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Viral and Cellular MARCH Ubiquitin Ligases and Cancer

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Abstract

Covalent conjugation of proteins with ubiquitin is one the most important post translational modifications because it controls intracellular protein trafficking typically resulting in protein degradation. Frequently ubiquitinated proteins are targeted to the proteasome for degradation in the cytosol. However, ubiquitinated membrane bound proteins can also be targeted for endocytosis and degradation in the lysosome. Ubiquitin-dependent degradation pathways have clear cancer relevance due to their integral involvement in protein quality control, regulation of immune responses, signal transduction, and cell cycle regulation. In spite of its fundamental importance, little is known regarding how proteins are specifically identified for ubiquitin-dependent degradation. In this article we review a newly discovered family of viral and cellular ubiquitin ligases called MARCH proteins. Recent studies of MARCH proteins define new paradigms showing how ubiquitin E3 ligases determine the intracellular location and fate of proteins.

Keywords

Degradation pathways; ubiquitination; intracellular trafficking; immune evasion; γ herpesvirus-68; Kaposi's sarcoma-associated herpes virus

1.Substrate ubiquitination

It has recently become appreciated that subtleties regarding the location, linkage and extent of protein ubiquitination can be key determinants controlling the fate of protein [1,2,3]. However, the basic process of ubiquitination has been highly conserved in evolution. Ubiquitination of substrates requires the catalytic cascade of three classes of proteins E1, E2, and E3 that interact sequentially to transfer ubiquitin moieties to substrates [4]. The E1, termed the ubiquitin-activating enzyme, forms a thioester bond between its active site cysteine and the carboxyl-terminal glycine of ubiquitin in an ATP-dependent manner. Although thought for many years that there was only one mammalian E1, a second E1 was recently discovered [5]. The ubiquitin is then transferred from the E1 to a cysteine residue of an ubiquitin-conjugating enzyme or E2. There are more than 30 mammalian E2s. Finally an E3 ubiquitin ligase facilitates the transfer of ubiquitin typically to an ϵ -NH₂ group of a lysine residue of the substrate thus making an isopeptide bond. There are hundreds of mammalian E3s, most of which contain RING domains composed of characteristically spaced conserved cysteine and histidine residues that form a zinc binding 'cross-brace structure' [6]. RING type E3s are non-enzymatic and are thought to facilitate the transfer of ubiquitin from the charged E2 to their substrates by bringing them in

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close proximity to each other [7]. Interestingly, there are three distinct subclasses of RING type E3s. These are the RING-HC (C3HC4), RING-H2 (C3H2C3), and RING-CH (C4HC3) that are distinguished from each other by the order of their metal binding cysteine and histidine residues within their RING domains. Ligases with RING-CH domains and multiple transmembrane domains are typically called MARCH (membrane-associated RING-CH) proteins, and are the topic of this review.

Although extensively and intensively studied, fundamental questions remain regarding the ubiquitination of proteins. For example, three outstanding questions are: 1) how are substrates specifically detected? 2) what determines which substrate residues get ubiquitinated, and 3) how are ubiquitin moieties transferred and assembled on the substrates? This last question is of particular relevance because linkage and extent (mono- vs polyubiquitination) can determine the fate of the substrate. For instance, monoubiquitination (one ubiquitin moiety attached to a single lysine residue of the substrate) is required for internalization of plasma membrane proteins or for the regulatory processes without a degradation outcome [8]. In contrast, polyubiquitination of at least 4 ubiquitin moieties via K48 linkage (conjugation of an ubiquitin chain formed through lysine48 of ubiquitin) frequently leads to degradation of the substrate in proteasome [9]. In mammalian cells, most short-lived proteins as well as ERAD substrates are turned over by this mechanism. However, membrane proteins can also be polyubiquitinated via K63 linkages or multiubiquitinated (a single ubiquitin moiety attached to multiple lysine residues on the substrate), which usually directs them to the late endosome/multivesicular body (MVB) for ultimate degradation in the lysosome [8,1].

2. Cancer relevance of ubiquitin-dependent degradation pathways

Ubiquitin-mediated degradation pathways are of clear relevance to cancer as discussed in recent reviews [10,11,12]. Furthermore, abnormal regulation of E3 ligases contribute to cancer development, which make E3 ligases extremely attractive targets for cancer therapy [13,14]. Recent advances in characterizing several ER resident E3 ligases are noteworthy here.

HRD1 is a ubiquitin ligase implicated in ER associated degradation (ERAD) in yeast and mammals [15,16,17]. Recently studies have also shown that HRD1 negatively regulates the function of tumor suppressor protein p53 by sequestering it in the cytoplasm and targeting it for ERAD [18]. Furthermore, HRD1 can be upregulated upon ER stress. Although the details of the molecular basis of this regulation remain unclear, these findings revealed a novel crosstalk between ERAD and p53-induced apoptosis under ER stress. **gp78** is another well-documented ER-resident E3 ligase implicated in ERAD in mammals [19]. Notably, gp78 was initially defined as a tumor autocrine motility factor receptor (AMFR) in the cytoplasmic membrane based on the observation that its expression correlates with the metastatic potential of a variety of tumors [20,21]. Very recently it was demonstrated that gp78 functions as an ubiquitin ligase that promotes human sarcoma metastasis by targeting metastasis suppressor KAI1 (CD82) for degradation [22]. **Rfp2** is a third ER-resident E3 ligase involved in ERAD and it has downregulated expression in various malignancies including chronic lymphocytic leukemia, myeloma, lymphoma and several solid tumors [23]. Thus identification of the physiological substrates of Rfp2 should be highly insightful. **TCR8** was originally identified as a tumor suppressor gene disrupted by chromosomal translocation t(3;8)(3p14.2;q24.1) that is associated with hereditary renal and thyroid cancers [24,25,26]. The TCR8 gene encodes an ER localized protein named Tcr8 that contains a sterol-sensing domain and a RING-H2 motif that confers ubiquitin ligase activity. Of note, dysregulation of lipid homeostasis is one of the characteristics of clear-cell renal cell carcinomas [27]. Thus studies of Tcr8 provide a regulatory link between growth control and lipid homeostasis. In sum these 4 examples provide convincing evidence of the cancer relevance of ERAD pathways and the need for better mechanistic understanding for translational applications.

3. Viral proteins as probes for degradation pathways

Unexpectedly, significant new insights into ubiquitin-dependent degradation pathways have come from studies of viral proteins [28]. Viruses have evolved for millions of years under the selective pressure of their host's immune system. Most notably CD8⁺ cytotoxic T lymphocytes, CTL, are a predominant force in controlling virus infection. CTL recognition of virus infected cells is dependent upon i) the processing the viral protein into 8-10 amino acid peptides, ii) the binding of peptides to MHC class I molecules in the ER lumen, iii) the display of class I/viral peptide complexes on the cell surface, and iv) CTL detection and lysis of the infected cells [29]. The importance of this process, referred to antigen processing and presentation, has not gone unnoticed by viruses [30]. Indeed, many viruses have elaborate immune evasion mechanisms that block various aspects of antigen processing or presentation. For example several viruses encode proteins that target class I molecules for destruction, thus preventing the recognition of virus infected cells by CTL. Such a mechanism is of particular importance for γ -herpesviruses that coexist in the presence of an active immune system in normal individuals, but are a significant health problem in immune compromised individuals. For example, Kaposi's sarcoma-associated herpesvirus (KSHV) is a major cause of malignancy in immune-deficient AIDS patients. The murine γ -herpesvirus γ HV68 shares homology with KSHV and provides a powerful mouse model amenable to studies of the pathogenesis of infection, and host-pathogen interactions [31].

Viral immune evasion proteins targeting class I have been tremendously informative probes for dissecting molecular pathways of degradation, especially ERAD. Clear examples of this are provided by the numerous published studies of the US2 and US11 immune evasion proteins of HCMV [30]. More recently, studies of mK3 protein of γ HV68 have added significant insights as detailed below [32]. What makes these 3 immune evasion genes potent probes of ERAD is the fact that they specifically target class I molecules and greatly expedite the kinetics of ERAD. Misassembled MHC molecules are substrates for ERAD, but this process typically takes hours. By contrast ERAD of MHC class I molecules in the presence of these 3 viral immune evasion proteins is initiated within minutes. Of these 3 viral proteins, mK3 is unique in that it is an E3 ligase [33,34]. By contrast, the ERAD associated with US11 is ubiquitin dependent, but the required E3s are undefined [35,36]. It is now clear from several recent studies of ERAD in yeast and mammals that the E3 ligase is a central and defining component of ERAD pathways [19,37]. Accordingly, studies of the viral mK3 ligase have been particularly insightful for mechanistic dissection of a mammalian ERAD pathway.

3.1. Viral MARCH proteins

The above mentioned mK3 protein of γ HV68 is one of the founding members of a family of viral proteins encoded by pox viruses or tumor-inducing herpesviruses [38]. mK3 was originally discovered by Stevenson et al. using a viral genomic library of γ HV68 clones to show that mK3 was solely responsible for the surface downregulation of class I expression (note prefix 'm' designates its origin from a murine virus) [33]. The K3 ORF is relatively conserved between MHV68 and KSHV, however the latter virus encodes two K3 homologs designated kK3 or kK5 (the prefix 'k' designate KSHV origin). Alternatively, in certain papers kK3 and kK5 have been designated MIR1 and MIR2 respectively. Several groups including Stevenson et al., Coscoy and Ganem, and Ishido et al. showed that kK5 and kK3 induced class I downregulation in human cells [33,39,40]. Significant insight into the mechanism of class I targeting used by these viral K3 homologs was gained by the recognition that they share a structurally conserved zinc RING finger motif. More specifically mK3, kK3 and kK5 possess a subclass of RING domain designated as a RING-CH domain, which differs from the classical RING-HC and RING-H2 domains in the organization of their zinc coordinating cysteine/histidine residues and the replacement of one small β -sheet strand with a single turn of a helix

[41]. Because mK3, kK3 and kK5 all contain RING-CH domains and multiple transmembrane domains, we call them here viral MARCH proteins. Importantly, all proteins found to contain these related RING-HC, RING-H2, and RING-CH motifs share a common function as E3 ligases [42] [7,43].

All viral MARCH proteins share a common domain organization of a type III protein. In this orientation the conserved N terminus containing the RING-CH domain is followed by two membrane passages, which is then followed by a C-terminal domain variable in length and sequence between viral MARCH proteins. This domain organization results in both the N- and C-terminal domains of viral MARCH proteins in the cytosol and a very short (about 12 amino acid) segment in the ER lumen. The exposure of the RING-CH domain to the cytosol allows the viral ligases to interact with the other ubiquitin components required for substrate ubiquitination. In spite of the properties shared by mK3, kK3 and kK5 including-- i) highly homologous RING-CH domain, ii) common topology and iii) targeting class I substrates, these three viral ligases have significant functional differences. Most notably, mK3 acts at an earlier point in MHC class I biosynthesis than kK3 and kK5 [34,44]. After translocon-mediated entry into the ER lumen, class I molecules acquire immature (Endo-H sensitive) N-linked glycans. The mK3 ligase targets these ER resident class I molecules for degradation by ERAD as detailed in the next section. By contrast the kK3 and kK5 proteins target class I molecules with mature glycans that have transited through the mid-Golgi (thereby gaining EndoH resistance) to the plasma membrane along the secretory pathway [39,40]. In the presence of kK3 and kK5, surface class I molecules are rapidly endocytosed and degraded [39,40]. Mechanistically, kK3 binds mature class I molecules and mediates the conjugation of K63-linked polyubiquitin chain to the cytoplasmic tail of HC by interacting with ubiquitin-conjugating enzymes Ubc5b/c and Ubc13 [45]. This ubiquitin chain then functions as a signal that triggers the clathrin-dependent internalization [39], TSG101-dependent late endosome sorting [46] and subsequent lysosome degradation [47,42]. In addition to their conserved RING-CH domain which is thought the major domain interacting with the E2s, a conserved tyrosine-based motif (YXX Φ , where X is any amino acid and Φ is a hydrophobic amino acid) in the C terminus of kK3 and kK5 is required for internalization of class I while the C-terminal diacidic clusters of kK3 and kK5 are involved in targeting class I molecules to the lysosomal compartment [48,49].

The viral ligases mK3, kK3 and kK5 are also distinguished by their differences in substrate specificity. mK3 primarily targets MHCI heavy chains (HC), although there can be concomitant collateral damage of TAP in certain cell lines [50]. By contrast, kK3 and kK5, especially kK5, target multiple immune regulatory molecules. For example both of them target classical HLA class I molecules as well as CD1d [51]. Interestingly, while kK3 appeared to have no selection on the HLA alleles kK5 primarily targets HLA-A and -B molecules and not certain HLA-C alleles [40]. In addition, kK5 also targets the degradation of costimulatory molecules ICAM-1 and B7.2, resulting in impaired T cell activation and NK cell detection of kK5 expressing cells [52]. Furthermore, kK5 also downregulates NKG2D and NKp80 ligands MICA, MICB and AICL by increased endocytosis to evade NK cell cytotoxicity [53]. Thus to avoid immune detection by the host the virus blocks the function of both CD8 T cells and NK cells. In addition of cell mediated immunity, kK3 and kK5 have more recently been shown to also target gamma interferon receptor 1 for endocytosis and lysosomal degradation [54]. In endothelial cells, kK5 additionally reduced expression of CD31/PECAM (platelet-endothelial cell adhesion molecule) and CD31-dependent endothelial cell migration, which is likely relevant to the KSHV-induced Kaposi's sarcomas [55].

3.2. The mK3 ERAD pathway

The mK3 viral ligase has been a highly effective probe for defining the molecular details of a mammalian ERAD pathway. Its effectiveness as a physiological probe for ERAD is based

largely on the following facts--- i) mK3 is an E3 ligase and E3s have a central role in determining substrate specificity, ii) MHC class I is the only primary substrate of mK3, and iii) ERAD induced by mK3 is substantially more rapid than ERAD of misassembled or mutated substrates. Thus far the greatest insights provided by mK3 concern the molecular basis of substrate recognition. Somewhat surprisingly, the major binding partner of mK3 is not its substrate HC, but TAP (Fig. 1A) [56,57,50]. TAP is an ER resident, ATP-dependent pump that translocates proteasome-generated peptides from the cytosol to the ER lumen. After assembly with $\beta 2m$, HC binds to TAP, an association bridged by the MHCI-specific ER chaperone tapasin (Fig. 1B). The association of HC with TAP/tapasin facilitates HC binding of suitable peptide ligands. Of note, binding of mK3 to TAP is HC independent and required for ubiquitination and ERAD of HC [56]. These combined findings raised the intriguing question of whether TAP binding or HC sequence determined mK3 substrate specificity. Several lines of evidence demonstrated that mK3 does not recognize a specific sequence in the cytoplasmic tail of class I. This evidence included swapping the tail of HC with the tails of other proteins or adding generic tails to HC [57]. Moreover, specific detection of HC by mK3 was maintained as long as ectodomain of HC was capable of binding TAP and the HC tail contained a potential ubiquitination site for mK3 [56].

The above evidence demonstrated that TAP association and not HC tail sequence determines the substrate specificity of mK3 ligase. Based on this conclusion we proposed a proximity model [58], whereby binding to TAP orients the RING domain of mK3 such that it only ubiquitinates HC (Fig. 1). The viral ligase mK3 exploits the host TAP protein to confer substrate-specific recognition of HC. Thus in this model, an adaptor protein (TAP), and not a substrate (HC)-specific sequence determines recognition by the E3 ligase (mK3). The attractiveness of this model is that it explains how mK3 can specifically target highly polymorphic class I molecules encoded by different loci and haplotypes with considerable tail sequence variation. On a more general level this proximity model establishes a paradigm by which E3 ligases can use adaptor proteins to limit the number of substrates detected without their sharing sequence similarity. And there are several examples (e.g. kK5 above and cellular MARCH proteins below) where known E3s have a limited number of substrates that share no obvious sequence motif.

To better understand the mechanism of substrate ubiquitination, we sought to identify the specific HC residues that were ubiquitinated. Lysine residues in the HC tail were the expected conjugation sites, but HCs with lysine free tails were still ubiquitinated and rapidly degraded in mouse cells expressing mK3 [58]. Although somewhat unexpected, this finding fit with one of the current models of how ERAD substrates are dislocated from the ER [32]. This model is referred to here as the partial dislocation model and postulates that the N terminus of membrane bound ERAD substrates is partially dislocated from the ER lumen, ubiquitinated and then fully extracted to the cytosol. If this were the case with mK3 substrates, then lysine residues in the HC ectodomain could be sites of ubiquitin modification. To test this hypothesis, a fully lysine-free HC was created by mutagenesis and surprisingly it was also ubiquitinated and rapidly degraded in mouse cells concomitantly expressing mK3 ligase [59]. N-terminal ubiquitination was then ruled out, strongly suggesting that non-lysine residues of HC are potential sites of ubiquitination. Indeed mutagenesis and chemical approaches unequivocally demonstrated that mK3 can form ester bonds with serine or threonine residues in the HC tail as well amide binds with lysine residues [59]. Furthermore, serine, threonine or lysine residues were preferentially ubiquitinated by mK3 when located toward the C terminus of the HC tail. These findings added additional credence to the proximity model. More specifically, what determines sites of substrate ubiquitination is the proximity of the mK3 RING domain with the HC tail as imposed by TAP binding and that mK3 interaction with HC is not dependent upon a specific sequence motif.

Findings with mK3 challenged two paradigms of ERAD substrate ubiquitination. The first paradigm shift concerns the notion that only lysine coupled ubiquitination is sufficiently stable to be physiological. The first demonstration of non-lysine ubiquitination of substrates was a recent study by Cadwell and Coscoy [60]. They reported that viral ligase kK3 of KSHV can ubiquitinate cysteine residues of the HC tail and induce surface downregulation. This finding implicates thioester bonds in ubiquitin conjugation of substrates, the same linkage used to transiently couple ubiquitin subunits to the E1 or E2 ubiquitination components. Thus it is very interesting that mK3 and its closest homolog kK3 both can perform non-lysine ubiquitinations [59], but the former via ester bonds to serine and threonine residues and the latter via thioester bonds to cysteine residues. These findings raise interesting questions of whether only viral E3 ligases can perform non-lysine conjugations, or whether non-lysine ubiquitination has physiological relevance. Although further experimental evidence will be required for definitive answers to these questions, there are notable examples of ERAD substrates lacking lysine residues being ubiquitinated [61,62,63,64]. And in one of these studies using mammalian cells, ester and/or thioester linkages are implicated suggesting the mammalian E3 ligases are capable of non-lysine ubiquitin linkages [65]. Interestingly, we have recently observed that ubiquitination of non-lysine residues can be facilitated by chimeric RING swap molecules consisting of a RING-CH domain from mouse cellular E3 ligase flanked by wt mK3 sequences (Herr et al in preparation). These findings imply that normal cellular environments have all of the ubiquitination machinery required for the ubiquitination of non-lysine residues, aside from the scaffold which the sequences outside of the RING-CH domain of mK3 provide. Thus, it is attractive to speculate that the ubiquitination of non-lysine residues may have physiological relevance. In any case the ability of viral ligases mK3 and kK3 to ubiquitinate non-lysine substrate residues provides a tremendous opportunity to define how E3 ligases interact with E2 conjugases to ubiquitinate specific substrate residues.

The second paradigm shift resulting from mK3 studies concerns how substrates are dislocated from the ER lumen to the cytosol. As mentioned above, most current mammalian models speculate the ERAD substrates need to be partially dislocated via their N terminus prior to ubiquitination and complete extraction (dislocation) from the ER lumen to the cytosol [66, 67]. However, the fact that mK3 ubiquitinates HC tail residues suggests a simpler model, namely that ubiquitination occurs prior to substrate dislocation. In this model ubiquitination could be a signal in the cytosol to initiate dislocation and substrate removal would be vectorial (ie via the C terminus) [59]. It will be interesting which of these models, partial dislocation vs vectorial dislocation, is more commonly used for removal of ERAD substrates. But regardless of the method of extraction, several fundamental questions remain regarding the mechanism of the dislocation of mammalian ERAD substrates. First, what molecular interactions in the ER lumen initiate or facilitate the removal of substrates and do these include ER chaperone proteins similar to ones implicated in yeast studies? Relevant to this question mK3 induces the dissociation of β 2m from HC and this event may introduce the ER chaperone calnexin and initiate HC dislocation from the luminal side (Fig 1C). Second, do substrates need to be denatured prior to ER dislocation? It seems unlikely that ERAD substrates need to be fully denatured since glycan moieties of glycosylated proteins are not removed prior to dislocation and ERAD substrates with bulky appendages are dislocated [68,69]. Third, what is the nature of the dislocation pore and are multiple membrane spanning ligases such as mK3 a component of the putative dislocation pore? Relevant to this question, the ERAD inducing HCMV protein US11 was found to be associated with the novel mammalian protein Derlin1 that is homologous to yeast ERAD associated protein Der1p [66,67]. Although it was proposed that Derlin1 may be a component of the dislocation channel, this has yet to be demonstrated. However, it is interesting that mK3 also associates with Derlin1 (Fig. 1D) [70].

In any case there is no doubt that viral proteins that induce ERAD will continue to provide key insights into outstanding questions of the mechanism of dislocation. In addition, the

degradation induced by these viral proteins clearly exploit physiological pathways as evidenced by the fact US11 and mK3 ultimately induce association with ATPase p97 [66,67,70]. This ATPase is highly conserved in evolution and is required for substrate dislocation to the cytosol for proteolysis by the proteasome (Fig. 1D) [71]. Furthermore, mK3 mediates K48 linked ubiquitination like physiological ERAD pathways (X. Wang in preparation), whereas kK3 mediates K63 linked ubiquitination like endocytosis/lysosomal degradation pathways [45].

4. Cellular MARCH proteins

As noted above, the viral E3 ligases of HV68 and KSHV have been incredibly useful probes in defining molecular pathways of degradation. Indeed the pathways defined by mK3, kK3 and kK5 are among the most well defined cellular mechanisms of protein degradation in mammals. As also noted above, degradation pathways have universal impact on diverse aspects of cell biology including cancer. The biological impact of this viral MARCH family of ligases has recently been extended to a large family of cellular E3 ligases. Based on the fact that viral MARCH proteins were identified from two divergent dsDNA virus families, *Herpesviridae* and *Poxviridae*, it was hypothesized that they were originally stolen from mammalian host genomes. The initial evidence to support this hypothesis was the identification and characterization of a cellular MARCH protein originally designated as c-MIR (cellular modifier of immune response) [72], but is now more commonly called MARCH8. Although overall sequence similarity between MARCH8 (c-MIR) and viral MARCH family members is low, they share interesting properties in addition to a common RING-CH and domain organization. For example, like kK5, overexpression of c-MIR specifically targets downregulation of B7.2 by ubiquitination, rapid endocytosis and lysosomal degradation [72]. Consistent with this finding, additional genome-wide searches based on common features of viral MARCH members identified total of 11 cellular homologs in eukaryotic genomes [73, 74,75] (Table1). All of these newly recognized cellular homologues, except MARCH7 and MARCH10, possess a RING-CH domain in the N terminus followed by two or more transmembrane domains [76,77,42]. MARCH 7 and MARCH 10 have no predicted transmembrane domains and their RING-CH domains are located towards the C-terminus. As expected most identified MARCH proteins have shown E3 ligase activity as conferred by their RING-CH domain. And notably, half of the cellular MARCH proteins appear to either directly or indirectly regulate immune functions by affecting surface turnover of immune regulatory molecules on the plasma membrane.

The RING-CH domains of viral and cellular E3 ligases share the highest level of sequence conservation of any domain within these molecules. This domain not only confers E3 ligase activity to these molecules, but also has been shown to contribute to the specific interaction with E2 conjugases [77,45]. Using amino acid sequences of viral and mouse cellular RING-CH domains that were previously aligned using the T-COFFEE algorithm, the Neighbor-Joining method was employed to generate a bootstrap consensus tree inferred from 5000 replicates in Fig. 2 [78,79,80]. A striking feature of this dendrogram is that the viral RING-CH domains form a separate clade from all of the cellular RING-CH domains. This observation suggests that the viral RING-CH domains have diverged evolutionarily from cellular RING-CH domains to provide a necessary or beneficial trait for viral survival within the host. Although the viral RING-CH domains differ in sequence from their cellular ancestors, they are presumably required to retain minimal functional/binding motifs to preserve their E3 ligase function in host cells. Indeed, alignment of the amino acid sequences of kK3, kK5 and mK3 RING-CH domains as well as the RING-CH domains from representative MARCH proteins reveals the conserved C4HC3 motif responsible for coordinating two zinc ions (blue shading Fig. 3).

The most rigorously characterized pair of mouse and human MARCH proteins are MARCH1 and MARCH8 (c-MIR). These homologs display high levels of sequence and functional similarity. In addition to the aforementioned downregulation of B7.2 by MARCH8, both MARCH8 and MARCH1 also down regulate CD95(Fas), TfR (transferrin receptor) and MHCII [77,81,82,83,84]. All of these substrates were initially defined in vitro by constitutive expression of the MARCH proteins in cell lines. These initial observations suggested a possible role for MARCH1 and MARCH8 in lymphocyte regulation and/or activation. In support of this notion, endogenous MARCH1 and MARCH8 expression is restricted to secondary lymphoid tissues such as lymph node and spleen with notable expression on the MHC II+ APC like B cells and DCs [84,77,82,72]. To probe mechanism, MARCH8 was overexpressed in several different B cell lines inducing surface MHCII downregulation in a ubiquitination and MHCII beta chain and cytoplasmic tail lysine dependant manner [84]. The mechanism underlying MARCH8-mediated MHCII and B7.2 down-regulation appears to be very similar to that of kK3 and kK5. For example, specific recognition of substrates by MARCH1 and MARCH8 is likely conferred by direct interaction with substrates through their respective transmembrane domains [72]. This interaction and subsequent ubiquitination of the substrates by MARCH8 takes place at the plasma membrane, triggering rapid internalization of the surface substrates in a clathrin-dependent manner [72].

Several recent studies support the physiological importance of both MARCH1 and MARCH8 in antigen presentation of MHCII to CD4 T cells [81,82,84]. For example, the effect of MARCH8 on immune response in vivo was recently demonstrated using a transgenic mouse model in which MARCH8 expression was controlled by the invariant chain promoter. The invariant chain promoter targeted MARCH8 expression largely to MHCII+ professional antigen presenting cells like B cells and DCs [84]. Consistent with the ability of MARCH8 to downregulate MHCII expression, DCs derived from these mice showed a diminished ability to present antigens. Furthermore, these transgenic mice were resistant to the onset of EAE and were impaired in their development of CD4 T cells in the thymus and the periphery [84]. Thus these findings provide support to the in vitro findings, and highlight the potential in vivo importance of MARCH8 in the regulation of class II-mediated antigen presentation.

Taking an alternative approach to probe in vivo function, mice were made with the genetic knockout of the MARCH1 gene. Importantly, B cells derived from these MARCH1-deficient mice showed a prolonged half-life of surface MHCII and a concomitant disappearance of ubiquitinated forms of MHCII. As a result, the amount of surface MHCII in MARCH1-deficient B cells was remarkably increased and antigen presentation by these cells was significantly enhanced [82]. These findings provided strong evidence supporting a physiological role of MARCH1 in the regulation of antigen presentation by APCs through ubiquitination of surface MHCII. In addition, it was recently demonstrated that MARCH1 plays a critical role in antigen presentation to human CD4 cells by human monocyte derived DCs [81]. It has been established that in response to TLR ligands such as LPS, DCs undergo a dramatic activation. Prominent in this DC activation is the upregulation of MHCII, the timing of which is critical for appropriate activation of CD4 T cells. Interestingly, down regulation of MARCH1 initiates the up regulation of MHCII expression following the activation of DC [81]. Thus MARCH1 and likely MARCH8 have physiological roles in immune regulation using mechanisms similar to their viral homologs. Although they share sequence homology and substrate specificities, it is currently unclear whether MARCH1 and MARCH8 have completely redundant in vivo functions. Minor differences in expression have been noted [77] and interestingly only expression of MARCH1 but not MARCH8 can be dramatically induced by the inhibitory cytokine IL-10 [83]. Thus the cumulative evidence is quite convincing that MARCH1 and MARCH8 use a post translational degradation pathway to temper unwanted immune responses.

Another interesting pair of closely related MARCH proteins is MARCH4 and MARCH9. Both of these MARCH proteins induce ubiquitin-dependent surface down-regulation and rapid endocytosis of MHC class I [77]. These findings strongly suggest that in a normal cellular environment, MARCH4 and MARCH9 regulate antigen presentation by MHC class I in a mechanistically similar manner to their viral homologs, kK3 and kK5 of KSHV. Interestingly, the MHC class I regulation by MARCH4 and MARCH9 is dependent upon lysine residues in the class I tail [77], differing from kK5 and kK3 that can also ubiquitinate cysteine residues [85,60]. In addition, over-expression of MARCH9 also results in down-regulation of surface ICAM1 [86]. ICAM1 is a co-stimulatory molecule for T and B cells and also plays important role in T cell activation and migration. For example, a low level of ICAM1 on tumors is associated with more aggressive disease. Although MARCH4 and 9 both facilitate the ubiquitination of class I molecules, their expression patterns as detected by real time PCR are quite different. MARCH9 is characterized by an abundant and ubiquitous expression pattern with elevated levels in lymph tissue and lung, whereas MARCH4 expression is restricted to brain and placenta [77]. These distinct expression profiles suggest that MARCH4 and MARCH9 may have distinct biological roles. Interestingly, the recombinant N termini of MARCH4 and MARCH9 require different E2 conjugases to facilitate their in vitro autoubiquitination, even though their RING-CH domains are highly homologous [77]. The significance of their different E2 requirements is not yet known, although it could be very important for defining functional differences between MARCH4 and MARCH9 proteins. This conclusion is based on the fact that E2-E3 interaction is known to play a pivotal role in determining the length and linkage of ubiquitin chain assembly on substrates, both of which are known to determine the trafficking and fate of ubiquitin conjugated substrates [87].

In contrast to MARCH1, 4, 8 and 9, less is known about the physiological substrates of other MARCH proteins. However, their subcellular localization, expression pattern and binding proteins have shed considerable light on potential physiological roles of these other MARCH proteins. For example, MARCH5 is detected in mitochondrial outer membrane where it interacts with mitofusin 2 (MFN2) and Drp1, two key components in mitochondrial membrane fusion and division [88]. Interestingly, overexpression of MARCH5 promotes ubiquitination of Drp1 and the formation of long tubular mitochondria in a MFN2- dependent manner, whereas mutations in the RING of MARCH5 cause fragmentation of mitochondria [89,90, 88]. These results suggest that MARCH5 is a novel mitochondrial E3 ligase and plays a crucial role in the control of mitochondrial morphology by regulating MFN2 and Drp1 activity via ubiquitination. Another example of a tissue-specific MARCH protein is the recently characterized MARCH11, that was found to be extremely abundant in developing spermatids [75]. This cellular E3 ligase, contains a tyrosine-based sorting motif and a PDZ binding motif (a consensus 3-7 residue motif at the C-terminal of transmembrane proteins recognized by PDZ domain [91]) in its C-terminal region that mediate its interaction with known components of trans-Golgi network (TGN)-MVB transport pathway, specifically adaptor protein complex-1 (AP-1) and Veli protein [75]. This finding is very intriguing because a major event in early spermatogenesis is acrosomal formation that is achieved by the continuous fusion of Golgi-derived vesicles with the immature acrosomal vesicle and ubiquitination is an important mechanism in regulation of vesicle trafficking. Thus, the implication of MARCH11 in mammalian spermatogenesis provides an additional novel function for this family of E3 ligases.

Based on the localization and structural similarity to yeast ER-resident E3 ligase Doa10, MARCH6 was proposed to be a mammalian ortholog of Doa10 [92]. Unlike most other MARCH proteins, MARCH6 is predicted to possess 13 [92]/14 [93] transmembrane domains and localize to the ER membrane. In in vitro ubiquitination assays, the MARCH6 RING interacts with Ubc7, a well defined ERAD-associated ubiquitin conjugating enzyme (E2), and promotes formation of ubiquitin dimers via K48 linkage [92]. These results are consistent with

a proposed role of MARCH6 in ERAD. Curiously, expression of MARCH6 is not induced by ER stress, and its physiological substrates have yet to be defined. Given that Doa10 is a central component of ERAD and ERQC in yeast [19], MARCH6 is likely to have a central function in ERAD and ERQC in mammals. Key to defining its physiological role will be the identification of MARCH6 binding partners and substrates.

Differing from other MARCH proteins, MARCH2 and MARCH3 were both reported to be ubiquitously expressed and localized to endosomal vesicles [77,94,95]. Furthermore, both of these homologs were found to directly bind syntaxin6 [94,95]. Syntaxin6 is a ubiquitously expressed SNARE that localizes to the TGN and endosomes and functions in multiple membrane-trafficking pathways [96,97]. Mutation in syntaxin is associated with an epithelial polarity defect which may directly contribute to tumor formation [98]. In addition, overexpression or depletion/mutation of MARCH2 and MARCH3 affected localization of syntaxin6 and TGN38/46 (a type I integral membrane protein predominantly localized to the TGN, and cycling between the TGN and the cell surface) [94,95]. MARCH2 and MARCH3 are thus thought to be novel regulators of trafficking between the TGN and endosomes. Unfortunately, the detailed mechanisms involved in this regulation and its functional significance remain elusive. However, the RING-CH domains in the N termini of MARCH2 and MARCH3 and their PDZ-binding motifs in their C termini have been shown to be essential for their effects on intracellular location of syntax6 and TGN38/46 [94,95]. More recently, MARCH2 was found to interact with DLG1, a PDZ domain containing molecular scaffold involved in determining cell polarity [99]. MARCH2 co-localizes with DLG1 at sites of cell-cell contact and promotes DLG1 ubiquitination. MARCH2 expression in some epithelial cancers such as bladder, prostate and colorectal was found to be reduced [99]. Taken together these suggest that MARCH2 and MARCH3 modulate epithelial polarity through regulation of endosomal recycling of polarity determinants. Whether and how their E3 ligase activity contributes to this regulation is not clear.

MARCH7 is also known as axotrophin. Its gene (*axot*) was initially identified in an in vitro screen for novel genes involved in neural differentiation from embryonic stem cells [100]. Detection of axotrophin mRNA in adult mouse tissues showed significant expression in brain, thymus, muscle and kidney, but little in spleen or liver [101]. Mice lacking axotrophin exhibit premature neural degeneration and defective development of corpus callosum [101]. In addition, T cell proliferation, T cell-derived leukemia inhibitory factor (LIF) and thymic Foxp3 in these *axot*^{-/-} mice were reduced in comparison to their wildtype littermates [101,102]. These findings implicated MARCH7 in thymic development of Foxp3⁺T cells (Tregs), which are known regulators of immune tolerance. Furthermore, MARCH7 readily undergoes autoubiquitination, however association with two deubiquitination enzymes, USP9X in the cytosol and USP7 in the nucleus, stabilizes MARCH7 [103]. So far, this is the first example of post-translational regulation of MARCH proteins. It will be worthwhile to see whether this type of regulation also applies to the other MARCH proteins given they appear to be important regulators of multiple fundamental cell processes dealing with membrane protein transport and/or degradation.

5. Concluding remarks

It is interesting that, where known, most cellular MARCH proteins use endocytosis/lysosomal degradation pathways similar to the kK3/kK5 proteins of KSHV. In contrast, MARCH6 is the only MARCH protein that has been implicated in proteasome-mediated degradation (ERAD). It is thus of considerable interest whether MARCH6 may have functional similarities to the mK3 protein of γ HV68. In any case an outstanding question is—what determines whether these highly homologous cellular and viral ligases induce degradation via the lysosomal or proteasome? It is not obviously due to differences in their RING domain, but most assuredly

has to do with host protein interactions (R. Herr in preparation). For example, the binding of mK3 to host protein TAP is a likely factor for why it induces ERAD. It is also possible the sequence juxtaposed to the RING influences the recruitment of different E2s that target different degradation pathways. Thus for both MARCH proteins and their viral descendents it remains critical to define further binding partners and critical interactions of substrates and ubiquitin components. A central question in all ubiquitination pathways will be defining the critical interactions between the E3 ligase, E2(s) and substrate that determine which substrate residues are ubiquitinated and the linkage and extent of this ubiquitination. Future studies of viral E3 ligases and their cellular homologs have a tremendous potential to address these outstanding questions and dissect novel aspects of ubiquitin-dependent degradation pathway many of which will have cancer relevance.

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Reference List

- Pickart CM, Fushman D. Polyubiquitin chains: polymeric protein signals. *Curr Opin Chem Biol* 2004;8:610–616. [PubMed: 15556404]
- Hochstrasser M. Lingering mysteries of ubiquitin-chain assembly. *Cell* 2006;124:27–34. [PubMed: 16413479]
- Fang S, Weissman AM. A field guide to ubiquitylation. *Cell Mol Life Sci* 2004;61:1546–1561. [PubMed: 15224180]
- Pickart CM. Mechanisms underlying ubiquitination. *Annu Rev Biochem* 2001;70:503–533. [PubMed: 11395416]
- Jin J, Li X, Gygi SP, Harper JW. Dual E1 activation systems for ubiquitin differentially regulate E2 enzyme charging. *Nature* 2007;447:1135–1138. [PubMed: 17597759]
- Freemont PS. RING for destruction? *Curr Biol* 2000;10:R84–R87. [PubMed: 10662664]
- Lorick KL, Jensen JP, Fang S, Ong AM, Hatakeyama S, Weissman AM. RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. *Proc Natl Acad Sci U S A* 1999;96:11364–11369. [PubMed: 10500182]
- Staub O, Rotin D. Role of ubiquitylation in cellular membrane transport. *Physiol Rev* 2006;86:669–707. [PubMed: 16601271]
- Thrower JS, Hoffman L, Rechsteiner M, Pickart CM. Recognition of the polyubiquitin proteolytic signal. *EMBO J* 2000;19:94–102. [PubMed: 10619848]
- Newton K, Vucic D. Ubiquitin ligases in cancer: ushers for degradation. *Cancer Invest* 2007;25:502–513. [PubMed: 17882664]
- Mani A, Gelmann EP. The ubiquitin-proteasome pathway and its role in cancer. *Journal of Clinical Oncology* 2005;23:4776–4789. [PubMed: 16034054]
- Fang S, Lorick KL, Jensen JP, Weissman AM. RING finger ubiquitin protein ligases: implications for tumorigenesis, metastasis and for molecular targets in cancer. *Semin Cancer Biol* 2003;13:5–14. [PubMed: 12507552]
- Sun Y. E3 ubiquitin ligases as cancer targets and biomarkers. *Neoplasia* 2006;8:645–654. [PubMed: 16925947]
- Burger AM, Seth AK. The ubiquitin-mediated protein degradation pathway in cancer: therapeutic implications. *Eur J Cancer* 2004;40:2217–2229. [PubMed: 15454246]
- Kikkert M, Doolman R, Dai M, Avner R, Hassink G, van Voorden S, et al. Human HRD1 is an E3 ubiquitin ligase involved in degradation of proteins from the endoplasmic reticulum. *J Biol Chem* 2004;279:3525–3534. [PubMed: 14593114]
- Bays NW, Gardner RG, Seelig LP, Joazeiro CA, Hampton RY. Hrd1p/Der3p is a membrane-anchored ubiquitin ligase required for ER-associated degradation. *Nat Cell Biol* 2001;3:24–29. [PubMed: 11146622]

17. Deak PM, Wolf DH. Membrane topology and function of Der3/Hrd1p as a ubiquitin-protein ligase (E3) involved in endoplasmic reticulum degradation. *J Biol Chem* 2001;276:10663–10669. [PubMed: 11139575]
18. Yamasaki S, Yagishita N, Sasaki T, Nakazawa M, Kato Y, Yamadera T, et al. Cytoplasmic destruction of p53 by the endoplasmic reticulum-resident ubiquitin ligase ‘Synoviolin’. *EMBO J* 2007;26:113–122. [PubMed: 17170702]
19. Kostova Z, Tsai YC, Weissman AM. Ubiquitin ligases, critical mediators of endoplasmic reticulum-associated degradation. *Semin Cell Dev Biol* 2007;18:770–779. [PubMed: 17950636]
20. Watanabe H, Nabi IR, Raz A. The relationship between motility factor receptor internalization and the lung colonization capacity of murine melanoma cells. *Cancer Res* 1991;51:2699–2705. [PubMed: 2021948]
21. Nabi IR, Watanabe H, Silletti S, Raz A. Tumor cell autocrine motility factor receptor. *EXS* 1991;59:163–177. [PubMed: 1655517]
22. Tsai YC, Mendoza A, Mariano JM, Zhou M, Kostova Z, Chen B, et al. The ubiquitin ligase gp78 promotes sarcoma metastasis by targeting KAI1 for degradation. *Nat Med* 2007;13:1504–1509. [PubMed: 18037895]
23. Lerner M, Corcoran M, Cepeda D, Nielsen ML, Zubarev R, Ponten F, et al. The RBCC gene RFP2 (Leu5) encodes a novel transmembrane E3 ubiquitin ligase involved in ERAD. *Mol Biol Cell* 2007;18:1670–1682. [PubMed: 17314412]
24. Cohen AJ, Li FP, Berg S, Marchetto DJ, Tsai S, Jacobs SC, et al. Hereditary renal-cell carcinoma associated with a chromosomal translocation. *N Engl J Med* 1979;301:592–595. [PubMed: 470981]
25. Gemmill RM, West JD, Boldog F, Tanaka N, Robinson LJ, Smith DI, et al. The hereditary renal cell carcinoma 3;8 translocation fuses FHIT to a patched-related gene, TRC8. *Proc Natl Acad Sci U S A* 1998;95:9572–9577. [PubMed: 9689122]
26. Li FP, Decker HJ, Zbar B, Stanton VP Jr, Kovacs G, Seizinger BR, et al. Clinical and genetic studies of renal cell carcinomas in a family with a constitutional chromosome 3;8 translocation. *Genetics of familial renal carcinoma. Ann Intern Med* 1993;118:106–111. [PubMed: 8416305]
27. Brauweiler A, Lorick KL, Lee JP, Tsai YC, Chan D, Weissman AM, et al. RING-dependent tumor suppression and G2/M arrest induced by the TRC8 hereditary kidney cancer gene. *Oncogene* 2007;26:2263–2271. [PubMed: 17016439]
28. Wiertz EJ, Jones TR, Sun L, Bogoy M, Geuze HJ, Ploegh HL. The human cytomegalovirus US11 gene product dislocates MHC class I heavy chains from the endoplasmic reticulum to the cytosol. *Cell* 1996;84:769–779. [PubMed: 8625414]
29. Bouvier M. Accessory proteins and the assembly of human class I MHC molecules: a molecular and structural perspective. *Mol Immunol* 2003;39:697–706. [PubMed: 12531281]
30. Lilley BN, Ploegh HL. Viral modulation of antigen presentation: manipulation of cellular targets in the ER and beyond. *Immunol Rev* 2005;207:126–144. [PubMed: 16181332]
31. Virgin HW, Latreille P, Wamsley P, Hallsworth K, Weck KE, DalCanto AJ, et al. Complete sequence and genomic analysis of murine gammaherpesvirus 68. *Journal of Virology* 1997;71:5894–5904. [PubMed: 9223479]
32. Lybarger L, Wang XL, Harris M, Hansen TH. Viral immune evasion molecules attack the ER peptide-loading complex and exploit ER-associated degradation pathways. *Current Opinion in Immunology* 2005;17:71–78. [PubMed: 15653314]
33. Stevenson PG, Efsthathiou S, Doherty PC, Lehner PJ. Inhibition of MHC class I-restricted antigen presentation by gamma 2-herpesviruses. *Proc Natl Acad Sci U S A* 2000;97:8455–8460. [PubMed: 10890918]
34. Boname JM, Stevenson PG. MHC class I ubiquitination by a viral PHD/LAP finger protein. *Immunity* 2001;15:627–636. [PubMed: 11672544]
35. Kikkert M, Hassink G, Barel M, Hirsch C, van der Wal FJ, Wiertz E. Ubiquitination is essential for human cytomegalovirus US11-mediated dislocation of MHC class I molecules from the endoplasmic reticulum to the cytosol. *Biochem J* 2001;358:369–377. [PubMed: 11513735]
36. Shamu CE, Flierman D, Ploegh HL, Rapoport TA, Chau V. Polyubiquitination is required for US11-dependent movement of MHC class I heavy chain from endoplasmic reticulum into cytosol. *Mol Biol Cell* 2001;12:2546–2555. [PubMed: 11514634]

37. Carvalho P, Goder V, Rapoport TA. Distinct ubiquitin-ligase complexes define convergent pathways for the degradation of ER proteins. *Cell* 2006;126:361–373. [PubMed: 16873066]
38. Fruh K, Bartee E, Gouveia K, Mansouri M. Immune evasion by a novel family of viral PHD/LAP-finger proteins of gamma-2 herpesviruses and poxviruses. *Virus Research* 2002;88:55–69. [PubMed: 12297327]
39. Coscoy L, Ganem D. Kaposi's sarcoma-associated herpesvirus encodes two proteins that block cell surface display of MHC class I chains by enhancing their endocytosis. *Proceedings of the National Academy of Sciences of the United States of America* 2000;97:8051–8056. [PubMed: 10859362]
40. Ishido S, Wang C, Lee BS, Cohen GB, Jung JU. Downregulation of major histocompatibility complex class I molecules by Kaposi's sarcoma-associated herpesvirus K3 and K5 proteins. *J Virol* 2000;74:5300–5309. [PubMed: 10799607]
41. Dodd RB, Allen MD, Brown SE, Sanderson CM, Duncan LM, Lehner PJ, et al. Solution structure of the Kaposi's sarcoma-associated herpesvirus K3 N-terminal domain reveals a Novel E2-binding C4HC3-type RING domain. *J Biol Chem* 2004;279:53840–53847. [PubMed: 15465811]
42. Lehner PJ, Hoer S, Dodd R, Duncan LM. Downregulation of cell surface receptors by the K3 family of viral and cellular ubiquitin E3 ligases. *Immunol Rev* 2005;207:112–125. [PubMed: 16181331]
43. Pickart CM. Ubiquitin enters the new millennium. *Mol Cell* 2001;8:499–504. [PubMed: 11583613]
44. Yu YYL, Harris MR, Lybarger L, Kimpler LA, Myers NB, Virgin HW, et al. Physical association of the K3 protein of gamma-2 herpesvirus 68 with major histocompatibility complex class I molecules with impaired peptide and beta(2)-microglobulin assembly. *Journal of Virology* 2002;76:2796–2803. [PubMed: 11861847]
45. Duncan LM, Piper S, Dodd RB, Saville MK, Sanderson CM, Luzio JP, et al. Lysine-63-linked ubiquitination is required for endolysosomal degradation of class I molecules. *Embo Journal* 2006;25:1635–1645. [PubMed: 16601694]
46. Hewitt EW, Duncan L, Mufti D, Baker J, Stevenson PG, Lehner PJ. Ubiquitylation of MHC class I by the K3 viral protein signals internalization and TSG101-dependent degradation. *EMBO J* 2002;21:2418–2429. [PubMed: 12006494]
47. Lorenzo ME, Jung JU, Ploegh HL. Kaposi's sarcoma-associated herpesvirus K3 utilizes the ubiquitin-proteasome system in routing class major histocompatibility complexes to late endocytic compartments. *J Virol* 2002;76:5522–5531. [PubMed: 11991980]
48. Means RE, Lang SM, Jung JU. The Kaposi's sarcoma-associated herpesvirus K5 E3 ubiquitin ligase modulates targets by multiple molecular mechanisms. *J Virol* 2007;81:6573–6583. [PubMed: 17409151]
49. Means RE, Ishido S, Alvarez X, Jung JU. Multiple endocytic trafficking pathways of MHC class I molecules induced by a Herpesvirus protein. *EMBO J* 2002;21:1638–1649. [PubMed: 11927548]
50. Boname JM, de Lima BD, Lehner PJ, Stevenson PG. Viral degradation of the MHC class I peptide loading complex. *Immunity* 2004;20:305–317. [PubMed: 15030774]
51. Sanchez DJ, Gumperz JE, Ganem D. Regulation of CD1d expression and function by a herpesvirus infection. *J Clin Invest* 2005;115:1369–1378. [PubMed: 15864354]
52. Ishido S, Choi JK, Lee BS, Wang C, DeMaria M, Johnson RP, et al. Inhibition of natural killer cell-mediated cytotoxicity by Kaposi's sarcoma-associated herpesvirus K5 protein. *Immunity* 2000;13:365–374. [PubMed: 11021534]
53. Thomas M, Boname JM, Field S, Nejentsev S, Salio M, Cerundolo V, et al. Down-regulation of NKG2D and NKp80 ligands by Kaposi's sarcoma-associated herpesvirus K5 protects against NK cell cytotoxicity. *Proc Natl Acad Sci U S A* 2008;105:1656–1661. [PubMed: 18230726]
54. Li Q, Means R, Lang S, Jung JU. Downregulation of gamma interferon receptor 1 by Kaposi's sarcoma-associated herpesvirus K3 and K5. *J Virol* 2007;81:2117–2127. [PubMed: 17166914]
55. Mansouri M, Douglas J, Rose PP, Gouveia K, Thomas G, Means RE, et al. Kaposi sarcoma herpesvirus K5 removes CD31/PECAM from endothelial cells. *Blood* 2006;108:1932–1940. [PubMed: 16601245]
56. Lybarger L, Wang XL, Harris MR, Virgin HW, Hansen TH. Virus subversion of the MHC class I peptide-loading complex. *Immunity* 2003;18:121–130. [PubMed: 12530981]

57. Wang XL, Lybarger L, Connors R, Harris MR, Hansen TH. Model for the interaction of gammaherpesvirus 68 RING-CH finger protein mK3 with major histocompatibility complex class I and the peptide-loading complex. *Journal of Virology* 2004;78:8673–8686. [PubMed: 15280476]
58. Wang XL, Connors R, Harris MR, Hansen TH, Lybarger L. Requirements for the selective degradation of endoplasmic reticulum-resident major histocompatibility complex class I proteins by the viral immune evasion molecule mK3. *Journal of Virology* 2005;79:4099–4108. [PubMed: 15767411]
59. Wang XL, Herr RA, Chua WJ, Lybarger L, Wiertz EJHJ, Hansen TH. Ubiquitination of serine, threonine, or lysine residues on the cytoplasmic tail can induce ERAD of MHC-I by viral E3 ligase mK3. *Journal of Cell Biology* 2007;177:613–624. [PubMed: 17502423]
60. Cadwell K, Coscoy L. Ubiquitination on nonlysine residues by a viral E3 ubiquitin ligase. *Science* 2005;309:127–130. [PubMed: 15994556]
61. Hassink GC, Barel MT, Van Voorden SB, Kikkert M, Wiertz EJ. Ubiquitination of MHC class I heavy chains is essential for dislocation by human cytomegalovirus-encoded US2 but not US11. *J Biol Chem* 2006;281:30063–30071. [PubMed: 16877758]
62. Binette J, Dube M, Mercier J, Halawani D, Latterich M, Cohen EA. Requirements for the selective degradation of CD4 receptor molecules by the human immunodeficiency virus type 1 Vpu protein in the endoplasmic reticulum. *Retrovirology* 2007;4:75. [PubMed: 17937819]
63. Shamu CE, Story CM, Rapoport TA, Ploegh HL. The pathway of US11-dependent degradation of MHC class I heavy chains involves a ubiquitin-conjugated intermediate. *J Cell Biol* 1999;147:45–58. [PubMed: 10508854]
64. Yu H, Kopito RR. The role of multiubiquitination in dislocation and degradation of the alpha subunit of the T cell antigen receptor. *J Biol Chem* 1999;274:36852–36858. [PubMed: 10601236]
65. Tait SW, de Vries E, Maas C, Keller AM, D'Santos CS, Borst J. Apoptosis induction by Bid requires unconventional ubiquitination and degradation of its N-terminal fragment. *J Cell Biol* 2007;179:1453–1466. [PubMed: 18166654]
66. Lilley BN, Ploegh HL. A membrane protein required for dislocation of misfolded proteins from the ER. *Nature* 2004;429:834–840. [PubMed: 15215855]
67. Ye Y, Shibata Y, Yun C, Ron D, Rapoport TA. A membrane protein complex mediates retrotranslocation from the ER lumen into the cytosol. *Nature* 2004;429:841–847. [PubMed: 15215856]
68. Fiebigler E, Story C, Ploegh HL, Tortorella D. Visualization of the ER-to-cytosol dislocation reaction of a type I membrane protein. *EMBO J* 2002;21:1041–1053. [PubMed: 11867532]
69. Tirosh B, Furman MH, Tortorella D, Ploegh HL. Protein unfolding is not a prerequisite for endoplasmic reticulum-to-cytosol dislocation. *J Biol Chem* 2003;278:6664–6672. [PubMed: 12482753]
70. Wang XL, Ye YH, Lencer W, Hansen TH. The viral E3 ubiquitin ligase mK3 uses the derlin/p97 endoplasmic reticulum-associated degradation pathway to mediate down-regulation of major histocompatibility complex class I proteins. *Journal of Biological Chemistry* 2006;281:8636–8644. [PubMed: 16446359]
71. Ye Y. Diverse functions with a common regulator: ubiquitin takes command of an AAA ATPase. *J Struct Biol* 2006;156:29–40. [PubMed: 16529947]
72. Goto E, Ishido S, Sato Y, Ohgimoto S, Ohgimoto K, Nagano-Fujii M, et al. c-MIR, a human E3 ubiquitin ligase, is a functional homolog of herpesvirus proteins MIR1 and MIR2 and has similar activity. *J Biol Chem* 2003;278:14657–14668. [PubMed: 12582153]
73. Holzerlandt R, Orengo C, Kellam P, Alba MM. Identification of new herpesvirus gene homologs in the human genome. *Genome Res* 2002;12:1739–1748. [PubMed: 12421761]
74. Jenner RG, Boshoff C. The molecular pathology of Kaposi's sarcoma-associated herpesvirus. *Biochim Biophys Acta* 2002;1602:1–22. [PubMed: 11960692]
75. Morokuma Y, Nakamura N, Kato A, Notoya M, Yamamoto Y, Sakai Y, et al. MARCH-XI, a novel transmembrane ubiquitin ligase implicated in ubiquitin-dependent protein sorting in developing spermatids. *Journal of Biological Chemistry* 2007;282:24806–24815. [PubMed: 17604280]
76. Ohmura-Hoshino M, Goto E, Matsuki Y, Aoki M, Mito M, Uematsu M, et al. A novel family of membrane-bound E3 ubiquitin ligases. *Journal of Biochemistry* 2006;140:147–154. [PubMed: 16954532]

77. Bartee E, Mansouri M, Nerenberg BTH, Gouveia K, Fruh K. Downregulation of major histocompatibility complex class I by human ubiquitin ligases related to viral immune evasion proteins. *Journal of Virology* 2004;78:1109–1120. [PubMed: 14722266]
78. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 1985;39:783–791.
79. Notredame C, Higgins DG, Heringa J. T-Coffee: A novel method for fast and accurate multiple sequence alignment. *J Mol Biol* 2000;302:205–217. [PubMed: 10964570]
80. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425. [PubMed: 3447015]
81. de Gassart A, Camosseto V, Thibodeau J, Ceppi M, Catalan N, Pierre P, et al. MHC class II stabilization at the surface of human dendritic cells is the result of maturation-dependent MARCH I down-regulation. *Proc Natl Acad Sci U S A* 2008;105:3491–3496. [PubMed: 18305173]
82. Matsuki Y, Ohmura-Hoshino M, Goto E, Aoki M, Mito-Yoshida M, Uematsu M, et al. Novel regulation of MHC class II function in B cells. *Embo Journal* 2007;26:846–854. [PubMed: 17255932]
83. Thibodeau J, Bourgeois-Daigneault MC, Huppe G, Tremblay J, Aumont A, Houde M, et al. Interleukin-10-induced MARCH1 mediates intracellular sequestration of MHC class II in monocytes. *Eur J Immunol* 2008;38:1225–1230. [PubMed: 18389477]
84. Ohmura-Hoshino M, Matsuki Y, Aoki M, Goto E, Mito M, Uematsu M, et al. Inhibition of MHC class II expression and immune responses by c-MIR. *Journal of Immunology* 2006;177:341–354.
85. Cadwell K, Coscoy L. The specificities of Kaposi's sarcoma-associated herpesvirus-encoded E3 ubiquitin ligases are determined by the positions of lysine or cysteine residues within the intracytoplasmic domains of their targets. *J Virol* 2008;82:4184–4189. [PubMed: 18272573]
86. Hoer S, Smith L, Lehner PJ. MARCH-IX mediates ubiquitination and downregulation of ICAM-1. *FEBS Lett* 2007;581:45–51. [PubMed: 17174307]
87. Christensen DE, Brzovic PS, Klevit RE. E2-BRCA1 RING interactions dictate synthesis of mono- or specific polyubiquitin chain linkages. *Nat Struct Mol Biol* 2007;14:941–948. [PubMed: 17873885]
88. Nakamura N, Kimura Y, Tokuda M, Honda S, Hirose S. MARCH-V is a novel mitofusin 2- and Drp1-binding protein able to change mitochondrial morphology. *EMBO Rep* 2006;7:1019–1022. [PubMed: 16936636]
89. Karbowski M, Neutzner A, Youle RJ. The mitochondrial E3 ubiquitin ligase MARCH5 is required for Drp1 dependent mitochondrial division. *J Cell Biol* 2007;178:71–84. [PubMed: 17606867]
90. Yonashiro R, Ishido S, Kyo S, Fukuda T, Goto E, Matsuki Y, et al. A novel mitochondrial ubiquitin ligase plays a critical role in mitochondrial dynamics. *EMBO J* 2006;25:3618–3626. [PubMed: 16874301]
91. Songyang Z, Fanning AS, Fu C, Xu J, Marfatia SM, Chishti AH, et al. Recognition of unique carboxyl-terminal motifs by distinct PDZ domains. *Science* 1997;275:73–77. [PubMed: 8974395]
92. Hassink G, Kikkert M, van Voorden S, Lee SJ, Spaapen R, van Laar T, et al. TEB4 is a C4HC3 RING finger-containing ubiquitin ligase of the endoplasmic reticulum. *Biochemical Journal* 2005;388:647–655. [PubMed: 15673284]
93. Kreft SG, Wang L, Hochstrasser M. Membrane topology of the yeast endoplasmic reticulum-localized ubiquitin ligase Doa10 and comparison with its human ortholog TEB4 (MARCH-VI). *J Biol Chem* 2006;281:4646–4653. [PubMed: 16373356]
94. Fukuda H, Nakamura N, Hirose S. MARCH-III is a novel component of endosomes with properties similar to those of MARCH-II. *Journal of Biochemistry* 2006;139:137–145. [PubMed: 16428329]
95. Nakamura N, Fukuda H, Kato A, Hirose S. MARCH-II is a syntaxin-6-binding protein involved in endosomal trafficking. *Mol Biol Cell* 2005;16:1696–1710. [PubMed: 15689499]
96. Bock JB, Klumperman J, Davanger S, Scheller RH. Syntaxin 6 functions in trans-Golgi network vesicle trafficking. *Mol Biol Cell* 1997;8:1261–1271. [PubMed: 9243506]
97. Bock JB, Lin RC, Scheller RH. A new syntaxin family member implicated in targeting of intracellular transport vesicles. *J Biol Chem* 1996;271:17961–17965. [PubMed: 8663448]
98. Lu H, Bilder D. Endocytic control of epithelial polarity and proliferation in *Drosophila*. *Nat Cell Biol* 2005;7:1232–1239. [PubMed: 16258546]

99. Cao Z, Huett A, Kuballa P, Giallourakis C, Xavier RJ. DLG1 is an anchor for the E3 ligase MARCH2 at sites of cell-cell contact. *Cell Signal* 2008;20:73–82. [PubMed: 17980554]
100. Baker RK, Haendel MA, Swanson BJ, Shambaugh JC, Micales BK, Lyons GE. In vitro preselection of gene-trapped embryonic stem cell clones for characterizing novel developmentally regulated genes in the mouse. *Dev Biol* 1997;185:201–214. [PubMed: 9187083]
101. Metcalfe SM, Muthukumarana PA, Thompson HL, Haendel MA, Lyons GE. Leukaemia inhibitory factor (LIF) is functionally linked to axotrophin and both LIF and axotrophin are linked to regulatory immune tolerance. *FEBS Lett* 2005;579:609–614. [PubMed: 15670816]
102. Muthukumarana PA, Lyons GE, Miura Y, Thompson LH, Watson T, Green CJ, et al. Evidence for functional inter-relationships between FOXP3, leukaemia inhibitory factor, and axotrophin/MARCH-7 in transplantation tolerance. *Int Immunopharmacol* 2006;6:1993–2001. [PubMed: 17161353]
103. Nathan JA, Sengupta S, Wood SA, Admon A, Markson G, Sanderson C, et al. The Ubiquitin E3 Ligase MARCH7 is Differentially Regulated by the Deubiquitylating Enzymes USP7 and USP9X. *Traffic*. 2008
104. Schwarz R, Dayhoff M. Matrices for detecting distant relationships. :1979353–358.
105. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 2007;24:1596–1599. [PubMed: 17488738]
106. Nagase T, Ishikawa K, Miyajima N, Tanaka A, Kotani H, Nomura N, et al. Prediction of the coding sequences of unidentified human genes. IX. The complete sequences of 100 new cDNA clones from brain which can code for large proteins in vitro. *DNA Res* 1998;5:31–39. [PubMed: 9628581]
107. Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA. “Stemness”: transcriptional profiling of embryonic and adult stem cells. *Science* 2002;298:597–600. [PubMed: 12228720]
108. Metcalfe SM. Axotrophin and leukaemia inhibitory factor (LIF) in transplantation tolerance. *Philos Trans R Soc Lond B Biol Sci* 2005;360:1687–1694. [PubMed: 16147533]
109. Muthukumarana P, Chae WJ, Maher S, Rosengard BR, Bothwell AL, Metcalfe SM. Regulatory transplantation tolerance and “stemness”: evidence that Foxp3 may play a regulatory role in SOCS-3 gene transcription. *Transplantation* 2007;84:S6–11. [PubMed: 17632414]
110. Su AI, Cooke MP, Ching KA, Hakak Y, Walker JR, Wiltshire T, et al. Large-scale analysis of the human and mouse transcriptomes. *Proc Natl Acad Sci U S A* 2002;99:4465–4470. [PubMed: 11904358]
111. Boll W, Ohno H, Songyang Z, Rapoport I, Cantley LC, Bonifacino JS, et al. Sequence requirements for the recognition of tyrosine-based endocytic signals by clathrin AP-2 complexes. *EMBO J* 1996;15:5789–5795. [PubMed: 8918456]
112. Fuhrer C, Geffen I, Huggel K, Spiess M. The two subunits of the asialoglycoprotein receptor contain different sorting information. *J Biol Chem* 1994;269:3277–3282. [PubMed: 8106365]
113. Owen DJ, Evans PR. A structural explanation for the recognition of tyrosine-based endocytotic signals. *Science* 1998;282:1327–1332. [PubMed: 9812899]

Abbreviations

β2m	beta-2 microglobulin
ER	endoplasmic reticulum
ERAD	ER-associated degradation
HC	heavy chain of MHC class I molecule
γHV68	gamma herpesvirus-68

KSHV	Kaposi's sarcoma-associated herpesvirus
MARCH	membrane associate RING-CH protein
MHCI	class I major histocompatibility molecule
MHCII	class II MHC molecule
MVB	multivesicular body
TAP	transporter associated with antigen processing
TGN	trans-Golgi network

Figure 1. ERAD pathway induced by viral ligase mK3

A. mK3 binds to its primary binding partner TAP that is associated with the class I specific chaperone tapasin. B. After assembly with $\beta 2m$, the MHC class I heavy chain (HC) binds the tapasin/TAP complex to await a suitable peptide ligand. C. The association of mK3 with TAP/tapasin induces the recruitment of an E2 conjugase and polyubiquitination of the HC tail. $\beta 2m$ also dissociates from HC perhaps bringing the ER chaperone calnexin (CXN) into the multimeric complex as shown in the next panel. D. Polyubiquitination of the HC initiates partial denaturation of HC and the recruitment of the ATPases p97. Then p97 facilitates the retro-translocation of HC to the cytosol via a putative dislocation channel that potentially includes Derlin and mK3. E. HC undergoes proteasome-mediated degradation in the cytosol as chaperoned by p97, resulting in ubiquitin monomers and peptides.

Figure 2. Evolutionary relationships of murine and viral RING-CH domains

Amino acid sequences of murine MARCH and viral RING-CH domains were aligned using the T-COFFEE algorithm, and the evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 5000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Dayhoff matrix based method and are in the units of the number of amino acid substitutions per site [104]. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons

(Pairwise deletion option). There were a total of 58 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 [105].

KSHV (kK5)	CWICREEV---G-NEGIHPCACTGELDVVHPQCLSTWLTVS-----RNTACQMC
KSHV (kK3)	CWICNEEL---G-NERFRACGCTGELENVHRSCLSTWLTIS-----RNTACQIC
γHV68 (mK3)	CWICHQPE---G-PL-KRFCGCKGSCAVSHQDCLRGWLETS-----RRQTCALC
mMARCH II	CRICHE-GA--NGENLLSPCGCTGTLGAVHKSCLEKWLSS-----SNTSYCELC
mMARCH IX	CRICFQ-GP--EQGELLSPCRCDGSVRCTHQPCLIRWISE-----RGSWSCELC
mMARCH VI	CRVCRSEGTPE--KPLYHPCVCTGSIKFIHQECLVQWLKH-----SRKEYCELC
mMARCH VIII	CRICHCEGDDE--SPLITPCHCTGSLHFVHQACLQQWIKS-----SDTRCCELC
mMARCH V	CWVCFATDEDDRTAEWVRPCRCRGSTKWVHQACLQRWVDEKQQRGNSTARVACPQC

RING-CH C-X₂-C-X₁₀₋₄₅-C-X₁-C-X₇-H-X₂-C-X₁₁₋₂₅-C-X₂-C

Figure 3.

Aligned amino acid sequences of viral K3 and murine RING-CH domains. Using the T-COFFEE algorithm, amino acid sequences of kK3, kK5 and mK3 RING-CH domains as well as the RING-CH domains from representative MARCH proteins were aligned. Blue shading reveals the conserved cysteine and histidine residues of the C4HC3 motif responsible for coordinating two zinc ions (blue shading Fig. 3).

Overview of cellular MARCH members

Name ^a	GeneID	Size/topology	Substrate	Subcellular location	Expression	Additional functional domains ^c	Binding proteins	Physiological function	Regulation
MARCH1	55016 (hu) 72925 (mo)	272aa, 30.9kDa, 285aa, 32.0kDa, 2TMs	MHCII (β chain) [77, 81,82,83], B7.2 [81, 77], Fas, TFR [77]	Endocytic or lysosomal vesicles [77]	Restricted to secondary lymphoid tissues, such as spleen and lymph node [77], particularly high in B cells [82]	Tyrosine-based motif (YXXΦ)*; PDZ binding motif*		Regulation of immune response by inhibition of MHCII expression on APCs (KO mouse) [82]	Maturation-dependent transcriptional down- regulation in DC [81] mRNA expression in monocytes can be induced by IL10 [83]
MARCH2	51257 (hu) 224703 (mo)	246aa, 27.0kDa, 287aa, 31.9kDa, 2TMs	TFR [77,95], DLGI [99], B7.2 [77]	Endosomal vesicles and plasma membrane [95]; cell-cell connections [99]	Abundantly and broadly expressed with elevated level in heart [77]	PDZ binding motif (localization) [95]; Tyrosine-based motif (YXXΦ)*	Syntaxin6 [95]	Regulation of trafficking between TGN and endosomes (suggested by RNAi) [95]	
MARCH3	115123 (hu) NP_796089 (mo)	253aa, 28.5kDa, 218aa, 24.5kDa, 2TMs	Causes relocalization of TGN46 & inhibition of Tf uptake when OE [94]	Early endosome (RING-dep) [94]	Broadly expressed, relatively high in spleen, colon and lung [94]	PDZ binding motif (localization and inhibition of Tf uptake) [94]; Tyrosine- based motif (YXXΦ)*	MARCH2, Syntaxin6 [94]	Regulation of vesicular trafficking in endosome (suggested by OE) [94]	
MARCH4	57574 (hu) 381270 (mo)	410aa, 45.5kDa, 409aa, 45.2kDa, 2TMs	MHCI, CD4 [77]	Golgi [77]	Mainly expressed in brain and placenta [77]	PDZ binding motif*; Tyrosine-based motif (YXXΦ)*		Regulation of MHCII antigen presentation (OE)?	
MARCH5 (MITOL)	54708 (hu) 69104 (mo)	278aa, 31.2kDa, 278aa, 31.2kDa, 4TMs	hFis1 [90], Drp1? [90, 88]	Mitochondrial outer membrane [88]	Ubiquitously expressed in multiple tissues [90]		Mitofusin 2 (MFN2) & ub- Drp1 [88]; hFis1 & Drp1 [90]	regulation of mitochondrial morphology (RNAi) [89,88,90]	Autoubiquitination [90]
MARCH6 (TEB4)	10299 (hu) 223455 (mo)	910aa, 102.5kDa 909aa, 102.3kDa 13TMs [92]/ 14TMs [93]	?	ER	Broadly expressed [106], not induced by ER stress [92]			ERAD?	Autoubiquitination [92]
MARCH7^b (axotrophin)	64844 (hu) 57438 (mo)	704aa, 78.0kDa, 693aa, 76.6kDa, 1TMs?	?	Cytosol, nucleus, and plasma membrane [103]	Preferentially expressed in cells of neuronal origin and stem cells [107]	Tyrosine-based motif (YXXΦ)*	USP7, USP9X [103]	Involved in neural differentiation and establishment of transplantation tolerance; influential in thymic Foxp3+T cell development (<i>axot</i> null mouse) [102,108,101,109]	Autoubiquitination; Stability and sublocalization are affected by USP7 and USP9X [103]
MARCH8 (c-MIR)	220972 (hu) 71779 (mo)	291aa, 33.0kDa, 286aa, 32.2kDa, 2TMs	MHCII (β chain) [84], B7.2 [77,72], Fas, TFR [77]	Endocytic or lysosomal vesicles [77]	High in lymph nodes [72]; increased predominantly in APCs in Tg mouse [84]	Tyrosine-based motif (YXXΦ)*; PDZ binding motif*		Influence CD4 T cell development in thymus and periphery (MARCH8 Tg mouse) and regulate immune response [84]	No maturation-dependent transcriptional down- regulation in DC [81] Not induced by IL10 [83]
MARCH9	92979 (hu) 216438 (mo)	346aa, 37.8kDa, 348aa, 37.9kDa, 2TMs	MHCI [77,86], CD4 [77], ICAM-1 [86]	Lysosome [86], TGN [77]	Abundantly and broadly expressed with elevated levels in lymph tissues and lungs [77]; elevated in DCs, T, and B cells [110]	TM (Substrate specificity) [77] PDZ binding motif*		Regulation of MHCII antigen presentation and T cell activation by targeting surface MHCII and ICAM for endocytosis [77,86]	
MARCH10^b	162333 (hu) 632687 (mo)?	808aa, 90.5kDa, 237aa, 26.3kDa, TM?	?						

Name ^a	GeneID	Size/topology	Substrate	Subcellular location	Expression	Additional functional domains ^c	Binding proteins	Physiological function	Regulation
MARCH11	441061 (hu) 211147 (mo)?	402aa, 43.9kDa, 2TMs 252aa, 27.2kDa, 0TM	CD4 [75]	TGN-derived vesicles and MVB [75]	Predominantly in testis and weakly pituitary [75]	Tyrosine-based motif (YXXΦ) (adaptin binding) and PDZ binding motif [75]	μ1-adaptin [75]	Involved in spermatogenesis in mammals (endogenous MARCH11) [75]	

^a Official symbols accepted by NCBI are MARCH for human and March for mouse. To avoid confusion we use MARCH for both human and mouse proteins in this table.

^b Unlike the other MARCHs the RING-CH domains of these two MARCH proteins are located proximal to their C termini and possess zero to one predicted transmembrane domain (TM).

^c Additional functionally uncharacterized motifs outside the RING-CH domain predicted by well recognized criteria are noted by an asterisk [99,111,112,113]