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The zDHHC family of S-acyltransferases

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ABSTRACT

The discovery of the zDHHC family of S-acyltransferase enzymes has been one of the major breakthroughs in the S-acylation field. Now, more than a decade since their discovery, major questions centre on profiling the substrates of individual zDHHC enzymes (there are twenty-four ZDHHC genes and several hundred S-acylated proteins), defining the mechanisms of enzyme-substrate specificity, and unravelling the importance of this enzyme family for cellular physiology and pathology.

S-acylation

The post-translational modification of cellular proteins by S-acylation, the reversible attachment of fatty acids onto cysteine residues, mediates a variety of regulatory effects on a broad range of proteins [1-4]. These effects include facilitating membrane attachment of otherwise soluble proteins [3], regulating protein sorting to specific intracellular compartments [3], modulating protein folding and stability [2], and affecting the lateral distribution of proteins in membranes [3]. These S-acylation-dependent processes impact key physiological molecules such as G protein-coupled receptors, heterotrimeric G proteins, ion channels and membrane receptors [5]. As a result, S-acylation is essential for normal cellular physiology, and there is growing evidence linking dysfunctional S-acylation to specific disease processes [6]. The enzymes that regulate S-acylation dynamics are therefore receiving increasing interest as potential new drug targets.

S-acylation enzymes

Nearly all S-acylation reactions in eukaryotic cells are mediated by enzymes defined by the presence of a conserved 51 amino acid zinc finger domain containing a central aspartate-histidine-cysteine (DHHC) tetrapeptide [7-12]. In mammals, these “zDHHC” enzymes are encoded by more than twenty distinct genes [13]. The process of zDHHC-catalysed S-acylation has been shown to occur in two steps: attachment of the acyl group to the zDHHC enzyme (autoacylation), followed by transfer of this acyl chain to a specific cysteine in the substrate protein [14, 15]. The importance of the cysteine within the conserved DHHC tetrapeptide for catalysis has been demonstrated in numerous studies, where its mutation has resulted in a loss of both autoacylation and substrate S-acylation [9].

All zDHHC enzyme isoforms are thought to share a similar membrane topology, having 4-6 membrane-spanning domains and with the catalytic DHHC-cysteine-rich domain present on a cytoplasmic loop [9, 16]. As zDHHC enzymes are membrane associated, the process of S-acylation can only occur at the cytosolic face of intracellular membranes. This distinguishes S-acylation from other types of lipidation events, such as myristoylation and prenylation, which are catalysed in the cell cytosol [17].

Although the vast majority of S-acylation reactions occurring in cells appear to be catalyzed by zDHHC enzymes [18], the process of protein deacylation is less well understood. Such dynamic regulation of S-acylation is essential for the function of many proteins, including signalling molecules such as RAS [19]. The cytosolic thioesterases enzymes APT1 and APT2 (acyl protein thioesterase) are generally thought to be responsible for deacylation reactions, and indeed APT1 has been shown to modulate RAS -acylation dynamics [20]. However it is likely that there are many other thioesterases enzymes that contribute to protein deacylation and which remain to be fully characterised.

zDHHC Localisation

The first comprehensive study of mammalian zDHHC enzyme localisation was undertaken by Ohno et al [21]. In this study, the distribution of zDHHC enzymes with an N-terminal His<sub>6</sub>-Myc or FLAG tag was examined in transfected HEK293T cells. It was clear from this study that the majority of human zDHHC enzymes localise to the endoplasmic reticulum (ER) and the Golgi apparatus, with a small number detected at the plasma membrane. This study serves as an important reference point for interested researchers. However, some of the zDHHC enzymes examined by Ohno et al have been reported to have different localisations in other studies; this might reflect cell-type specific differences (e.g. in expression of specific accessory factors) or might be due to the generally high expression levels of transfected proteins in HEK293T cells, which could affect trafficking/folding of certain zDHHC enzymes. Some examples include zDHHC4, which was reported to be localised to the Golgi
in HEK293T cells but which was subsequently identified at the endoplasmic reticulum in hippocampal neurons [22]. PC12 cells [23] and indeed HEK293T cells [23]. zDHHC2 was described as an ER/Golgi protein by Ohno et al, however other studies showed localisation of zDHHC2 to the plasma membrane and recycling endosomes in PC12 cells [24], HEK293 cells [24] and hippocampal neurons [25]. It will be important in future work to verify the localisations of all endogenously-expressed zDHHC enzymes by antibody staining. Thus far this has only been achieved for a small number of the enzymes, e.g. [26], mainly reflecting an absence of suitable and specific antibodies against zDHHC enzymes. There are seven zDHHC genes in the yeast Saccharomyces cerevisiae, and the resulting proteins have been reported to localise in an isoform-specific manner on the ER, Golgi, plasma membrane or vacuole [8, 21, 27-29].

Existing knowledge about the targeting signals within zDHHC enzymes that mediate their intracellular targeting is somewhat limited. zDHHC4 and zDHHC6 utilise C-terminal lysine-based signals to allow ER retention [23]. Mutational analysis identified a KKXX motif in zDHHC6 and a KXX motif in zDHHC4 that are important for ER retention; these signals occur at the extreme C-termini of the zDHHC enzymes [23]. Removing the KXX motif from zDHHC4 led to its redistribution onto Golgi membranes, whereas zDHHC6 moved towards the plasma membrane and a perinuclear Golgi-like compartment. Furthermore, the lysine signals from both zDHHC4 and zDHHC6 were sufficient to relocalise zDHHC3 from the Golgi to the ER when they were appended to the C-terminus of this protein. Although these studies provide key information on how the localisations of zDHHC4/6 are achieved, other ER-localised zDHHC enzymes do not contain obvious lysine-based sorting signals, implying that these other isoforms use distinct signals to become localised to the ER. These localisation signals might be contained within the transmembrane domains, or involve cytoplasmic signals, such as arginine-based motifs [30].

In PC12 cells and neurons, zDHHC2 exhibits a dynamic localisation, being present on mobile vesicles that traffic between recycling endosomes and the plasma membrane [24, 25, 31]. This dynamic cycling of zDHHC2 is proposed to require the cytoplasmic C-terminus of this enzyme [24] and is important functionally for controlling activity-dependent S-acylation and synaptic clustering of PSD95 [31].

zDHHC Substrate Specificity

A major outstanding question in the protein S-acylation field relates to the specificity of interaction between zDHHC enzymes and substrate proteins. Numerous S-acylation assays where S-acyl-transferases and potential substrates were co-expressed in the same cell reveal various degrees of selectivity/promiscuity for both zDHHC enzymes and substrates [11]. Kinetics of membrane localization/S-acylation between stereoisomeric semisynthetic NRas peptides have suggested that zDHHC enzymes do not recognize the surrounding to the S-acylated cysteine(s) residues on substrates [32]. Therefore, zDHHC enzymes that exhibit selective interactions must recognise regions on substrates that are distal to the S-acylated cysteine(s). Indeed, the yeast Pfa3 recognises a region downstream of the palmitoylated SH4 domain of Vac8 [33], whereas zDHHC17 recognises a hydrophobic region downstream of the cysteine-rich domain of SNAP25 [34], as well as a region outside the palmitoylated cISTID domain of NMNAT2 [35]. zDHHC-substrate interactions seem to be mediated by particular modules found on certain zDHHC enzymes: zDHHC5 and zDHHC8 recognise GRIP1b via their C-terminal PDZ ligands and simultaneous depletion of these enzymes almost abolishes GRIP1 S-acylation [36]; zDHHC17 binds to huntingtin (HTT) via its N-terminal ankyrin-repeat (ANK) domain, and RNAi-mediated depletion of this enzyme reduced HTT S-acylation [37]. Furthermore, appending the ankyrin-repeats from zDHHC17 onto zDHHC3, enabled the latter enzyme to S-acylate huntingtin [37]. Recently, the ANK domain of zDHHC17 was also shown to recruit CSP and SNAP25, and its deletion rendered zDHHC17 unable to S-acylate these two substrates [38]. The above indicate that PDZ-binding and ANK modules are indeed substrate-recruiting regions of zDHHCs; nevertheless, PDZ or ANK mediated interactions do not seem to always result in substrate S-acylation, and this has been observed for PSD95 recruitment by zDHHC5’s PDZ interaction motif [39], and also for SNAP25/CSP recruitment by zDHHC13’s ANK domain [38]. It would be interesting to see whether the predicted PDZ binding motifs on zDHHC3, 14 and 23 also bind PDZ-containing proteins, and whether these interactions result in substrate S-acylation.
The notion that the above zDHHC enzymes exhibit specific substrate profiles is also supported by the clear phenotypes of respective zDHHC mutant mice. zDHHC8 knockout mice have deficits in pre-pulse inhibition (PPI) and exploratory behaviour and underlying changes in synapse formation and dendritic growth [40]. zDHHC17 genetrap mice exhibit motor coordination deficits and decreased PPI, and display a decreased volume of striatum, cerebral cortex and hippocampus, decreased density of medium spiny neurons, and a decreased number of excitatory synapses [41]. zDHHC17 mice also have a deficit in hippocampal-dependent learning and exhibit motor abnormalities [42]. Collectively, these phenotypes show that individual zDHHC enzymes have important physiological functions, although it will be important in follow-up work to test whether these changes specifically reflect a loss of S-acylation activity or are related to other functions of these enzymes; such S-acyl-transferase-independent functions have been demonstrated for zDHHC17 [43] and the yeast zDHHC enzymes Swf1 [44] and Ak1 [45].

Certain zDHHC enzymes (such as the Golgi-localised zDHHC3 and zDHHC7) do not seem to be very selective in the substrates that they can acylate, which is reflected from the fact that these commonly appear as highly active against a plethora of proteins, upon co-expression within the same cell. Indeed, zDHHC3 and zDHHC7 do not seem to recognise their apparent substrates (CSP/SNAP25), but are able to S-acylate them more robustly upon co-expression, compared to (the also Golgi-localized) zDHHC17 and zDHHC13 [38]. Similarly, zDHHC7 could S-acylate NMNAT2 more robustly than zDHHC17, despite the fact that only the latter, but not the former, could bind to NMNAT2 lacking its palmitoylated cISTID domain [35]. Hence, at least within the Golgi region, where most S-acylation events tend to occur [32], two classes of zDHHC enzymes seem to exist: a class with low substrate affinity but very high S-acyl-transferase activity (zDHHC3/zDHHC7), and another one with high substrate affinity but low S-acyl-transferase activity (zDHHC17/zDHHC13); the first class may exist to facilitate S-acylation of multiple substrates which are already weakly attached to membranes (due to either intrinsic affinity, or myristoylation/ prenylation), while the second class ensures substrate-specific S-acylation of proteins with no, or very weak membrane affinity; the latter are recruited for S-acylation by specific modules within certain zDHHC enzymes.

Diseases Associated with S-acylation Deficits

There is strong evidence linking S-acylation by zDHHC9 with intellectual disability (ID). One study identified mutations in ZDHHC9 as the cause of ID in 4 out of 250 families studied [46], and importantly, two of these mutations resulted in single amino acid changes in the DHH-CR domain (R148W and P150S). These amino substitutions have since been shown to affect the auto-acylation of zDHHC9, albeit in different ways [47]. Although it is possible that these mutations cause ID by affecting other functions of zDHHC9, it is most likely that deficits in autoacylation and subsequent substrate S-acylation are responsible for this disorder in the individuals with ZDHHC9 mutations. Key substrates of zDHHC9 are thought to be the S-acylated RAS isoforms (H- and N-RAS) [48], and knockdown of this enzyme reportedly results in mis-localisation of H-RAS in neurons [49]. Importantly, mutations in RAS proteins are associated with a range of disorders many of which have ID as a feature [46], suggesting that loss of RAS S-acylation could be a contributing factor leading to ID in the affected individuals. Clearly an important area of research will be to map the substrates of zDHHC9, which could be achieved by employing quantitative proteomics on wild-type and knockout mouse models or following RNA-mediated knockdown of zDHHC9 in cultured cells.

Mutations in the enzymes that mediate deacylation of proteins are also known to cause disease. Removal of acyl chains from S-acylated proteins is mediated by thioesterase enzymes with a characteristic α/β-hydrolase fold [50]. Of these, the cytosolic enzymes APT1 and APT2 are thought to be responsible for S-acylation/deacylation turnover of numerous proteins. A related protein, PPT1 (protein palmitoyl thioesterase 1) is localised to lysosomes, where it functions in the deacylation of proteins during their degradation [51-53]. Mutations in PPT1 are known to cause infantile neuronal ceroid lipofuscinosis (INCL) [54] an early-onset neurodegenerative disorder, characterised by blindness, seizures, and cognitive and motor deficits [55]. Symptoms of INCL typically appear around 18 months of age and are followed by death in early childhood; there is currently no cure for this disorder.

There is also evidence that mutations that disrupt the S-acylation of specific substrates can lead to neuronal dysfunction. Of interest, recent work identified mutations in the
DNAJC5 gene, encoding cysteine-string protein (CSP), as the cause of adult-onset NCL (ANCL) in several unrelated families [56-58]. Two disease-causing mutations were identified that resulted in a Leucine-to-Arginine substitution of amino acid 115 (L115R) or a deletion of the neighbouring cysteine residue (ΔL116). These mutations occur in a region of the CSP protein that is highly S-acylated [59] and were shown to lead to a loss of S-acylated monomers of CSP (on SDS gels) and the presence of high molecular weight SDS-resistant aggregates [60]. Formation of these aggregates was promoted by co-expression of zDHHC enzymes that are active against CSP (zDHHC-3, -7 and -17) but not by inactive forms of these enzymes [60]. Furthermore, the aggregates were solubilised by hydroxylamine, a chemical which deacylates proteins [60]. This work suggested that the aggregation of ANCL mutants is promoted by their S-acylation and also that the aggregates are stabilised by this modification. Recent work has shown that the mutants (particularly L115R) can form aggregates in the absence of S-acylation [61]. It was also suggested that hydroxylamine treatment did not solubilise the aggregates, although the experimental conditions employed in this study did not lead to efficient deacylation of wild-type CSP, questioning this conclusion [61]. Irrespective of these differences it is clear that enhanced aggregation of CSP mutants might be a key feature of ANCL perhaps leading to disruption of protein degradation pathways.

In addition to these clear examples of where defects in S-acylation cause disease, there are a number of other suggested links between zDHHC enzymes and disease: (i) a ZDHHC8 SNP and schizophrenia [62], which has not been consistently seen in different studies; (ii) reduction of zDHHC17 activity has been proposed as a possible contributing factor to Huntington’s disease [6, 41, 63], although as yet there is no clinical evidence to support this idea; and (iii) expression changes in zDHHC2, zDHHC9, zDHHC11 and zDHHC14 have been reported in different cancers [64].

Perspective

The identification of the zDHHC enzyme family was a major breakthrough for the S-acylation field, and recent evidence is revealing the importance of these enzymes for physiology and pathology. Continued progress in this area is likely to come from the further characterisation of mechanisms underlying zDHHC-substrate specificity, profiling of the substrates of individual zDHHC enzymes, and understanding how zDHHC dysfunction (or hyper-function) contributes to physiology and pathology.

References


