

Study on the regulation of immunity by palmitoylation

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Abstract. Palmitoylation is a reversible enzymatic post-translational modification of a protein, where the palmitoyl chain connects to cysteine residues via thioester bonds. Palmitoylation affects protein function by affecting protein activity, stability, protein transport, and protein-protein interactions. Palmitoylation occurs in lymphocytes that are critical to both innate and acquired immune responses. Palmitoylation can also occur in the receptors of immune cells, thereby regulating immunity. In this paper, the role of palmitoylation in immunity is summarized by better combining the immune-related signaling pathways affected by palmitoylation.

Keywords: Palmitoylation, Immune cells, Signaling pathway.

1. Introduce

Palmitoylation modification (S-palmitoylation, or S-acylation) refers to long chain fatty acids (usually 16 carbon palmitic acid) covalent modification to protein cysteine residues, is a widespread dynamic, reversible post-translational modification, has a very important role in regulating protein transport, cellular localization and stability, participate in many biological processes, and is closely related to the occurrence and development of many diseases. Overall, S-palmitoylation is involved in a range of different physiological processes, including cell signaling transduction, cell differentiation, transcriptional regulation, metabolism, etc

Lipid metabolism disorder is associated with many diseases, such as metabolic disorders, cancer, atherosclerosis, cardiovascular disease, infections, and inflammatory diseases. It has been shown that lipid metabolites play an important role in the regulation of the host immune system. Bioactive lipid mediators regulate a wide range of immune responses involving multiple inflammatory processes that coordinate immune cell function, leading to the onset of metabolic-related diseases.

In this review, we will focus on the role and mechanisms of S-palmitoylation in the functional regulation of immune cells and immune-related proteins. In particular, the ways in which palmitoylation and palmitoylation of proteins involved in adaptive and natural immunity regulate certain important immune-relevant signaling pathways.

2. Palmitoylation affects immune cell function

Immune cells are cells involved in or associated with the immune response. Including lymphocytes, dendritic cells, monocytes / macrophages, granulocytes, mast cells, etc. Immune cells can be divided into multiple types, and various immune cells play important roles in the human body. Recently, S-palmitoylation of key proteins has been identified successively in several cells. However, limited studies on functional aspects of these cellular protein-palmitoylation.

2.1 T cell

Palmitoylation affects T cell function mainly by affecting TCR receptors on the T cell surface. Efficient TCR signaling requires the S-palmitoylation of the Src family signaling kinases, LCK and FYN, as well as of signaling molecules such as the accessory receptors CD4 and CD8. Abnormal palmitoylation causes these proteins to lose their ability to bind to membrane rafts causing the disappearance of TCR signaling.

2.2 DCs

Palmitoylation has an important role in host defense against viral pathogens. During viral infection, palmitoylation of several receptors on the dendrite cell surface can affect antigen uptake or dendritic cell activation, such as CD36 and the interferon γ receptor.

2.3 Macrophage

Macrophages act as phagocytes in the immune system, engulfing pathogens and cell fragments for digestion in lysosomes. Membrane remodeling involved in macrophagocytosis and other immunomodulatory functions (e. g., cytokine secretion) may involve S-palmitoylated proteins, such as Synxin-7.

Meanwhile, palmitoylation can also help with the apoptotic in macrophages. There are many palmitoylated proteins in the mitochondria, such as PLSCR3. Given the key role of mitochondria in initiating programmed cell death, the investigators investigated the role of PLSCR3 in apoptosis. Overexpression of PLSCR3 sensitizes macrophages to etoposide-induced apoptosis, which is largely dependent on the S-palmitoylation of PLSCR3.

2.4 B cell

The primary function of the B cells is to recognize the antigen via the B cell receptor (BCR), and then to produce and secrete antigen-specific antibodies. B cells mediate humoral immune responses and are important for resistance against pathogens. B cells have long been recognized as important model systems for studying Tetraspanin-rich membranes, characterized by the presence of numerous S-palmitoylated Tetraspanin proteins that provide an important basis for receptor signaling to intracellular molecules. We found that palmitoylation of CD81 is necessary to stabilize the CD19/CD21/CD81/BCR complex that leads to an enhanced signal of BCR in the presence of antigen binding, so palmitoylation is very important for the function of the BCR of B cells.

3. Palmitoylation affects immune-related receptors

The innate immune system responds to microbial invasion by using pattern recognition receptors (PRRs) identifying conserved microbial features or associated molecules. PRRs can be divided into several relevant molecular families. These families include Toll-like receptors (TLRs), type C lectin receptors, RIG-I-like receptor, and Nod-like receptors. Several of these molecules and their aptamer proteins have been shown to be palmitoylated in recent years. In addition, several important immune-related receptors, such as TCR, have also detected palmitoylation. As we will review below, the already detected S-palmitoylation, often modified by the activation of immune signaling by these receptors.

3.1 TCR receptors

CD4 is palmitoylated at the transmembrane domain and at the cytoplasmic domains, Cys394 and Cys397. Palmitoylation of CD4 modulates TCR enrichment in the lipid raft. The co-receptor CD8 is a heterodimer of CD8 α and CD8 β . In humans, both CD8 α and CD8 β are s-palmitoylated, whereas in mice, only CD8 β is s-palmitoylated. Mouse CD8 β s-palmitoylation is critical for CD8 coreceptor function, which increases the association of CD8 with Lck in the lipid raft.

3.2 The Src family of kinases

The Src family kinase Lck is S-palmitoylated at Cys3 and Cys5, promoting the binding of Lck to the plasma membrane. Loss of Lck S-palmitoylation attenuates Lck and CD4 binding, and also weakens downstream signaling. Further studies showed that S-palmitoylation specific for Cys3 is important for the localization of Lck lipid rafts. The S-palmitoylation of Fyn, another Src family kinase involved in T cell signaling, has also been shown to play an important role in membrane binding. Fyn can undergo S-palmitoylation on Cys3 and Cys6, where Cys3, as the primary site, is essential for lipid raft binding. Activation of Fyn by Lck in lipid rafts causes TCR/CD3 activation,

which is required for downstream signaling. The common palmitoyase DHHC2, 3, 7, 10, 15, 20, 21 all has a role in mediating Fyn S-palmitoylation.

3.3 Fas and FasL

Apoptosis of T cells that recognize autoantigens is required for the prevention of autoimmune diseases. Fas and Fas ligands (FasL) are key regulators of T cell apoptosis. Binding of Fas to FasL causes the recruitment of DISC in cells carrying Fas, and the complex activates caspase-3-mediated apoptosis. The S-palmitoylation of Fas is required for its stability. Mutations in the Fas S-palmitoylation site can reduce the stability of binding to lipid rafts and cause a decay of the apoptotic signal. The S-palmitoylation can regulate regulating FasL lipid raft distribution and proteolytic cleavage of ADAM10 to efficiently induce Fas-mediated cell death.

3.4 PD-1 and PD-L1

PD-1 is a T cell surface receptor that inhibits T cell proliferation and cytokine production upon activation. The ligands, PD-L1 and PD-L2, of PD-1, will be expressed in antigen-presenting cells and in tumor cells. An S-palmitoylation of PD-1 between the transmembrane and cytoplasmic regions of Cys192 was found. The S-palmitoylation reaction of PD-1 is catalyzed by DHHC9, and the palmitoylation of PD1 plays an important role for its stability. Some cancer cells also express PD-1 on the surface and can promote tumor growth without relying on acquired immunity. Meanwhile, the S-palmitoylation of PD-1 in the tumor cells can regulate the downstream signaling and proliferation of the mammalian rapamycin (MTOR).

The PD-L1 expressed on the tumor cells is also S-palmitoylated, and this modification inhibits the ubiquitination of PD-L1 and the degradation of PD-L1 performed in lysosomes. DHHC3 was found to catalyze the PD-L1 palmitoylation reaction. Inhibition of PD-L1 palmitoylation using 2-bromopalmitate or silencing by DHHC3 silencing increased antitumor immunity in cells and mice. Studies of palmitoylation of PD-1 and PD-L1, which can specifically modulate immune responses in cancer T cells, provide a good research basis for combinatorial therapy and immune checkpoint therapy.

3.5 TLR

The toll-like receptors (TLRs) were one of the first microbial-sensing PRRs to be identified. Binding of TLR to its ligand results in signals from adapter proteins (including MyD88) associated with TLR. The TLR2 in the TLR is a palmitoylated protein, and the proper localization and antibacterial of Cys609 by the TLR2 palmitoylation is required for. The finding that this modification is highly conserved in the TLR2 protein throughout evolution further supports an important role in antimicrobial function.

3.6 MyD88

Palmitoylation of MyD88 is an essential regulatory modification that can reduce TLR-mediated inflammation by directly inhibiting the enzymatic palmitoylation of MyD88, or by reducing endogenous palmitates that can be used for protein modification. By studying the activation of NF- κ B after the overexpression of various signaling molecules downstream of TLRs, it was found that the inhibitory effect of C75 on TLR signaling is associated with MyD88. Mass spectrometry analysis identified the candidate s-palmitoyl cysteine Cys113 and Cys274 on MyD88. Both single mutant Cys113 or Cys274 significantly reduced MyD88s-palmitoylation levels, while further reduction in double mutations did not. For NF- κ B and MAPK activation, mutations in Cys113 reduced the responsiveness to the TLR4 ligand LPS, while mutations in Cys274 had no significant effect. The failure of the Ala MyD88 mutant Cys113 to recruit IRAK4 signaling molecules in response to TLR stimulation suggests that s-palmitoylation is required to recruit Myddosome signaling complexes downstream of TLR ligand binding. There have also been attempts to determine whether the MyD88s-palmitoylation promotes the localization of the TLR (eg, TLR2), as the TLR is also

palmitoylated. Some studies have identified DHHC6 as the major candidate palmitoyltransferase for MyD88 because its overexpression will increase s-palmitoylation of MyD88 as well as high expression in myeloid cell types with strong TLR activity. Meanwhile, the downregulation of DHHC6 reduced the macrophage MyD88s-palmitoylation and LPS reactivity. So the MyD88 promotes the localization of the TLR.

3.7 STING

STING is a signaling protein involved in the DNA response in the cytoplasm. Ligand-activated STING transfers from the ER to the Golgi and recruits signaling molecules to activate NF- κ B and interferon regulators 3 (IRF3) to produce pro-inflammatory cytokines and type I interferon (ifn). Mukai et al speculated that STING was posttranslationally modified in the Golgi, and found that STING could be modified by radiolabeled palmitate after cell activation by the chemical STING agonist DMXAA. In addition, overexpression of the Golgi localization of dhhc 3, 7 and 15 increased STING s palmitylation. In contrast, the 2-BP-treated cells eliminated STING palmitoylation and prevented the production of cytokines upon DMXAA stimulation, suggesting that palmitoylation is necessary for STING-induced inflammatory cytokines.

The primary modification sites of STING were localized to Cys88 and Cys91. Upon stimulation with DMXAA, a STING mutant with serine substitutions at these positions was transmitted from the ER to the Golgi, similar to WT STING, but could not induce NF- κ B and IRF3 activation, and therefore could not induce proinflammatory downstream gene products, including type I interferon. These results suggest that the s-palmitoylation of STING on a specific cysteine is the required for its inflammatory signaling function.

3.8 NOD1/2

NOD1 and NOD2 are mainly dissolved in the cytoplasm. However, when intracellular bacteria are infected, they are quickly reassigned to the phagosomal membrane to activate NF- κ B and MAPK signaling. Since NOD1/2 lack lipid-binding motifs in the transmembrane region, it is speculated that their rapid membrane binding may be mediated by dynamic s-palmitylation. Screening of the NOD1 and NOD2-interacting proteins revealed that DHHC5 may be a candidate for the modification of these immune effectors. DHHC5 knockdown or knockdown resulted in decreased s-palmitoylation of NOD1 and NOD2, decreased NOD membrane association, and decreased activation of the NF- κ B and MAPK pathways in response to NOD ligands. Furthermore, the DHHC5 and the intact NOD s-palmitoylation sites both require NOD1/2 recruitment to the Salmonella-containing phagosome. NOD2 variants with reduced peptidoglycan reactivity are associated with pathology, such as Crohn's disease. Of the six disease variants tested, five showed a significant reduction in s-palmitoylation, suggesting that defective palmitoylation leads to a decrease in its function.

4. Palmitoylation affects immune-related signaling pathways

Immune-related receptors of palmitoylation activate signaling pathways that produce pro-inflammatory cytokines, including tumor necrosis factor (TNF), interferon (IFNs), chemokines, and immune effector proteins, to eliminate microbial pathogens.

4.1 IFN

Interferons bind to interferon receptors on the cell surface, activate the JAK-STAT pathway, and induce the expression of interferon-stimulated genes (ISGs). [3H] Palmitate acid radiolabeling identified the S-palmitoylation reaction for IFNAR1 and IFNAR2. IFNAR1 is S-palmitoylated and located in the cytoplasmic domain proximal to the transmembrane region. Mutagenesis of A L A by Cys463 does not affect receptor stability, endocytosis. However, the loss of IFNAR1 S-palmitoylation results in reduced STAT phosphorylation, which thus affects interferon-stimulated gene transcription. Palmitoyl group profiling of adipocytes revealed that four proteins in the JAK/STAT

pathway JAK1, STAT1, STAT3 and STAT5A undergo S-palmitoylation response. Among them, STAT3 palmitoylation is thought to be implicated in regulating TH17 cell differentiation. TH17 cells are pro-inflammatory T cells that express interleukin 17 (IL-17) and γ T (ROR γ t). STAT3 is mediated by DHHC7 and APT2, respectively, and undergoes a palmitoylation-dealdehyylation cycle on Cys108. This cycle accelerates the differentiation of TH17 cells by promoting membrane binding, phosphorylation, and cell cycle regulation.

4.2 TNF

TNF signaling transduced TNF factors (TNFs) form another important superfamily of cytokines. Tumor necrosis factors regulate multiple cellular processes, including inflammation, proliferation, differentiation, and can induce multiple forms of cell death. Tumor necrosis factor (TNF) is synthesized as the transmembrane protein (TmTNF), is present on the plasma membrane and released as soluble TNF (sTNF) after being cleaved on the plasma membrane. The membrane-bound tumor necrosis factor N terminal fragment (NTF) was further cleaved by signal peptidase 2b to generate the intracellular region of the (ICD-TNF α) of the tumor necrosis factor. All forms of TNF are biologically active. [3H] Palmitate metabolic markers identified the S-palmitoylation reaction of tmTNF. Recent studies have shown that palmitoylation plays an important role in the segmentation of tmTNF lipid rafts, the stability of NTF, and the formation of an efficient cleavage of ICD-TNF α . The interaction of S-palmitoylated tmTNF in lipid rafts with TNFR1 reduces the sensitivity to sTNF and thereby regulates the downstream NF κ B and ERK1/2 signaling pathways. Dynamic S-palmitoylation of TNFR1 also regulates TNF signaling by. TNFR activation in the plasma membrane results in the recruitment of the complex 1 aptamer protein to the TNFR, thereby activating NF-B signaling. On the other hand, K63 ubiquitination can trigger apoptotic signaling. TNFR1 can be palmitoylated on multiple Cys, but the dynamic S-palmitoylation on the proximal Cys248 transmembrane would play a major role in regulating the localization of its plasma membrane. The demitavage of APT2 on activated TNFR 1 is required to enhance NF-B signaling, whereas knockdown of APT2 enhances Caspase8-mediated cell death and decreases NF-B signaling.

5. Sum up

S-palmitoylation is involved in many immune pathways and processes, similar to phosphorylation and ubiquitination. Besides its extensive abundance, palmitoylation has some interesting features. First, although palmitoylation modifications and regulates a large number of proteins, there are only 23 known palmitoyltransferases to date. Second, the major known function of palmitoylation can be generalized as promoting membrane binding of cytoplasmic proteins, or targeting integrated membrane or peripheral membrane proteins to specific membrane domains or lipid rafts. Other roles of palmitoylation, such as regulating protein stability and activity, may be secondary roles. Finally, palmitoylation effects generally promote the function of modified proteins and promote the immune signaling response. This makes palmitoylation a promising target for treating immune-related diseases. Promoting palmitoylation may help to combat infection, and inhibition of palmitoylation may help in the treatment of autoimmune diseases.

Given that palmitoylation generally promotes immune signaling, an interesting question is how the activity of the palmitoyl transferase and dealdehydase enzymes are regulated. This is important because too much palmitoylation may lead to uncontrolled inflammation, and too little palmitoylation may lead to immunodeficiency. Although some regulatory mechanisms are known, such as regulating ZDHHC13, by phosphorylation regulates ZDHHC6 and APT1/APT2, through palmitoylation, more needs to be understood.

Understanding the regulatory mechanisms of palmitoyltransferases and dealdehydases is another challenge to be addressed in future studies. Due to the number of techniques developed to study S-palmitoylation, Such as the acyl-biotin exchange, acyl-RAC, acyl-PEG exchange metabolic labeling with clickable palmitate analogs, site-specific chemical acylation as well as the ability to rapidly

generate knockout genes in mice using CRISPR technology, Addressing the above challenges is therefore important in S-palmitoylation studies. We should therefore expect more exciting new developments in the field over the next decade.

In recent years, we have recognized the role of S-palmitoylation and modifying enzymes in immune cells extends from antimicrobial functions to cancer and autoimmune diseases. Furthermore, as discussed in this review, their potential as attractive targets for therapeutic interventions is increasingly recognized.

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