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REVIEW

MHC class II fine tuning by ubiquitination: lesson from MARCHs

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Abstract
Ubiquitination, a posttranscriptional modification, has been known to contribute to many aspects of cellular event (e.g., protein quality control, signal transduction). In 2007 and 2016, we reported physiological E3 ubiquitin ligases for MHC class II; these are membrane-associated ring-CH-type finger (MARCH)-1 and MARCH-8. Importantly, MARCH-1 and -8 are structurally close to each other, but have different expression profiles. MARCH-1 and -8 are expressed at secondary lymphoid organs and thymic epithelial cells, respectively. These findings suggest contribution of MARCHs to immunological disorders in human; however, its contribution remains to be elucidated. In this review, recent progress on MARCHs will be summarized from molecular and/or immunological point of view and future direction would be discussed.

Keywords MHC class II · Ubiquitin · E3 ubiquitin ligase

Brief history of MARCHs

In 2000, two groups including us reported modulator of immune recognitions (MIRs) which are encoded by Kaposi's sarcoma-associated herpesvirus (Ishido et al. 2000b; Coscoy and Ganem 2000). MIRs are MIR1 and MIR2, designated by Ganem's group (Coscoy and Ganem 2000). Since both MIRs have the ability to inhibit surface expression of MHC class I, these are thought to contribute to viral immune evasion. In 2002, Ganem's group demonstrated that MIRs belong to RING type E3 ubiquitin ligase by examining zing finger domain of MIRs (Coscoy et al. 2001). In addition to zing finger domain as a catalytic domain, MIRs were shown to have two transmembrane regions (TM) which are involved in recognition of their substrates; MIR2's TM could target MIR2's substrate, which is CD86/B7-2, in the context of MIR1 (Sanchez et al. 2002). In 2004, membrane-associated ring-CH-type fingers (MARCHs) were discovered by searching functional homologues of viral MIRs in the database. These works were initiated by two groups: ours and Fruh's group (Goto et al. 2003; Bartee et al. 2004). Since large DNA virus (e.g., Herpesvirus) encodes several immune modulators presumably derived from host genome, viral MIRs are thought to be derived from MARCHs (Choi et al. 2001). Though, at present, several MARCHs were reported, in this review MARCH-1 and MARCH-8 will be highlighted, because these MARCHs were intensively examined from molecular and immunological point of view (Ohmura-Hoshino et al. 2006; Bauer et al. 2017). Hereafter, "MARCH-1 and MARCH-8" are described as "MARCHs."

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MARCH-1 and -8

Similar to MIRs, MARCH-1 and -8 are structurally and functionally close to each other; therefore, they might be evolved by gene duplication. Both MARCHs regulate peptide-MHC class II complex (pMHC II), which are critical for "acquired immunity," through ubiquitination of MHC II beta chain's cytoplasmic tail (Ishido et al. 2009). Therefore, ubiquitination by both MARCHs contributes to surface expression of pMHC II, but not to quality control of MHC II protein itself (Ishido et al. 2010). In addition to these molecular features, both MARCHs have individual expression profile; MARCH-1 and -8 are expressed at antigen-presenting cells (e.g., dendritic

cells, B cells) in secondary lymphoid organs (e.g., lymph node, spleen) and at thymic epithelial cells, respectively, indicating individual contribution to immune regulation (Matsuki et al. 2007; von Rohrscheidt et al. 2016; Liu et al. 2016).

MARCH-1's function in vivo

To examine how MARCHs contribute to immune regulation, our group examined how MARCHs are regulated in the context of immune responses and generated and analyzed genetically modified mice (e.g., MARCHs knock-out mice) (Matsuki et al. 2007). MARCH-1 was shown to be expressed stably in immature dendritic cells, but downregulated quickly by stimuli (e.g., LPS) (De Gassart et al. 2008; Young et al. 2008). MARCH-1 downregulation correlated with loss of pMHC II ubiquitination (Young et al. 2008). Since ubiquitination inhibits the function of membrane proteins (e.g., EGF receptor) through lysosomal degradation, downregulation of MARCH-1 was suggested to be able to initiate proper immune responses and maintain immunological consequences (Ishido et al. 2009; Foot et al. 2017). In this line, deletion of MARCH-1 in immature dendritic cells showed augmented expression of pMHC II and enhanced antigen presentation (Matsuki et al. 2007; Ishikawa et al. 2014). However, these phenomena did not lead to make "super DCs," instead suppress DC function in terms of ability of cytokine secretion and stimulation of naïve CD4 T cells (Ohmura-Hoshino et al. 2009; Ishikawa et al. 2014). These findings led to different direction; sustained pMHC II or augmented pMHC II may be unwelcome for immunity (Ishido et al. 2010). This idea was proved recently; deletion of MARCH-1 inhibits the development of thymic Treg through accumulated pMHC II-mediated disruption of lipid rafts (Oh et al. 2018). Thus, deleterious effects of accumulated pMHC II in dendritic cells will be an obstacle to analyze MARCH's function. However, this may not be the case in germinal center (GC) B cells (Bannard et al. 2016). GC B cells consist of centrocyte and centroblast, which contribute to affinity maturation of antibody (Bannard and Cyster 2017). It is known that BCR-mediated antigen-uptake and MHC II-mediated antigen presentation are critical for GC B cell generation (Bannard and Cyster 2017). Interestingly, pMHC II was continuously degraded by MARCH-1 in centroblast, but not in centrocyte (Bannard et al. 2016). These findings led to examination about whether MHC II regulation contributes to GC generation. In this experiment, loss of pMHC II ubiquitination did not interfere initial GC response, but disturbed long-term GC response for influenza virus infection (Bannard et al. 2016). These findings suggest that MARCH-1-mediated continuous replacement of pMHC II on GC B cells are required for long-term GC response (Fig. 1).

MARCH-8's function in vivo

Even though MARCH-8 is similar to MARCH-1 in terms of structure and function, MARCH-8 is expressed in thymic epithelial cells, but not in peripheral antigen-presenting cells (e.g., dendritic cells, B cells) (Matsuki et al. 2007; von Rohrscheidt et al. 2016; Liu et al. 2016). Evidence showing MARCH-8 as physiological E3 ubiquitin ligase was recently obtained from analysis of CD83-deficient mice (Liu et al. 2016; von Rohrscheidt et al. 2016). CD83 was shown to contribute to positive selection for CD4 T cells in the thymus; CD83 was mainly expressed at cortical thymic epithelial cells and deletion of CD83 interfered positive selection, but not negative selection for CD4 T cells (von Rohrscheidt et al. 2016). Previous report describing CD83 as a competitor of MARCH-1 in the periphery suggested the presence of equivalent interaction in the thymus (Tze et al. 2011). Since MARCH-1 was reported not to be expressed in the thymus, MARCH-8, a functional homologue of MARCH-1, was subjected to examination. Consistent with speculation, deletion of MARCH-8 canceled deleterious effect by CD83 defect (von Rohrscheidt et al. 2016). Importantly, MARCH-8 was mainly expressed in cortical thymic epithelial cells, but not in medullary thymic epithelial cells (von Rohrscheidt et al. 2016). Thus, balanced activity of CD83 and MARCH-8 regulates positive selection at cortical thymic epithelial cells (Fig. 2).

Struggle with MARCHs in immunology

Even though the characters of MARCHs are extremely attractive, MARCHs are not highlighted from clinical point of view. Does impaired function of MARCHs lead to disease in human? The answer is still under the ground. In addition, does stimuli-induced MARCH-1 downregulation promote beneficial immune responses against pathogens (Ishido et al. 2009)? Given that accumulated pMHC II has deleterious effect on dendritic cells, it will be hard to prove this concept by using MARCH-1-deficient mice. Alternatively, it will be an effective way to inhibit the machinery of MARCH-1 downregulation; continuous pMHC II ubiquitination in stimulated/activated dendritic cells will show aberrant immunological consequences. Therefore, the machinery of transcriptional or posttranscriptional regulation of MARCH-1 should be examined. Recently, Roche's group has tackled this important issue (e.g., transcriptional regulation) and demonstrated responsible region for stimuli-induced transcriptional suppression by using in vitro generated dendritic cells (Kaul et al. 2018). Moreover, life span of MARCH-1 protein was shown to be short by using exogenously expressed MARCH-1, suggesting posttranscriptional regulation is present in vivo (Jabbour et al. 2009). It is necessary to test whether these are the case in vivo although detection of MARCH-1 protein is not easy.

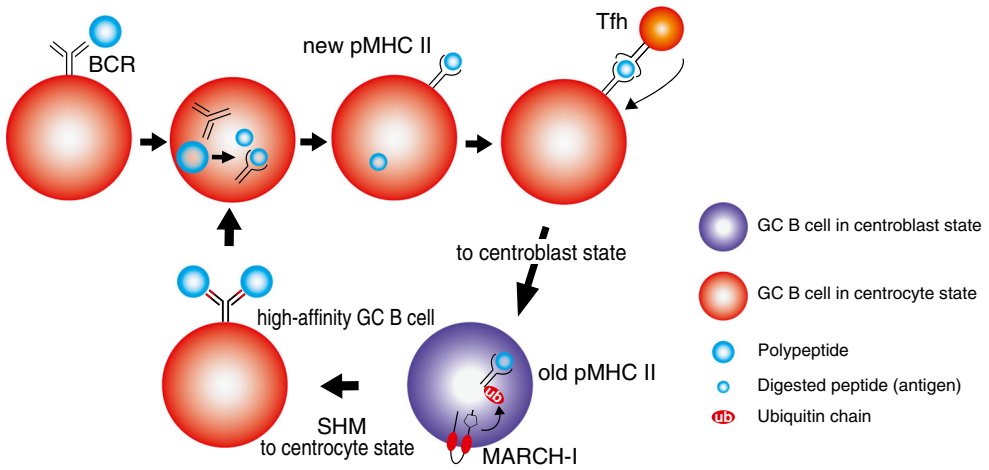

Fig. 1 Constitutive replacement of pMHC II by MARCH-1 for long-term GC response. Polypeptides captured by BCR are digested and converted to peptides/antigens in germinal center (GC) B cell in centrocyte state, then peptides/antigens make GC B cells to be ready to receive stimuli from peptide/antigen-specific follicular helper T cells (Tfh) by generating new peptide-MHC class II complexes (pMHC II). Once received Tfh's stimuli, GC B cells will go to centroblast state and undergo somatic hypermutation (SHM) to be high-affinity GC B cells. If they have a higher-affinity BCR by SHM, they more efficiently present antigens than before and support long-term GC responses. Since "old" pMHC II will hamper generation of new pMHC II, which are necessary to receive "fresh" Tfh's stimuli and support long-term GC responses, MARCH-1 is expressed in GC B cell in centroblast state to throw away "old" pMHC II

Recognition mode of MARCHs

MARCHs are extremely attractive from another point of view, because they have favorites; MARCHs effectively ubiquitinate MHC II and CD86/B7-2, but not MHC class I and ICAM-1 by their exogenous expression (Ishido et al. 2000a). Exploration of the way how they determine favorites was initiated by studies of MIRs: MIR1 and MIR2, since CD86/B7-2 is recognized by MIR2 but not MIR1. Region-swapping experiment revealed that TM of CD86/B7-2 is sufficient to be ubiquitinated by MIR2 (Ishido et al. 2000a). In addition, TM of MIR2 was shown to recognize CD86/B7-2 in the context of MIR1, suggesting that MIRs determine their favorites by TM-TM interaction (Sanchez et al. 2002). Similar to MIRs, MARCHs also recognize TM of MHC II and CD86/B7-2 (Jahnke et al. 2012). Interestingly, inhibition of MHC II and CD86/B7-2 by MARCH-1 is interfered by exogenous expression of CD83's TM (Tze et al. 2011). However, at present, molecular basis of TM-TM interaction by MARCHs remains obscure, because charge pair interaction and GXXXG motif-mediated interaction, which are well-recognized modes for TM-TM interaction, were shown not to be involved in recognition by MARCHs (Russ and Engelman 2000; Jahnke et al. 2012; Walther and Ulrich 2014). In addition to TM-mediated recognition, different mode of MARCH's recognition has come up; extracellular juxtamembrane (JM) region of B7-2 was shown to be recognized by short intertransmembrane (ITM) loop of MIR2 (Kajikawa et al. 2012). Mutational analysis suggested that ITM of MIR2 recognizes aspartic acid located at CD86/B7-2's JM (Kajikawa et al. 2012). This seems to be the case with MARCH-8; ITM loop of MARCH-8 recognizes a lysine residue located at JM region of HLA DR beta chain (Jahnke et al. 2012). Computational structural prediction and mutagenesis analysis indicated that aromatic ring of MIR2's Phe119 allows MIR2's ITM to recognize CD86/B7-2's JM by anchoring MIR2's ITM on plasma membrane (Kajikawa et al. 2012).

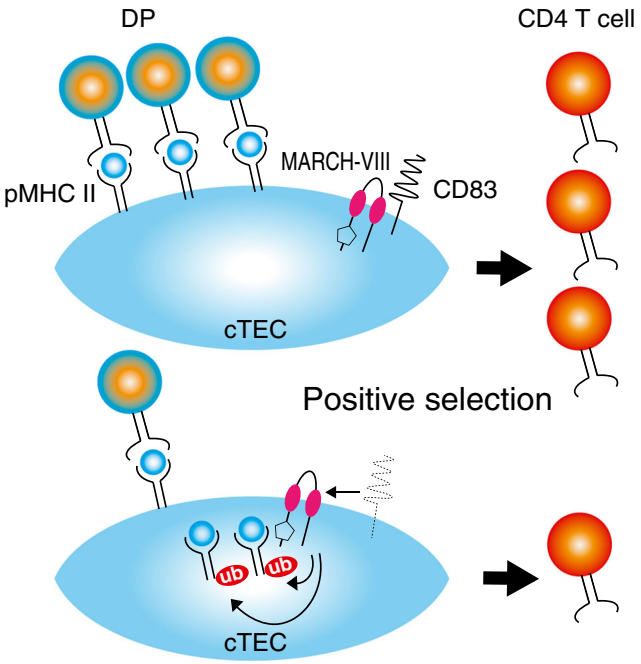

Fig. 2 Positive selection by balanced activity of MARCH-8 and CD83. In cortical thymic epithelial cells (cTEC), MARCH-8 is expressed. In the presence of CD83, MARCH-8 is captured by CD83's TM and inactivated, then pMHC II are able to promote positive selection for CD4 T cells. In contrast, suppression of CD83 leads to activation of MARCH-8 by releasing from CD83 and inhibit positive selection from double-positive T cells (DP)

Interestingly, ITM of MARCH-8 also has an aromatic amino acid, but this location is different from MIR2's Phe119. Thus, ITM-mediated recognition may be common between MIRs and MARCHs; however, details might be different (Fig. 3).

Struggle with MARCHs in molecular cellular biology

So far, proposed model for recognition by MARCHs is based on direct interaction model between MARCHs and their substrates. On the other hand, proteolipid protein 2 (PLP2) was identified as co-factor for MIRs, rising possibility of indirect interaction mode (Timms et al. 2013). This is not the case with MARCHs; PLP2 was not shown to contribute to recognition by MARCHs (Timms et al. 2013). In vitro re-constitution system is definitely required to verify the proposed modes, but even in vitro preparation of MIRs and MARCHs has been failed so far. Indeed, there are a few Protein Data Bank (PDB)-registered three-dimensional structures of two-transmembrane proteins at present, compared with seven-helix transmembrane receptors (Kozma et al. 2013). Given hopeless structural experiments for MARCHs at present, other approaches (e.g., fluorescence resonance energy transfer (FRET) or proximity ligation assay (PLA)) are strongly considered (Khadria and Senes 2015; Ota et al. 2017).

Concluding remarks

Even though almost ten years has passed since we reported MARCH-1 as a physiological E3 ubiquitin ligase of MHC II by genetical approach, pathological relevance of MARCHs remains obscure. Intensive examination of MARCHs expression at mRNA and protein level is definitely required at least in animal diseases. In addition, MARCH's recognition mode seems to be more complicated than expected. Given that structural analyses of MARCHs are technically hard at present, cell biological experiments combined with careful mutational analysis must be carried on.

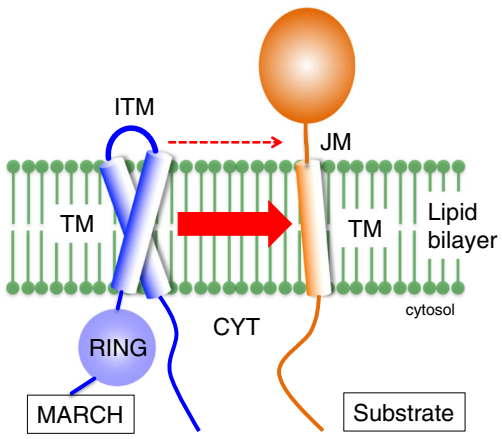

Fig. 3 Schematic model for direct recognition by MARCHs. Transmembrane regions (TM) of MARCHs recognize TM of substrate (large arrow) in lipid bilayer. ITM also contributes to recognition (dashed arrow); ITM of MARCHs recognize juxtamembrane (JM) of substrate

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