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The Role of Lipid-Protein Dynamics in Disease Development: Focus on S-Palmitoylation and Cancer

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Abstract

Protein lipidation, a crucial post-translational modification, plays a fundamental role in regulating protein function, localization, and stability. Among the different lipidation types, S-palmitoylation is one of the most extensively studied due to its dynamic and reversible nature. Hence, this review explores the enzymatic regulation and catalytic mechanisms of S-palmitoylation, with a focus on its physiological functions and pathological implications, particularly in cancer. Dysregulation of S-palmitoylation has been linked to enhanced tumour progression, metabolic adaptation, and immune evasion in various cancers. Conversely, it also exhibits tumor-suppressive effects by promoting the degradation of oncogenic proteins. Despite promising preclinical findings, targeted therapies leveraging S-palmitoylation regulation remain underdeveloped. This review consolidates current knowledge on the role of S-palmitoylation in disease development, highlighting its therapeutic potential and the need for further clinical research.

1. Introduction

Over the years, living organisms have developed various mechanisms to enhance protein complexity and diversity beyond their genetic limitations. Processes such as alternative splicing, cotranslational modifications, and posttranslational modifications (PTMs) enable the transformation of a genome containing a wide number of genes into a proteome exceeding one million proteins [1-3]. This adaptability allows proteins to respond effectively to both internal and external stimuli.

PTMs, which occur after protein biosynthesis, involve covalent or enzymatic modifications at specific amino acid side chains. These modifications can take several forms, including the attachment of functional groups, polypeptide chains, complex molecules, and direct amino acid alterations [4]. Scientists have identified over 620 different PTMs that regulate protein activity, with strong connection to various diseases such as cancers, diabetes, and neurodegenerative diseases. The study that breast cancers have the largest number of associated PTMs.

Among the widely researched PTMs, protein lipidation is particularly notable. This process facilitates the attachment of up to seven types of lipids such as fatty acids, sterols, and glycosylphosphatidylinositol (GPI) anchors to proteins [5,6]. Depending on their location, lipid modifications fall into two broad categories: those occurring in the cytoplasm or on the cytoplasmic side of membranes, including S-palmitoylation, N-myristoylation, and S-prenylation; and those within secretory organelles, such as GPI anchoring and cholesterylation [7].

Studies have shown that one of the best-studied lipidation types is S-palmitoylation, in which a 16-carbon palmitate molecule is covalently attached to cysteine residues through a thioester bond [8,9]. Researchers use various techniques to detect this modification, such as bioorthogonal probes, radioactive isotope-labelled palmitic acid, and acyl-biotin exchange [10,11]. Other, less common forms of palmitoylation include O-palmitoylation (attachment to serine residues) and N-palmitoylation (attachment to the N-terminus). Another key lipidation process is N-myristoylation, where a 14-carbon myristic acid is linked to the N-terminal glycine of a protein through an amide bond. This modification, either cotranslational or posttranslational, was first discovered in 1982 and is facilitated by the enzyme N-myristoyltransferase (NMT) [12]. Traditional detection methods include the use of radioactive-labeled fatty acids and bioorthogonal probes like azido- or alkynyl myristate analogues [13]. Unlike S-palmitoylation, canonical

N-myristoylation is generally irreversible. However, when myristic acid attaches to lysine residues, the fatty acyl group can be removed by enzymes such as sirtuins and histone deacetylases (HDACs), making lysine myristoylation a reversible lipidation process [14].

S-prenylation, another lipidation type, involves the addition of either a 15-carbon farnesyl or a 20-carbon geranylgeranyl lipid to cysteine residues via a stable thioether bond [15]. Detection methods often involve alkynyl and azido-isoprenoid probes [16]. According , most prenylated proteins belong to the small GTPase family, which includes subfamilies such as Ras, Rab, and Rho [17]. This modification typically occurs at the C-terminal CAAX motif of proteins, where enzymatic recognition leads to further processing, including cleavage of the -AAX residues and methylation of the prenylated cysteine.

The GPI anchor is another lipid modification, where the amino group of ethanolamine phosphate (EtNP) at the end of a GPI molecule is linked to the carboxyl group at a protein's C-terminus via an amide bond. GPI-anchored proteins (GPI-APs) undergo multiple remodelling steps while being transported from the endoplasmic reticulum (ER) to the plasma membrane [18,19]. Detection of GPI-APs is possible through chemical probes that target conserved Glucosaminyl phosphatidylinositol motifs [20,21]. In eukaryotic cells, over 250 different GPI-APs have been identified, playing key roles as enzymes, receptors, antigens, and adhesion molecules in various biological processes [22,23].

Cholesterylation is another important lipidation type, characterized by the covalent attachment of cholesterol to proteins through an autoprocessing mechanism. This modification can be detected using azido- or alkyne-modified cholesterol analogues. Key cholesterylated proteins include Hedgehog (Hh) and Smoothened (SMO), which are essential regulators in the Hedgehog signalling pathway and influence various physiological processes, including embryonic development [24,25].

However, the significance of protein lipidation extends beyond its biochemical modifications, as it plays a crucial role in protein stability, localization, conformation, signal transduction, and interactions. Lipidation is a major regulatory mechanism in cellular functions by increasing the hydrophobicity of proteins. Recent studies have highlighted the involvement of lipidation dysregulation in numerous diseases, including cancer, and infectious diseases among others [26-29]. Understanding the mechanisms governing lipidation is vital for developing novel therapeutic strategies.

However, because of time limitations, this review explores the enzymatic regulation and catalytic mechanisms of S-Palmitoylation only, examines its roles in normal physiology and disease and discusses potential therapeutic targets. Studies emphasised that Palmitoylation appears to affect key aspects of cancer, including cancer cell proliferation and survival, cell invasion and metastasis, and antitumor immunity [30-33]. By consolidating key research findings, this study aims to provide a comprehensive overview of lipid-protein and its biomedical significance.

2. S-Palmitoylation: Enzymatic Regulation and Catalytic Mechanisms

2.1. Enzymes Involved and Their Catalytic Function

S-palmitoylation is facilitated by a distinct group of enzymes known as DHHC-palmitoyl acyltransferases (DHHC-PATs). These enzymes are characterized by a highly conserved Asp–His–His–Cys (DHHC) motif within a cysteine-rich region, which serves as the catalytic site for palmitoylation [34,35]. Currently, 23 DHHC-PATs have been identified in humans, with additional variants found across different species [36]. Structurally, DHHC-PATs are integral membrane proteins containing between four to seven transmembrane domains (TMDs). While most of these enzymes are predominantly localized within the Golgi apparatus and endoplasmic reticulum (ER), some are also found in the plasma membrane, mitochondria, and perinuclear regions. Their localization is influenced by specific structural motifs, such as the lysine-based sorting signals present in DHHC4 and DHHC6, which facilitate their transport to the ER [37]. Furthermore, DHHC1, 2, 4, 9, 12, 14, 20, and 22 have been observed to shuttle between the ER and Golgi. Notably, DHHC20 is distributed widely across various cellular compartments, including the plasma membrane, ER, Golgi, and perinuclear regions.

2.2. Structural and Functional Complexity of DHHC-PATs

The complexity of DHHC-PAT functionality is heightening ed by the presence of overlapping substrate specificities and enzyme redundancy. Some DHHC-PATs can catalyze the palmitoylation of multiple substrates, raising questions about whether distinct enzyme-substrate recognition sites exist. Researchers have identified structural domains such as PDZ motifs in substrate proteins and ankyrin repeats and SH3 domains in DHHC-PATs, which may facilitate selective binding [38,39]. For instance, the S-palmitoylation of GRIP1b by DHHC5/8 depends on a PDZ ligand-mediated recognition process, while PSD93 palmitoylation is governed by DHHC14 [40,41].

Moreover, ankyrin repeats in DHHC13 and DHHC17, as well as the type II PDZ-binding motif in DHHC6, contribute to substrate selection and interaction [42]. These structural elements are critical in mediating specific protein modifications. Interestingly, the presence of ankyrin repeats in DHHC3 enables it to regulate the palmitoylation and trafficking of huntingtin proteins, a function it would otherwise lack [43].

While functional redundancy complicates efforts to delineate individual DHHC-PAT roles, this redundancy serves a biological purpose. It ensures that proteins can be regulated in different cellular compartments and enables single DHHC-PATs to modify multiple substrates, thereby fulfilling physiological demands [44].

2.3. Inhibition of DHHC-PAT Activity

To date, no highly specific small-molecule inhibitors have been developed to selectively target DHHC-PATs. The most commonly

used inhibitor, 2-bromopalmitate (2-BP), acts as a broad-spectrum lipid-based inhibitor by irreversibly occupying the DHHC domain's lipid-binding cavity [45,46]. However, its lack of specificity presents challenges, as 2-BP also interferes with lipid metabolism and inhibits depalmitoylases such as acyl-protein thioesterase 1 (APT1) and APT2.

To address the limitations of 2-BP, a novel DHHC inhibitor known as cyanomethyl-N-myracrylamide (CMA) was developed. Studies have shown that CMA effectively reduces protein palmitoylation at lower concentrations than 2-BP and does not inhibit APT1 or APT2 activity [47]. However, more experimental data are required to validate CMA's efficacy and specificity. The development of highly selective DHHC inhibitors remains a crucial goal for advancing the study of S-palmitoylation and its physiological and pathological implications.

2.4. Depalmitoylation and Its Regulation

The reversal of protein palmitoylation is mediated by the hydrolysis of thioester bonds by depalmitoylating enzymes. These enzymes fall into three major categories: acyl protein thioesterases (APTs), palmitoyl-protein thioesterases (PPTs), and the ABHD17 family of thioesterases [48]. All three enzyme classes belong to the metabolic serine hydrolase superfamily and share an active site serine responsible for substrate hydrolysis. Their primary function is to cleave ester, amide, or thioester bonds on palmitoylated proteins, thereby regulating protein localization and function.

3. Physiological Function

S-palmitoylation plays a crucial role in the regulation of protein function, affecting various aspects such as trafficking, localisation, stability, degradation, and protein interactions. This post-translational modification enhances protein affinity for membranes by attaching a hydrophobic palmitate group, thereby influencing intracellular distribution and functionality [49]. The dynamic nature of S-palmitoylation-depalmitoylation cycles ensures proper protein movement across cellular compartments, including the Golgi apparatus, plasma membrane, and endosomal recycling pathways [50,51].

A well-studied example is the fatty acid transporter CD36, a glycoprotein involved in metabolic regulation. The modification increases CD36's integration into the plasma membrane by enhancing its hydrophobicity. However, when S-palmitoylation is inhibited, CD36 accumulates in the endoplasmic reticulum instead of reaching the membrane [52]. The enzymes DHHC4 and DHHC5 play distinct roles in this process. DHHC4 facilitates CD36 trafficking from the Golgi to the plasma membrane, whereas DHHC5 ensures its retention at the plasma membrane [53]. Additionally, dynamic S-palmitoylation of CD36 is crucial during fatty acid uptake, where phosphorylation of DHHC5 by the downstream kinase LYN disrupts its enzymatic activity, leading to CD36 depalmitoylation and subsequent endocytosis of fatty acids [54,55].

Beyond metabolic functions, S-palmitoylation is integral to maintaining cell polarity, which governs cell proliferation, apoptosis, and migration. Studies emphasised that DHHC7-mediated SCRIB palmitoylation is critical for SCRIB membrane targeting, cell polarity in prostate cancer and other tumour suppression [56,57]. However, disruption in this polarity can contribute to uncontrolled growth and cancer progression. SCRIB, a key protein involved in epithelial cell junctions, requires S-palmitoylation for proper localisation. Mutations preventing its palmitoylation result in mislocalisation to the cytoplasm, leading to structural disarray and polarity loss [58]. Similarly, in neuronal cells, synaptic function and protein targeting rely on this modification. The glutamate receptor AMPAR, critical for synaptic plasticity, undergoes palmitoylation at two sites, one within the transmembrane domain and another at the C-terminal region [59]. These modifications regulate its internalisation, retention in the Golgi, and interactions with associated proteins. Interestingly, S-palmitoylation affects not only AMPAR but also its interacting proteins, such as PSD95 and PICK1, sometimes producing opposing effects, underscoring the complexity of this regulatory mechanism [60-62]. Any perturbations in this network can lead to synaptic dysfunction and associated neurological disorders.

The precise mechanisms through which S-palmitoylation drives protein transport remain an active area of investigation. Studies suggest that palmitoylated proteins cluster in Golgi membrane regions with high curvature, where they interact with sorting adapters [63,64]. These interactions facilitate vesicle formation, enabling movement to various intracellular sites. Upon reaching destinations such as axons, proteins are released from transport vesicles through depalmitoylation [65]. Additionally, some proteins, such as H-Ras, N-Ras, and K-Ras4a, require other lipid modifications alongside S-palmitoylation to ensure proper localisation. H-Ras, for instance, needs two palmitoyl groups for endosomal targeting and subsequent plasma membrane anchoring, highlighting the role of multiple lipid modifications in membrane association.

Apart from trafficking and localisation, S-palmitoylation influences protein stability and degradation. This modification can either enhance protein lifespan by preventing degradation or, conversely, promote degradation under certain conditions. One example is PD-L1, a key immune checkpoint protein. Its stability increases upon S-palmitoylation, thereby maintaining its availability for immune regulation [66]. Therapeutic strategies targeting PD-L1 often involve antibodies that block its function at the membrane, but these fail to counteract its intracellular storage. However, preventing its palmitoylation leads to PD-L1 ubiquitination and subsequent lysosomal degradation, suggesting a novel immunotherapeutic approach to reducing PD-L1-mediated immune evasion [67].

Similar stabilising effects are observed in proteins such as Oct4, NOD2, CLDN3, and Fas, where S-palmitoylation shields them from lysosomal degradation. On the other hand, certain conditions trigger degradation upon palmitoylation. In hepatocellular

carcinoma cells, reducing the palmitoylation of AEG-1 stabilises the protein, as the modified form shows reduced binding to ubiquitin ligases, thereby evading degradation. A similar mechanism is seen in NLRP3, a component of the inflammasome, where palmitoylation facilitates its lysosomal degradation [68,69].

4. Pathological Implications of S-Palmitoylation in Cancer

Recent studies have established a strong link between S-palmitoylation and cancer. This correlation is evident in a clinical study conducted by who conducted a comprehensive PanCancer and PanSoftware analysis of 9,423 tumour exomes across all 33 Cancer Genome Atlas projects [70]. It was found that 299 driver genes were linked to specific cancer types. Likewise, the experiment identified over 300 missense driver tumours associated with high PD-1/PD-L1 expression, and 57% of analysed tumours contain potentially actionable clinical events.

Furthermore, research suggests that S-palmitoylation can function as both a tumour promoter and a suppressor, depending on the type of cancer [71]. Despite these insights, the precise role of palmitoylation-regulating enzymes in cancer-related proteins and their effects on tumour development remains largely unexplored. This review examines the impact of S-palmitoylation across different cancer types to shed light on its complex role in oncogenesis.

4.1. Mechanism of Enhanced S-Palmitoylation in Tumor-Associated Proteins

Dysregulation of PAT and APT enzymes, elevated palmitic acid levels, and strengthened interactions between enzymes and substrate proteins are known to increase S-palmitoylation. For example, in clear cell renal cell carcinoma resistant to tyrosine kinase inhibitors (TKIs), DHHC2 is significantly upregulated [72]. This upregulation enhances the S-palmitoylation of AGK, facilitating its relocation to the plasma membrane and activating the PI3K/Akt/mTOR pathway, ultimately contributing to TKI resistance [73-75].

Similarly, in colorectal cancer, depletion of ACOX1 leads to palmitic acid accumulation, which enhances S-palmitoylation of β-catenin [76]. This modification protects β-catenin from proteasomal degradation and promotes cancer progression through c-Myc-mediated upregulation of DUSP14 [77,78].

In epithelial ovarian cancer, tumour cells rely on oxidative phosphorylation and the tricarboxylic acid (TCA) cycle for anabolic growth [79]. S-palmitoylation enhances the function of MDH2, a key TCA cycle enzyme, supporting mitochondrial respiration and tumor proliferation [80]. This process is facilitated by the increased binding affinity of DHHC18 to MDH2.

4.2. S-Palmitoylation and Metabolic Adaptation in Cancer Cells

Numerous tumour-associated proteins depend on S-palmitoylation for accurate localization to the plasma membrane, where they play a crucial role in oncogenic signalling and nutrient uptake. For instance, GLUT1, essential for glucose transport, undergoes S-palmitoylation at Cys207 by DHHC9 before being properly positioned at the plasma membrane [81]. However, disrupting GLUT1 S-palmitoylation impairs tumor glycolysis, hindering glioblastoma progression.

In hepatocellular carcinoma, a similar role is observed with hexokinase 1 (HK1). HK1 undergoes S-palmitoylation in hepatic stellate cells before being secreted via vesicles containing TSG101 [82]. These vesicles are then absorbed by hepatocellular carcinoma cells, where HK1 enhances glycolysis and tumour progression.

4.3. Tumor Suppression Through S-Palmitoylation

S-palmitoylation can also exert tumour-suppressive effects by either activating tumour suppressor pathways or inhibiting oncogenic signalling. A well-documented example is the melanocortin-1 receptor (MC1R) in melanoma. Studies highlighted that the activation of MC1R by α-MSH reduces ultraviolet-induced damage through cAMP signalling and melanin production [83,84]. However, individuals carrying the MC1R RHC variant exhibit impaired function. Nonetheless, S-palmitoylation of MC1R restores its activity, enhancing pigmentation and mitigating melanoma genesis [85]. Additionally, inhibition of APT2, an enzyme responsible for MC1R depalmitoylation, has been shown to sustain MC1R S-palmitoylation thereby reinforcing its tumor-suppressive effects [86]. Furthermore, AMPK-mediated phosphorylation of DHHC13 at S208 has been identified as a strategy to enhance MC1R S-palmitoylation and melanoma suppression.

Another mechanism by which S-palmitoylation suppresses tumour progression is by promoting the degradation or mislocalization of oncogenic proteins. In breast cancer, DHHC22 functions as a tumour suppressor. This enzyme mediates the S-palmitoylation of mTOR, influencing its stability over time and thereby disrupting the PI3K/Akt/mTOR pathway [87]. As a result, breast cancer cell proliferation is inhibited, and resistance to neratinib is reduced. Targeting DHHC22 may thus represent a promising therapeutic approach for breast cancer treatment.

S-palmitoylation can also regulate tumour-associated protein localization to suppress oncogenic signalling. In acute myeloid leukaemia (AML), the FLT3-ITD mutation is a common genetic alteration associated with poor prognosis [88-90]. Normally, FLT3-ITD remains localized in the endoplasmic reticulum (ER) due to S-palmitoylation. However, mutations disrupting FLT3-ITD palmitoylation, such as the C563S substitution, lead to its translocation to the plasma membrane, where it activates Akt and ERK signalling, promoting leukaemia progression [91]. In many cancers, S-palmitoylation exerts its tumour-suppressive function by being downregulated. Consequently, strategies aimed at enhancing the S-palmitoylation of tumour-associated proteins, such as activating PAT enzymes or inhibiting depalmitoylases may offer promising therapeutic avenues [92,93].

5. Therapeutic Targets and Clinical Research Progress

Despite extensive preclinical evaluation of various S-palmitoylation-targeting agents, none have advanced to clinical trials. In cancer research, two primary methods have been employed to disrupt S-palmitoylation in tumour-bearing animal models. This includes the administration of 2-BP and the suppression of DHHC-PATs. One notable example is the inhibition of PD-L1 S-palmitoylation using 2-BP, which has been reported to trigger antitumour immune responses in MC38 tumour-bearing mice [94]. However, due to its high toxicity and broad activity, 2-BP is unsuitable for widespread therapeutic use. As an alternative, the researchers designed a competitive peptide inhibitor based on PD-L1's palmitoylation motif, which has demonstrated effectiveness in reducing PD-L1 expression.

Recent findings by suggest that certain widely used clinical drugs also possess the ability to inhibit protein S-palmitoylation. One example is artemisinin, a well-known antimalarial drug, which has been shown to form a covalent bond with DHHC6, disrupting its enzymatic activity. This interference reduces N-Ras S-palmitoylation, potentially weakening its oncogenic signalling and contributing to anticancer effects.

Similarly, local anaesthetics like proparacaine have been found to lower GP130 S-palmitoylation by downregulating DHHC15 transcripts. This reduction interferes with the IL-6/STAT3 signalling pathway, ultimately suppressing both the proliferation and self-renewal capacity of glioblastoma stem cells [95].

6. Conclusion

A growing body of genetic, structural, and biomedical research strongly indicates that lipidation is a key factor influencing various physiological and pathological processes. Among these modifications, S-palmitoylation plays a pivotal role in regulating protein interactions, membrane trafficking, and intracellular signalling [96-98]. Its dysregulation is increasingly recognized as a contributor to disease progression, particularly in cancer, where it can either promote tumor growth or suppress oncogenesis depending on the specific protein target. Despite significant advances in understanding the enzymatic mechanisms underlying S-palmitoylation, therapeutic strategies targeting this modification remain in their infancy. Current inhibitors, such as 2-BP, lack specificity and exhibit undesirable side effects, underscoring the urgent need for the development of selective DHHC inhibitors and depalmitoylase modulators. As research continues to uncover novel functions and disease associations of S-palmitoylation, future studies should focus on translating these findings into clinically viable treatments, ultimately paving the way for innovative therapeutic interventions.

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