CARD14-Mediated Activation of Paracaspase MALT1 in Keratinocytes: Implications for Psoriasis

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Mutations in *caspase recruitment domain-containing protein 14 (CARD14)* have been linked to susceptibility to psoriasis. CARD14 is an intracellular scaffold protein that regulates proinflammatory gene expression. Recent studies have offered novel insights into the mechanisms of CARD14-mediated signaling in keratinocytes and the molecular impact of psoriasis-associated CARD14 mutations. CARD14 forms a signaling complex with BCL10 and the paracaspase MALT1, and this process is enhanced upon pathogenic CARD14 mutation, culminating in the activation of MALT1 protease activity and psoriasis-associated gene expression. This review summarizes the current knowledge of CARD14/MALT1-mediated signaling in keratinocytes and its therapeutic implications in psoriasis.

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PSORIASIS, A SKIN DISEASE WITH A STRONG GENETIC COMPONENT

Psoriasis is a common chronic autoinflammatory skin disease that affects 2–3% of the world's population and greatly impairs the quality of life of affected individuals. Psoriasis vulgaris, the most prevalent disease type, is characterized by well-demarcated red, scaly plaques. More rare types of psoriasis also exist, such as pustular, palmoplantar, inverse, erythrodermic, and guttate psoriasis (Deng et al., 2016; Lowes et al., 2014; Nestle et al., 2009). Furthermore, psoriasis is associated with several comorbidities, and almost 30% of patients suffer from psoriatic arthritis, indicating that this disease is not only restricted to the skin (Mease et al., 2013).

Psoriasis-affected skin is characterized by a thickened epidermis with scaly patches, due to excessive proliferation and aberrant differentiation of keratinocytes, as well as redness caused by increased dilatation of the dermal blood vessels and infiltration of immune cells (Lowes et al., 2014). Although the pathogenesis of psoriasis has not yet been completely elucidated, it is generally believed to arise from a complex interplay between hyperproliferating keratinocytes and infiltrating, activated immune cells, mainly dendritic cells and T cells. Skin injury or associated infections trigger keratinocytes to elicit IL-23 and IL-12 production in dendritic cells. These cytokines in turn activate T cells and induce the production of several psoriatic cytokines, such as IL-17, IFN-γ, tumor necrosis factor (TNF), and IL-22, which further induce keratinocyte hyperproliferation as well as the production of chemokines to sustain the recruitment and activation of immune cells (Lowes et al., 2014).

Even though the etiology of psoriasis is still largely unknown, the concordance rate of psoriasis in monozygotic twins of approximately 70% illustrates that there is a strong genetic component. Through linkage disequilibrium studies in psoriasis-affected families, multiple psoriasis susceptibility (*PSORS*) loci have been identified (Lowes et al., 2014). However, most of the genes responsible for the observed susceptibility are not known (Harden et al., 2015; Lowes et al., 2014). Recently, mutations in *caspase recruitment domain-containing protein 14* (*CARD14*), a gene located in the *PSORS2* locus, have been linked to psoriasis susceptibility (Jordan et al., 2012a, 2012b). Here, we review the role of CARD14-mediated signaling in keratinocytes and its potential implications for psoriasis therapy.

CARD14 structure and function

CARD14, also known as CARD-containing MAGUK protein 2 (CARMA2) and Bimp2, is a member of the CARMA family of proteins, which also includes CARD11/CARMA1 and CARD10/CARMA3 (Bertin et al., 2001; Gaide et al., 2001; McAllister-Lucas et al., 2001; Scudiero et al., 2014). Similar to CARD10 and CARD11, CARD14 acts as a scaffolding protein that can activate the inflammatory transcription factor NF-κB (Bertin et al., 2001). The CARMA proteins have a uniform domain structure consisting of an N-terminal CARD domain followed by a coiled-coil (CC) domain, a linker region, and a C-terminal membrane-associated guanylate

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Abbreviations: BCL10, B-cell lymphoma/leukemia 10; CARD14, caspase recruitment domain-containing protein 14; CARMA, CARD-containing MAGUK protein; CC, coiled coil; MAGUK, membrane-associated guanylate kinase; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; PKC, protein kinase C; TNF, tumor necrosis factor

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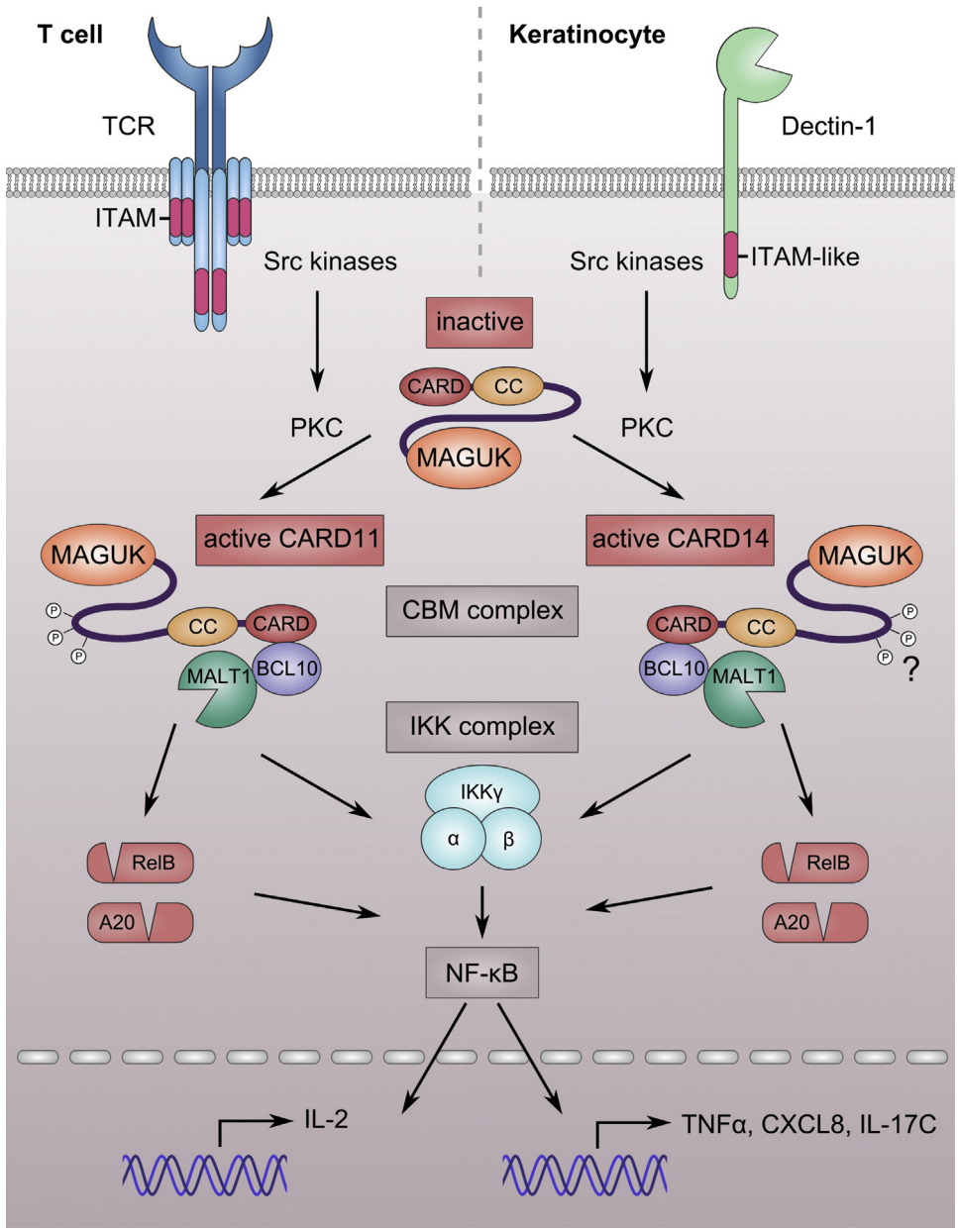

Figure 1. Comparison of CBM complex formation in T cells and keratinocytes. Triggering of the ITAM-containing receptors TCR and dectin-1 activates kinases from the Src and PKC families, which are believed to be required for the assembly of a CARD-BCL10-MALT1 (CBM) signaling complex. Whereas BCL10 and MALT1 are recruited to CARD11 in lymphocytes, a CARD14-containing complex is formed in keratinocytes. Both CARD11 and CARD14 interact with BCL10 via their CARD domains, which is only accessible in CARD11 after PKC-mediated phosphorylation of its linker region. The CBM complex subsequently activates the transcription factor NF-κB in two ways. First, it acts as a scaffold to recruit and activate the IKK complex, which phosphorylates and thus targets IκBα for proteasomal degradation, allowing the nuclear translocation of NF-κB. Secondly, it promotes optimal NF-κB activation by MALT1-mediated cleavage of RelB and A20. In both T cells and keratinocytes, NF-κB regulates the expression of proinflammatory cytokines. BCL10, B-cell lymphoma/leukemia 10; CARD14, caspase recruitment domain-containing protein 14; CBM, CARD-BCL10-MALT1; CC, coiled coil; ITAM, immunoreceptor tyrosine-based activation motif; IκB, inhibitor of κB; IKK, IκB kinase; MAGUK, membrane-associated guanylate kinase; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; PKC, protein kinase C; TCR, T cell receptor.

kinase domain (MAGUK) comprising PDZ, SH3, and GUK subdomains (Figure 1) (Bertin et al., 2001; Gaide et al., 2001; McAllister-Lucas et al., 2001). Whereas the CARD and CC domains are necessary for NF-κB activation and self-oligomerization, the linker region might exert an auto-inhibitory function (Bertin et al., 2001; Howes et al., 2016; Matsumoto et al., 2005; Sommer et al., 2005; Tanner et al., 2007). The MAGUK domain targets proteins to the membrane and is involved in various processes, such as signal transduction, tight junction formation, cell proliferation, apoptosis, and differentiation (te Velthuis et al., 2007). However, its specific role in CARD14-mediated signaling is still unclear.

The *CARD14* gene gives rise to several splice variants. In addition to the full-length form (CARD14fl), a shorter splice variant (CARD14sh), which lacks a part of the membrane-associated guanylate kinase domain, has been described (Scudiero et al., 2011). The functional differences between CARD14fl and CARD14sh have remained elusive thus far, as they seem equally potent in mounting an NF-κB response (Afonina et al., 2016). A third splice variant, CARD14cardless, lacks the CARD domain as well as part of the CC domain and the SH3 and GUK domains. Because of the missing CARD domain, CARD14cardless is not able to activate NF-κB and may function as a dominant-negative regulator of CARD14 signaling (Scudiero et al., 2011).

The three CARD14 splice variants are predominantly expressed in placenta and skin tissue (Jordan et al., 2012b). In healthy skin, CARD14 is primarily expressed in the keratinocytes of the basal layer of the epidermis. In contrast, psoriatic skin lesions show increased levels of CARD14 in the upper layers of the epidermis and reduced CARD14 levels in the basal layer (Jordan et al., 2012b). This expression pattern might reflect the deregulated differentiation of keratinocytes

in psoriasis-affected skin and could suggest a role for CARD14 in keratinocyte differentiation. Harden et al. (2014) also showed that CARD14 expressed in dermal endothelial cells could modulate the expression of chemokines such as CXCL8, CXCL10, and CCL2, which sensitize the skin vasculature of psoriasis patients bearing *CARD14* mutations to inflammation. Because psoriasis is often associated with cardiovascular comorbidities, these observations might also imply a role for CARD14 in cardiovascular disease (Yim and Armstrong, 2016).

CARD14 variants in psoriasis

In 2012, Jordan et al. described several common and rare variants of *CARD14* that are directly associated with psoriasis in familial and nonfamilial cases (Jordan et al., 2012a). Since then, several studies have reported associations of *CARD14* variants with psoriasis vulgaris, psoriatic arthritis, generalized pustular psoriasis, and palmoplantar pustular psoriasis (Ammar et al., 2013, 2016; Eskin-Schwartz et al., 2016; Feng et al., 2016; Gonzalez-Lara et al., 2013; Inoue et al., 2016; Korber et al., 2013; Mossner et al., 2015; Qin et al., 2014; Sugiura et al., 2014, 2015; Zhu et al., 2016). Furthermore, *CARD14* variants have also been associated with pityriasis rubra pilaris, a distinct inflammatory skin disease characterized by keratotic follicular papules and salmon-colored erythematous plaques (Eytan et al., 2014; Fuchs-Telem et al., 2012; Has et al., 2016; Inoue et al., 2016; Li et al., 2015). An overview of all *CARD14* variants identified in patients with psoriasis and pityriasis rubra pilaris is listed in Table 1.

Most of the observed *CARD14* missense variants are heterozygous. Interestingly, exon 4, which encodes part of the CC domain, seems to be a hotspot for missense variants (Table 1). In addition, the *CARD14* variants that were shown to be most pathogenic, such as p.Glu138Ala, p.Glu142Lys, and p.Glu142Gly, are also encoded by exon 4 (Jordan et al., 2012a). Another strongly pathogenic variant, p.Gly117Ser, is encoded by exon 3 and leads to altered splicing of *CARD14*, resulting in the insertion of 22 additional amino acids between exons 3 and 4 (Jordan et al., 2012b). Overexpression of the pathogenic missense CARD14 variants in primary keratinocytes resulted in enhanced NF-κB activation and increased production of several psoriasis-associated chemokines, such as CXCL8 and CCL20 (Afonina et al., 2016; Jordan et al., 2012b). Therefore, it is generally believed that excessive activation of NF-κB and expression of NF-κB-responsive genes in keratinocytes by psoriasis-associated CARD14 variants can initiate an inflammatory reaction that attracts immune cells to the skin and culminates in psoriasis development. However, not all CARD14 variants that have been identified in patients with psoriasis and pityriasis rubra pilaris lead to excessive NF-κB activation. For instance, the variant p.Arg69Trp reduces NF-κB activation sevenfold compared with wild-type CARD14 (Ammar et al., 2016). These ambiguous effects on NF-κB suggest that CARD14 could be involved in additional signaling pathways or that basal NF-κB levels might be crucial to preserve skin homeostasis. Finally, p.Arg820Trp, a common polymorphism of *CARD14* that is associated with psoriasis susceptibility, was shown to be enriched in patients who responded well to anti-TNF therapy, indicating that *CARD14* variants might be used to stratify patients for optimal treatment strategies (Coto-Segura et al., 2016; Feng et al., 2016; Gonzalez-Lara et al., 2013; Jordan et al., 2012a; Sugiura et al., 2015). Although further research is necessary to identify pathogenic *CARD14* variants and their effects on CARD14 function, recent studies have provided some insight into CARD14-induced signaling events and how psoriasis-associated CARD14 variants may affect NF-κB activation.

THE CARD14-BCL10-MALT1 COMPLEX IN KERATINOCYTES

Activated CARD14 and its homologues are known to bind the adapter protein B-cell lymphoma 10 (BCL10) via a CARD-CARD-mediated interaction (Bertin et al., 2001; McAllister-Lucas et al., 2001; Wang et al., 2001). BCL10 is constitutively bound to mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) and has recently been shown to recruit MALT1 to activated CARD14, resulting in the formation of a CBM (CARD14-BLC10-MALT1) complex (Afonina et al., 2016; Howes et al., 2016; Lucas et al., 2001; Uren et al., 2000) (Figure 1). CARD14-induced CBM complex formation further culminates in the activation of NF-κB as well as of the MAP kinases p38 and JNK (Afonina et al., 2016). The formation of a CARD14-BCL10-MALT1 signaling complex in keratinocytes is reminiscent of the well-described CARD11-BCL10-MALT1 signaling complex that is formed in antigen receptor-stimulated lymphocytes. CBM complex assembly in lymphocytes leads to the recruitment of the ubiquitin ligase TRAF6, which mediates K63-linked polyubiquitination of itself, MALT1 and BCL10 (Deng et al., 2000; Oeckinghaus et al., 2007; Sun et al., 2004). These polyubiquitin chains serve as docking sites for the inhibitor of κB kinase complex (IKK), the linear ubiquitin chain assembly complex, and a TAK1-containing complex, resulting in the optimal activation of the IKK complex (Afonina et al., 2015; Thome, 2008). The activated IKK complex phosphorylates IκB, which is subsequently degraded by the proteasome, thus releasing NF-κB subunits into the nucleus to activate the transcription of target genes. Although the molecular signaling events downstream of the CARD14-BCL10-MALT1 signaling complex have not yet been described in keratinocytes, it can be expected that they are similar to those downstream of the CARD11-induced signaling described above (Figure 1).

The molecular events that lead to the formation of a CARD14-BCL10-MALT1 signaling complex are still largely unclear. It has been suggested that, in unstimulated cells, CARD14 is kept in an autoinhibitory conformation by the inhibitory linker domain located between the CC and the PDZ domains (Howes et al., 2016). Removal of the inhibitory domain of CARD14 abrogates the effect of psoriasis-associated activating point mutations on NF-κB induction (Howes et al., 2016). Compared to wild-type CARD14 expression, overexpression of the p.Glu138Ala and p.Gly117Ser mutants enhances CBM complex formation, leading to increased NF-κB activation (Afonina et al., 2016; Howes et al., 2016). Collectively, these data indicate that single point mutations in the CC domain of CARD14 result in

Table 1. Overview of CARD14 variants associated with psoriasis or pityriasis rubra pilaris

Mutation	Exon	Domain	Disease	Effect on NF-κB[1]	Reference
p.Arg38Cys	2	CARD	PsV	0.11	(Jordan et al., 2012a)
p.Arg62Gln	2	CARD	PsV	1.06	(Ammar et al., 2016; Jordan et al., 2012a)
p.Arg69Trp	2	CARD	PsV/PsA	0.144	(Ammar et al., 2016)
p.Gly117Ser	3	Between CARD and CC	PsV/PsA/GPP	3.71	(Ammar et al., 2013, 2016; Eskin-Schwartz et al., 2016; Jordan et al., 2012a, 2012b; Korber et al., 2013)
c.349+5G>A	3	Between CARD and CC	PsV	ND	(Jordan et al., 2012a, 2012b)
c.349+1G>A	3	Between CARD and CC	PRP type V	ND	(Fuchs-Telem et al., 2012)
p.Met119Val	4	Between CARD and CC	GPP	ND	(Qin et al., 2014)
p.Leu124Pro	4	Between CARD and CC	PRP	ND	(Eytan et al., 2014)
p.Glu138Ala	4	CC	GPP	8.95	(Jordan et al., 2012a, 2012b)
p.Glu138Lys	4	CC	PRP type V	ND	(Has et al., 2016; Inoue et al., 2016)
p.Glu138del	4	CC	PRP type V	ND	(Fuchs-Telem et al., 2012)
p.Glu142Lys	4	CC	PsV	4.03	(Jordan et al., 2012a)
p.Glu142Gly	4	CC	PsV	5.00	(Jordan et al., 2012a)
p.Leu150Arg	4	CC	PsV	1.79	(Ammar et al., 2016; Jordan et al., 2012a)
p.Arg151Gln	4	CC	PsV	1.766	(Ammar et al., 2016)
p.Arg151Trp	4	CC	PsV	0.576	(Ammar et al., 2016)
p.Leu156Pro	4	CC	PRP type V	ND	(Fuchs-Telem et al., 2012)
p.Arg166His	4	CC	GPP	ND	(Qin et al., 2014)
p.His171Asn	4	CC	PsV	0.68	(Jordan et al., 2012a)
p.Asp176His	4	CC	PsV/GPP + PsV/PPP	2.78	(Jordan et al., 2012a; Mossner et al., 2015; Sugiura et al., 2014; Zhu et al., 2016)
p.Arg179His	4	CC	PsV/PPP	1.38	(Jordan et al., 2012a; Mossner et al., 2015)
p.Val191Leu	4	CC	PsV	1.02	(Jordan et al., 2012a)
p.Glu197Lys	4	CC	PPP/PsV/PsA	1.667	(Ammar et al., 2016; Mossner et al., 2015)
p.Ser200Asn	4	CC	PsV/GPP/PPP	0.67	(Ammar et al., 2016; Jordan et al., 2012a; Korber et al., 2013; Mossner et al., 2015)
p.Leu209Pro	4	CC	PsV	0.785	(Ammar et al., 2016)
p.Ala216Thr	4	CC	PsV	0.575	(Ammar et al., 2016; Qin et al., 2014; Zhu et al., 2016)
p.Asp285Gly	6	None	PsV	1.14	(Jordan et al., 2012a)
p.Met338Val	7	CC	PsV	0.914	(Ammar et al., 2016)
p.Thr420Ala	9	none	PsV	0.663	(Ammar et al., 2016)
c.1356+5G>A	9	CC	PsV	ND	(Ammar et al., 2016)
p.Thr591Met	13	PDZ	PsV	ND	(Qin et al., 2014)
p.Ile593 Asn	13	PDZ	PsV	1.30	Jordan et al. (2012a)
p.Ser602Leu	13	PDZ	PsV/GPP/PPP	1.196	(Ammar et al., 2016)
p.Arg682Trp	15	SH3	PsV/GPP	0.95	(Jordan et al., 2012a; Qin et al., 2014)
p.Gly714Ser	15	SH3	PsV	1.02	(Jordan et al., 2012a)
p.Arg820Trp	18	GUK	PsV/PsA	ND	(Feng et al., 2016; Gonzalez-Lara et al., 2013; Jordan et al., 2012a; Sugiura et al., 2015)
p.Asp973Glu	21	GUK	PsV	ND	(Jordan et al., 2012a)

Details on the location of the mutations, their disease occurrence, and their effect on NF-κB activation is shown. The exon sequence was determined using transcript CARD14-201 (ENST00000344227).

Abbreviations: CARD, caspase recruitment domain; CC, coiled coil; GPP, generalized pustular psoriasis; GUK, guanylate kinase-like; ND, not determined; PDZ, postsynaptic density 95/disk large/zona occludens 1; PPP, palmoplantar pustular psoriasis; PsA, psoriatic arthritis; PsV, psoriasis vulgaris; PRP, pityriasis rubra pilaris; SH3, SRC homology 3.

[1]Fold change compared with unstimulated CARD14 WT.

conformational changes that affect inter- or intramolecular interactions crucial for CBM assembly.

It has been shown that MALT1 can be activated by treatment of keratinocytes with the fungal cell wall component zymosan. Moreover, silencing of the zymosan-detecting C-type lectin receptor dectin-1, but not of TLR2 or its adaptor MyD88, strongly reduces MALT1 activity in keratinocytes (Schmitt et al., 2016). Interestingly, stimulation of dectin-1 in myeloid cells leads to the formation of a CARD9-containing CBM complex that is crucial for antifungal immune responses (Gross et al., 2006), suggesting the potential formation of a related CARD14-containing CBM complex in dectin-1-stimulated keratinocytes. How exactly dectin-1 activates the CBM complex in keratinocytes is thus far unclear. Dectin-1, like other immune receptors (e.g., T cell receptor, B cell receptor, and NKG2D), employs immunoreceptor tyrosine-based activation motifs (ITAMs) to initiate downstream signaling via Src kinases (Thome, 2008). In T cells, Src kinase activity eventually leads to the activation of PKCθ, which in turn phosphorylates and activates CARD11

(Matsumoto et al., 2005; Sommer et al., 2005; Thome, 2008). In keratinocytes, PKC activation upon treatment with phorbol 12-myristate 13-acetate results in MALT1 activation that can be prevented by CARD14 silencing (Schmitt et al., 2016), suggesting also a regulatory role for PKC in the activation of CARD14. In addition, Src and PKC inhibition interferes with zymosan-induced MALT1 activation in keratinocytes. It is therefore tempting to speculate that dectin-1 leads to PKC-mediated phosphorylation and activation of CARD14, facilitating the formation of a CBM complex (Figure 1).

PROTEASE ACTIVITY AND SUBSTRATES OF MALT1

Aside from its function as a scaffold protein in the CBM complex, MALT1 also acts as an arginine-specific protease and further fine-tunes the activation of the proinflammatory cascade. Although the caspase-like domain of MALT1 had already been described in 2000 (Uren et al., 2000), its first substrates were only identified in 2008 by two independent groups (Coornaert et al., 2008; Rebeaud et al., 2008). Because of its unique protease activity—MALT1 is the only known human paracaspase (Hulpiau et al., 2016)—it seems to be a promising drug target for dampening excessive inflammatory signaling.

So far, eight substrates of MALT1 have been described in stimulated lymphocytes: A20, BCL10, CYLD, RelB, regnase-1, roquin, HOIL-1, and MALT1 itself (Afonina et al., 2015; Hailfinger et al., 2014; Klein et al., 2015). Cleavage of these substrates affects various processes, such as NF-κB (A20, RelB, HOIL-1) and JNK (CYLD) activation, linear ubiquitination (HOIL-1), mRNA stability (regnase-1, roquin), and cell adhesion (BCL10). Because MALT1 can regulate inflammatory signaling and immune responses by cleaving these substrates, its proteolytic activity is subject to several regulatory mechanisms. Dimerization and monoubiquitination of MALT1 are necessary to adopt and maintain its catalytically active conformation (Cabalzar et al., 2013; Pelzer et al., 2013; Wiesmann et al., 2012). Thus, formation of supramolecular filamentous CBM complexes not only activates NF-κB signaling but also drives MALT1 proteolytic activity through oligomerization of MALT1 (Qiao et al., 2013).

Recently, a distinct role for MALT1 protease activity has emerged in inflammatory signaling in keratinocytes. Certain stimuli, such as zymosan and *Staphylococcus aureus*, were able to induce MALT1 proteolytic activity in keratinocytes, resulting in the cleavage of CYLD, RelB, A20, and regnase-1 (Schmitt et al., 2016) (Figure 1). In addition, overexpression of CARD14 in keratinocytes was shown to promote processing of MALT1 substrates (Afonina et al., 2016), which was further enhanced by the psoriasis-associated CARD14 versions p.Gly117Ser and p.Glu138Ala, highlighting the pathological relevance of *CARD14* mutations in psoriasis (Afonina et al., 2016; Howes et al., 2016). Inhibition of MALT1 protease activity in keratinocytes reduced the expression of important CARD14-regulated proinflammatory cytokines (e.g., TNF, IL-1β, and IL-17C), chemokines (e.g., CXCL8 and CCL20), and antimicrobial peptides (e.g., HBD-2 and S100A7), pointing to an important role for MALT1 in the immune response in the skin (Afonina et al., 2016; Schmitt et al., 2016). Interestingly, it was also shown that A20 and CYLD, two substrates of MALT1 that have been associated with psoriasis (Nititham et al., 2015; Oudot et al., 2009; Tejasvi et al., 2012), can inhibit CARD14-mediated signaling (Afonina et al., 2016). In this way, the cleavage of these negative regulators by MALT1 may promote optimal CARD14-mediated signaling. These findings imply that the proteolytic activity of MALT1 could contribute to the pathology of psoriasis.

THERAPEUTIC POTENTIAL OF MALT1 INHIBITORS IN PSORIASIS AND FUTURE PERSPECTIVES

Currently, there is no cure for psoriasis, but several treatments targeting the immune response and the differentiation status of keratinocytes can alleviate its symptoms. Conventional therapies, including glucocorticoids, fumarates, vitamin D derivatives, and phototherapy, are effective in treating mild cases but often induce unwanted side effects in patients. Recently, biological entities that target central cytokines in psoriasis, such as TNF, IL-17, and IL-12/IL-23, have emerged and often show better efficacy than conventional therapies in severe cases. However, not all patients are responsive to these cost-intensive treatments, highlighting the need for alternative treatment options (Deng et al., 2016).

The ability of CARD14 and particularly its psoriasis-associated mutants to activate the protease function of MALT1 and proinflammatory gene expression in keratinocytes provides a rationale for MALT1 inhibitors in psoriasis treatment (Afonina et al., 2016; Howes et al., 2016). Many CARD14-regulated genes are key players in the pathogenesis of psoriasis and are targets of current standard treatment regimens (e.g., anti-TNF biologics). Although there are at present no MALT1 inhibitors in the clinic, several groups of small molecule inhibitors have been published and patented, thus providing a pool of potential lead structures for further clinical development (Fontan et al., 2012; Lim et al., 2015; Nagel et al., 2012). MALT1 inhibitors have been successfully tested preclinically in vitro and in vivo in the treatment of a subtype of diffuse large B-cell lymphoma and in a mouse model of multiple sclerosis (Fontan et al., 2012; Mc Guire et al., 2014; Nagel et al., 2012). Because CBM activation in keratinocytes leads to MALT1-dependent inactivation of A20, CYLD, RelB, and the endonuclease regnase-1, MALT1 inhibition will likely result in reduced NF-κB and AP-1 activity and decreased mRNA stability of various proinflammatory target genes.

Potential side effects of MALT1 inhibition can be predicted from MALT1 activity deficient mice. *MALT1* knockout mice are viable and develop normally but suffer from severe immunodeficiency, including proliferation and activation defects in B and T cells after antigen receptor stimulation as well as reduced numbers of marginal zone and peritoneal B1 cells (Ruefli-Brasse et al., 2003; Ruland et al., 2003). Surprisingly, mice expressing protease-inactive MALT1 additionally suffer from spontaneous autoimmunity (Bornancin et al., 2015; Gewies et al., 2014; Jaworski et al., 2014; Yu et al., 2015). Whereas T cells from *MALT1* knockout mice show no proliferation and IL-2 production upon stimulation, T cells from *MALT1* protease-dead knock-in mice still respond partially due to the preserved scaffold function of MALT1. Because the development of regulatory T cells is

strongly decreased in the knock-in mice, the residual activity of T cells might be enough to drive autoimmunity. Whether the effect on regulatory T cell development is relevant in human patients is unclear but could most likely be circumvented by the topical administration of a therapeutic MALT1 inhibitor. Thus, further studies are needed to validate the potential of MALT1 inhibitors in vivo in psoriasis-like mouse models. New insights could also come from *CARD14* knockout or *CARD14* mutant-expressing mice or from the keratinocyte-specific expression of a protease-inactive MALT1. In addition, the identification of other receptors driving CBM complex formation will help to further elucidate the role of MALT1 in keratinocytes. Because *CARD14* mutations in psoriasis are relatively rare, the analysis of human psoriasis biopsies for active MALT1 will clarify the importance of the CBM pathway in this disease. MALT1 inhibition seems to be a particularly promising treatment strategy because it can tackle psoriasis in two ways: first by decreasing the expression of proinflammatory target genes in keratinocytes and secondly by blocking the activation of immune cells in the skin.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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REFERENCES

Afonina IS, Elton L, Carpentier I, Beyaert R. MALT1—a universal soldier: multiple strategies to ensure NF-kappaB activation and target gene expression. FEBS J 2015;282:3286–97.

Afonina IS, Van Nuffel E, Baudelet G, Driege Y, Kreike M, Staal J, et al. The paracaspase MALT1 mediates CARD14-induced signaling in keratinocytes. EMBO Rep 2016;17:914–27.

Ammar M, Bouchlaka-Souissi C, Helms CA, Zaraa I, Jordan CT, Anbunathan H, et al. Genome-wide linkage scan for psoriasis susceptibility loci in multiplex Tunisian families. Br J Dermatol 2013;168:583–7.

Ammar M, Jordan CT, Cao L, Lim E, Bouchlaka Souissi C, Jrad A, et al. CARD14 alterations in Tunisian patients with psoriasis and further characterization in European cohorts. Br J Dermatol 2016;174:330–7.

Bertin J, Wang L, Guo Y, Jacobson MD, Poyet JL, Srinivasula SM, et al. CARD11 and CARD14 are novel caspase recruitment domain (CARD)/membrane-associated guanylate kinase (MAGUK) family members that interact with BCL10 and activate NF-kappa B. J Biol Chem 2001;276:11877–82.

Bornancin F, Renner F, Touil R, Sic H, Kolb Y, Touil-Allaoui I, et al. Deficiency of MALT1 paracaspase activity results in unbalanced regulatory and effector T and B cell responses leading to multiorgan inflammation. J Immunol 2015;194:3723–34.

Cabalzar K, Pelzer C, Wolf A, Lenz G, Iwaszkiewicz J, Zoete V, et al. Monoubiquitination and activity of the paracaspase MALT1 requires glutamate 549 in the dimerization interface. PLoS One 2013;8:e72051.

Coornaert B, Baens M, Heyninck K, Bekaert T, Haegman M, Staal J, et al. T cell antigen receptor stimulation induces MALT1 paracaspase-mediated cleavage of the NF-kappaB inhibitor A20. Nat Immunol 2008;9:263–71.

Coto-Segura P, Gonzalez-Fernandez D, Batalla A, Gomez J, Gonzalez-Lara L, Queiro R, et al. Common and rare CARD14 gene variants affect the anti-TNF response among Psoriasis patients. Br J Dermatol 2016;175:134–41.

Deng L, Wang C, Spencer E, Yang L, Braun A, You J, et al. Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. Cell 2000;103:351–61.

Deng Y, Chang C, Lu Q. The inflammatory response in psoriasis: a comprehensive review. Clin Rev Allergy Immunol 2016;50:377–89.

Eskin-Schwartz M, Basel-Vanagaite L, David M, Lagovsky I, Ben-Amitai D, Smirin-Yosef P, et al. Intra-familial variation in clinical phenotype of CARD14-related psoriasis. Acta Derm Venereol 2016;96:885–7.

Eytan O, Sarig O, Sprecher E, van Steensel MA. Clinical response to ustekinumab in familial pityriasis rubra pilaris caused by a novel mutation in CARD14. Br J Dermatol 2014;171:420–2.

Feng C, Wang T, Li SJ, Fan YM, Shi G, Zhu KJ. CARD14 gene polymorphism c.C2458T (p.Arg820Trp) is associated with clinical features of psoriasis vulgaris in a Chinese cohort. J Dermatol 2016;43:294–7.

Fontan L, Yang C, Kabaleeswaran V, Volpon L, Osborne MJ, Beltran E, et al. MALT1 small molecule inhibitors specifically suppress ABC-DLBCL in vitro and in vivo. Cancer Cell 2012;22:812–24.

Fuchs-Telem D, Sarig O, van Steensel MA, Isakov O, Israeli S, Nousbeck J, et al. Familial pityriasis rubra pilaris is caused by mutations in CARD14. Am J Hum Genet 2012;91:163–70.

Gaide O, Martinon F, Micheau O, Bonnet D, Thome M, Tschopp J. Carma1, a CARD-containing binding partner of Bcl10, induces Bcl10 phosphorylation and NF-kappaB activation. FEBS Lett 2001;496:121–7.

Gewies A, Gorka O, Bergmann H, Pechloff K, Petermann F, Jeltsch KM, et al. Uncoupling Malt1 threshold function from paracaspase activity results in destructive autoimmune inflammation. Cell Rep 2014;9:1292–305.

Gonzalez-Lara L, Coto-Segura P, Penedo A, Eiris N, Diaz M, Santos-Juanes J, et al. SNP rs11652075 in the CARD14 gene as a risk factor for psoriasis (PSORS2) in a Spanish cohort. DNA Cell Biol 2013;32:601–4.

Gross O, Gewies A, Finger K, Schafer M, Sparwasser T, Peschel C, et al. Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. Nature 2006;442:651–6.

Hailfinger S, Lenz G, Thome M. Targeting B-cell lymphomas with inhibitors of the MALT1 paracaspase. Curr Opin Chem Biol 2014;23:47–55.

Harden JL, Krueger JG, Bowcock AM. The immunogenetics of psoriasis: a comprehensive review. J Autoimmun 2015;64:66–73.

Harden JL, Lewis SM, Pierson KC, Suarez-Farinas M, Lentini T, Ortenzio FS, et al. CARD14 expression in dermal endothelial cells in psoriasis. PLoS One 2014;9:e111255.

Has C, Schwieger-Briel A, Schlipf N, Hausser I, Chmel N, Rosler B, et al. Target-sequence capture and high throughput sequencing identify a de novo CARD14 mutation in an infant with erythrodermic pityriasis rubra pilaris. Acta Derm Venereol 2016;96:989–90.

Howes A, O'Sullivan PA, Breyer F, Ghose A, Cao L, Krappmann D, et al. Psoriasis mutations disrupt CARD14 autoinhibition promoting BCL10-MALT1-dependent NF-kappaB activation. Biochem J 2016;473:1759–68.

Hulpiau P, Driege Y, Staal J, Beyaert R. MALT1 is not alone after all: identification of novel paracaspases. Cell Mol Life Sci 2016;73:1103–16.

Inoue N, Dainichi T, Fujisawa A, Nakano H, Sawamura D, Kabashima K. CARD14 Glu138 mutation in familial pityriasis rubra pilaris does not warrant differentiation from familial psoriasis. J Dermatol 2016;43:187–9.

Jaworski M, Marsland BJ, Gehrig J, Held W, Favre S, Luther SA, et al. Malt1 protease inactivation efficiently dampens immune responses but causes spontaneous autoimmunity. EMBO J 2014;33:2765–81.

Jordan CT, Cao L, Roberson ED, Duan S, Helms CA, Nair RP, et al. Rare and common variants in CARD14, encoding an epidermal regulator of NF-kappaB, in psoriasis. Am J Hum Genet 2012a;90:796–808.

Jordan CT, Cao L, Roberson ED, Pierson KC, Yang CF, Joyce CE, et al. PSORS2 is due to mutations in CARD14. Am J Hum Genet 2012b;90:784–95.

Klein T, Fung SY, Renner F, Blank MA, Dufour A, Kang S, et al. The paracaspase MALT1 cleaves HOIL1 reducing linear ubiquitination by LUBAC to dampen lymphocyte NF-kappaB signalling. Nat Commun 2015;6:8777.

Korber A, Mossner R, Renner R, Sticht H, Wilsmann-Theis D, Schulz P, et al. Mutations in IL36RN in patients with generalized pustular psoriasis. J Invest Dermatol 2013;133:2634–7.

Li Q, Jin Chung H, Ross N, Keller M, Andrews J, Kingman J, et al. Analysis of CARD14 polymorphisms in pityriasis rubra pilaris: activation of NF-kappaB. J Invest Dermatol 2015;135:1905–8.

Lim SM, Jeong Y, Lee S, Im H, Tae HS, Kim BG, et al. Identification of beta-lapachone analogs as novel MALT1 inhibitors to treat an aggressive subtype of diffuse large B-cell lymphoma. J Med Chem 2015;58:8491–502.

Lowes MA, Suarez-Farinas M, Krueger JG. Immunology of psoriasis. Annu Rev Immunol 2014;32:227–55.

Lucas PC, Yonezumi M, Inohara N, McAllister-Lucas LM, Abazeed ME, Chen FF, et al. Bcl10 and MALT1, independent targets of chromosomal translocation in malt lymphoma, cooperate in a novel NF-kappa B signaling pathway. J Biol Chem 2001;276:19012–9.

Matsumoto R, Wang D, Blonska M, Li H, Kobayashi M, Pappu B, et al. Phosphorylation of CARMA1 plays a critical role in T cell receptor-mediated NF-kappaB activation. Immunity 2005;23:575–85.

Mc Guire C, Elton L, Wieghofer P, Staal J, Voet S, Demeyer A, et al. Pharmacological inhibition of MALT1 protease activity protects mice in a mouse model of multiple sclerosis. J Neuroinflammation 2014;11:124.

McAllister-Lucas LM, Inohara N, Lucas PC, Ruland J, Benito A, Li Q, et al. Bimp1, a MAGUK family member linking protein kinase C activation to Bcl10-mediated NF-kappaB induction. J Biol Chem 2001;276:30589–97.

Mease PJ, Gladman DD, Papp KA, Khraishi MM, Thaci D, Behrens F, et al. Prevalence of rheumatologist-diagnosed psoriatic arthritis in patients with psoriasis in European/North American dermatology clinics. J Am Acad Dermatol 2013;69:729–35.

Mossner R, Frambach Y, Wilsmann-Theis D, Lohr S, Jacobi A, Weyergraf A, et al. Palmoplantar pustular psoriasis is associated with missense variants in CARD14, but not with loss-of-function mutations in IL36RN in European patients. J Invest Dermatol 2015;135:2538–41.

Nagel D, Spranger S, Vincendeau M, Grau M, Raffegerst S, Kloo B, et al. Pharmacologic inhibition of MALT1 protease by phenothiazines as a therapeutic approach for the treatment of aggressive ABC-DLBCL. Cancer cell 2012;22:825–37.

Nestle FO, Kaplan DH, Barker J. Psoriasis. N Engl J Med 2009;361:496–509.

Nititham J, Taylor KE, Gupta R, Chen H, Ahn R, Liu J, et al. Meta-analysis of the TNFAIP3 region in psoriasis reveals a risk haplotype that is distinct from other autoimmune diseases. Genes Immun 2015;16:120–6.

Oeckinghaus A, Wegener E, Welteke V, Ferch U, Arslan SC, Ruland J, et al. Malt1 ubiquitination triggers NF-kappaB signaling upon T-cell activation. EMBO J 2007;26:4634–45.

Oudot T, Lesueur F, Guedj M, de Cid R, McGinn S, Heath S, et al. An association study of 22 candidate genes in psoriasis families reveals shared genetic factors with other autoimmune and skin disorders. J Invest Dermatol 2009;129:2637–45.

Pelzer C, Cabalzar K, Wolf A, Gonzalez M, Lenz G, Thome M. The protease activity of the paracaspase MALT1 is controlled by monoubiquitination. Nat Immunol 2013;14:337–45.

Qiao Q, Yang C, Zheng C, Fontan L, David L, Yu X, et al. Structural architecture of the CARMA1/Bcl10/MALT1 signalosome: nucleation-induced filamentous assembly. Mol Cell 2013;51:766–79.

Qin P, Zhang Q, Chen M, Fu X, Wang C, Wang Z, et al. Variant analysis of CARD14 in a Chinese Han population with psoriasis vulgaris and generalized pustular psoriasis. J Invest Dermatol 2014;134:2994–6.

Rebeaud F, Hailfinger S, Posevitz-Fejfar A, Tapernoux M, Moser R, Rueda D, et al. The proteolytic activity of the paracaspase MALT1 is key in T cell activation. Nat Immunol 2008;9:272–81.

Ruefli-Brasse AA, French DM, Dixit VM. Regulation of NF-kappaB-dependent lymphocyte activation and development by paracaspase. Science 2003;302:1581–4.

Ruland J, Duncan GS, Wakeham A, Mak TW. Differential requirement for Malt1 in T and B cell antigen receptor signaling. Immunity 2003;19:749–58.

Schmitt A, Grondona P, Maier T, Brandle M, Schonfeld C, Jager G, et al. MALT1 Protease activity controls the expression of inflammatory genes in keratinocytes upon zymosan stimulation. J Invest Dermatol 2016;136:788–97.

Scudiero I, Vito P, Stilo R. The three CARMA sisters: so different, so similar: a portrait of the three CARMA proteins and their involvement in human disorders. J Cell Physiol 2014;229:990–7.

Scudiero I, Zotti T, Ferravante A, Vessichelli M, Vito P, Stilo R. Alternative splicing of CARMA2/CARD14 transcripts generates protein variants with differential effect on NF-kappaB activation and endoplasmic reticulum stress-induced cell death. J Cell Physiol 2011;226:3121–31.

Sommer K, Guo B, Pomerantz JL, Bandaranayake AD, Moreno-Garcia ME, Ovechkina YL, et al. Phosphorylation of the CARMA1 linker controls NF-kappaB activation. Immunity 2005;23:561–74.

Sugiura K, Kitoh T, Watanabe D, Muto M, Akiyama M. Childhood-onset PsA in Down syndrome with psoriasis susceptibility variant CARD14 rs11652075. Rheumatology (Oxford) 2015;54:197–9.

Sugiura K, Muto M, Akiyama M. CARD14 c.526G>C (p.Asp176His) is a significant risk factor for generalized pustular psoriasis with psoriasis vulgaris in the Japanese cohort. J Invest Dermatol 2014;134:1755–7.

Sun L, Deng L, Ea CK, Xia ZP, Chen ZJ. The TRAF6 ubiquitin ligase and TAK1 kinase mediate IKK activation by BCL10 and MALT1 in T lymphocytes. Mol Cell 2004;14:289–301.

Tanner MJ, Hanel W, Gaffen SL, Lin X. CARMA1 coiled-coil domain is involved in the oligomerization and subcellular localization of CARMA1 and is required for T cell receptor-induced NF-kappaB activation. J Biol Chem 2007;282:17141–7.

te Velthuis AJ, Admiraal JF, Bagowski CP. Molecular evolution of the MAGUK family in metazoan genomes. BMC Evol Biol 2007;7:129.

Tejasvi T, Stuart PE, Chandran V, Voorhees JJ, Gladman DD, Rahman P, et al. TNFAIP3 gene polymorphisms are associated with response to TNF blockade in psoriasis. J Invest Dermatol 2012;132(Pt 1):593–600.

Thome M. Multifunctional roles for MALT1 in T-cell activation. Nat Rev Immunol 2008;8:495–500.

Uren AG, O'Rourke K, Aravind LA, Pisabarro MT, Seshagiri S, Koonin EV, et al. Identification of paracaspases and metacaspases: two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma. Mol Cell 2000;6:961–7.

Wang L, Guo Y, Huang WJ, Ke X, Poyet JL, Manji GA, et al. Card10 is a novel caspase recruitment domain/membrane-associated guanylate kinase family member that interacts with BCL10 and activates NF-kappa B. J Biol Chem 2001;276:21405–9.

Wiesmann C, Leder L, Blank J, Bernardi A, Melkko S, Decock A, et al. Structural determinants of MALT1 protease activity. J Mol Biol 2012;419:4–21.

Yim KM, Armstrong AW. Updates on cardiovascular comorbidities associated with psoriatic diseases: epidemiology and mechanisms [e-pub ahead of print]. Rheumatol Int 2016; http://dx.doi.org/10.1007/s00296-016-3487-2.

Yu JW, Hoffman S, Beal AM, Dykon A, Ringenberg MA, Hughes AC, et al. MALT1 protease activity is required for innate and adaptive immune responses. PLoS One 2015;10:e0127083.

Zhu K, Shi G, Liu H, Zhu C, Fan Y. Variants of CARD14 gene and psoriasis vulgaris in southern Chinese cohort. An Bras Dermatol 2016;91:45–8.