ligase, Science 309 (2005) 127–130, https://doi.org/10.1126/science.1110340.
[25] X. Wang, R.A. Herr, W.J. Chua, L. Lybarger, E.J. Wiertz, T.H. Hansen, Ubiquitination of serine, threonine, or lysine residues on the cytoplasmic tail can induce ERAD of MHC-I by viral E3 ligase mK3, J. Cell Biol. 177 (2007) 613–624, https://doi.org/10.1083/jcb.200611063.
[26] A. Hinz, N. Miguet, G. Natrajan, Y. Usami, H. Yamanaka, P. Renesto, B. Hartlieb, A. A. McCarthy, J.P. Simorre, H. Göttlinger, W. Weissenhorn, Structural basis of HIV-1 tethering to membranes by the BST-2/tetherin ectodomain, Cell Host Microbe 7 (2010) 314–323, https://doi.org/10.1016/j.chom.2010.03.005.
[27] C. Pardieu, R. Vigan, S.J. Wilson, A. Calvi, T. Zang, P. Bieniasz, P. Kellam, G. J. Towers, S.J. Neil, The RING-CH ligase K5 antagonizes restriction of KSHV and HIV-1 particle release by mediating ubiquitin-dependent endosomal degradation of tetherin, PLoS Pathog. 6 (2010), e1000843, https://doi.org/10.1371/journal.ppat.1000843.
[28] L.J. Cocka, P. Bates, Identification of alternatively translated Tetherin isoforms with differing antiviral and signaling activities, PLoS Pathog. 8 (2012), e1002931, https://doi.org/10.1371/journal.ppat.1002931.
[29] A. Bachmair, A. Varshavsky, The degradation signal in a short-lived protein, Cell 56 (1989) 1019–1032, https://doi.org/10.1016/0092-8674(89)90635-1.
[30] V. Akimov, I. Barrio-Hernandez, S.V.F. Hansen, P. Hallenborg, A.K. Pedersen, D. B. Bekker-Jensen, M. Puglia, S.D.K. Christensen, J.T. Vanselow, M.M. Nielsen, I. Kratchmarova, C.D. Kelstrup, J.V. Olsen, B. Blagoev, UbiSite approach for comprehensive mapping of lysine and N-terminal ubiquitination sites, Nat. Struct. Mol. Biol. 25 (2018) 631–640, https://doi.org/10.1038/s41594-018-0084-y.