Published in final edited form as:

J Membr Biol. 2015 December ; 248(6): 929–941. doi:10.1007/s00232-015-9835-4.

Pharmacological Inhibition of Protein Lipidation

Lakshmi Ganesan and Ilya Levental

University of Texas Medical School, Houston, TX USA

Abstract

Lipid modifications of mammalian proteins are widespread, modifying thousands of targets centrally involved in cellular physiology. Broadly, these serve to increase protein hydrophobicity and association with cellular membranes. Often, these modifications are absolutely essential for protein stability and localization, and serve critical roles in dynamic regulation of protein function. A number of proteins affected by lipidation are associated with diseases, including parasite infections, neurological diseases, diabetes and cancer, suggesting that lipid modifications represent potentially attractive targets for pharmacological intervention. This review briefly describes the various types of post-translational protein lipid modifications, proteins modified by them, and the enzymatic machinery associated with these. We then discuss several case studies demonstrating successful development of lipidation inhibitors of potential (and more rarely, realized) clinical value. Although this field remains in its infancy, we believe these examples demonstrate the potential utility of targeting protein lipidation as a viable strategy for inhibiting the function of pathogenic proteins.

Keywords

Protein lipidation; post-translational modification; subcellular localization; membrane binding; lipid raft; microdomain

OVERVIEW OF PROTEIN LIPIDATION

Post-translational modifications are ubiquitous mechanisms for regulation of protein structure, activity, and localization. Many of these modifications (e.g. disulfide oxidation) are co-translational, serving a necessary role for folding the protein into its native conformation, and remain with the protein for its functional lifetime. A smaller, but still significant, fraction are reversible modifications that are added and removed many times before a protein is degraded, and therefore play a key role in rapid, reversible regulation of protein function. Among dozens of different chemical moieties used for protein modification, a small subset serves to increase protein hydrophobicity by attaching lipid anchors: fatty acids, isoprenoids, sterols, and/or phospholipids. The common outcome of such lipid modifications is increased affinity for cellular membranes; however, they can also facilitate intra and inter-molecular protein-protein interactions and confer stability to the modified protein⁽¹⁾. However, the various lipidations are carried out by distinct enzymes at distinct cellular locations, ultimately conferring distinct properties to the modified proteins. The modifying enzymes can be cytoplasmic (S-prenylation and N-myristoylation) or membrane-bound (S-palmitoylation)⁽²⁾, and modify proteins on the cytoplasmic face of the membranes (myristoylation, S-palmitoylation, and prenylation) or in the lumen of the

secretory pathway (GPI anchor). Moreover, lipids modifications can serve as guides for targeting of proteins to specific membrane subdomains, with saturated fatty acids (incl. GPI-anchors and palmitate) and sterols potentially targeting the tightly packed ‘lipid raft’ environment, while unsaturated and branched hydrocarbon anchors (e.g. in prenylated proteins such as G-protein $\beta\gamma$, K-ras 4B, Rab family proteins) favor the less ordered non-raft membrane regions⁽³⁾.

A large number and broad variety of disease-relevant proteins are lipid modified, with these modifications often critical for protein function, suggesting that pharmacological intervention of protein lipidation may be an efficient – though currently underexplored – approach to drug discovery. In this review, we will briefly introduce the various post-translational protein lipidations with a focus on disease-associated proteins, and discuss several case studies of drugs designed to interfere with these modifications.

Cholesterylation

Cholesterol is an abundant plasma membrane lipid in eukaryotes and is therefore vital for membrane physiology. In addition, it serves as a precursor for a variety of steroid-based endocrine signaling molecules (e.g. glucocorticoids, bile acids, and estrogens) and is also used, albeit infrequently, for protein modification. Remarkably, to our knowledge, there remains only a single well-characterized example of a protein being modified by cholesterol: the patterning morphogens of the Sonic Hedgehog (Shh) family⁽⁴⁾. During biosynthesis, these proteins undergo autocatalytic cleavage wherein a catalytic thiol attacks the carbonyl of the preceding amino acid, resulting in the formation of a thioester, which then reacts with cholesterol as a nucleophile to produce a cholesterol-modified N-terminal fragment. This lipidated fragment is the signaling-active, secreted form of the protein. Such cholesterol modification is a critical regulator of its spatial restriction/localized signaling effects⁽⁵⁾. Although the Hedgehog family is the only one that has been definitively identified to be modified in this way, chemical tagging approaches suggest many others remain to be detailed^(6, 7).

Glycophosphatidylinositol anchors

Glycophosphatidylinositol-anchored proteins (GPI-AP) constitute ~0.5% of all proteins in eukaryotes⁽⁸⁾. The GPI anchor is comprised of a diacyl phosphatidylinositol lipid covalently coupled to a trimannosyl-glucosaminyl glycan via a phosphodiester, then to the protein via a C-terminal ethanolamine. Assembly of the anchor and transfer to the protein occur in the endoplasmic reticulum (ER), mediated by nearly two-dozen different enzymes. GPI-modified proteins are transferred from the ER to the plasma membrane via the Golgi, where they are exclusively localized in the extracellular leaflet⁽⁹⁾. In this membrane, the GPI anchor is believed to confer specific association with specialized membrane microdomains known as lipid rafts, because of the preferential interactions between its saturated acyl chains and the ordered environment of the membrane raft⁽¹⁰⁾. Finally, a functionality unique to GPI-anchored proteins in lower and higher eukaryotes is that they can be released from the cell surface by cleavage of the lipid anchor by phosphatidylinositol-specific endogenous hydrolases⁽¹¹⁾. Such phospholipases show specificity for specific GPI-APs⁽¹²⁾, for example, phospholipase C acts on acetylcholinesterase⁽¹³⁾ and phospholipase D on alkaline

phosphatase⁽¹⁴⁾. By controlling the release of GPI-APs from the membrane, these enzymes play a critical regulatory role in their expression and function⁽¹⁵⁾.

Myristoylation

Approximately 0.5 – 0.8% of all eukaryotic proteins are covalently modified with myristate, a 14-carbon saturated fatty acid. For this modification, an amino peptidase cleaves the initiator methionine to expose an N-terminal glycine (part of a (GXXXXS/T motif) that gets myristoylated through a stable amide linkage by one of two (in humans) cytosolic N-myristoyl transferases (NMT1 and 2). Myristoylation is usually co-translational, but can also occur post-translationally, when caspase cleavage activated by apoptosis exposes a cryptic myristoylation site in previously unmodified protein⁽¹⁶⁾.

The main function of myristoylation appears to be membrane targeting; however, myristate is believed to be generally insufficient to stably anchor protein to membranes. Rather, this modification is hypothesized to be one part of a two-signal mechanism, wherein myristate confers weak membrane binding, which is then stabilized by a second signal:

1. A polybasic stretch of amino acids that interacts electrostatically with the negative charged phospholipids (phosphatidyl serine or phosphatidyl inositol) prevalent on the cytosolic face of many cellular membranes (e.g. c-Src, HIV-gag)
2. Hydrophobic residues proximal to the myristoylation site (small G-protein Arf1)
3. An additional lipid modification, such palmitoylation (Src-family kinases, and Gα-subunits)

Myristoylation offers an interesting example of regulation of membrane binding without post-translational control of protein modification, via the “myristoyl switch”. Myristate is buried in a hydrophobic cleft in certain proteins and its exposure is regulated by ligand binding or protein multimerization. An example is the HIV matrix protein myr-MA, which guides the intracellular localization of HIV-Gag proteins to membrane rafts during viral assembly and its dissociation during infectivity using the “myristoyl switch” mechanism. The equilibrium between the ‘myr-exposed’ and ‘myr-sequestered’ is entropically driven: self-associating Gag subdomains promote the exposed state, while the cleavage of Gag by HIV-1 protease triggers the sequestered state, causing the release of the protein from the membrane⁽¹⁷⁾.

Prenylation

The most abundant and evolutionarily conserved proteins modified by prenylation are small GTPases, including those of the Ras and Rab families. This modification involves the post-translational addition of a C₁₅ (farnesylation) or C₂₀ (geranyl-geranylation) isoprenoid via a thioether to the C-terminal cysteine of a cytosolic protein (detailed review in⁽¹⁸⁾). The recognized signal for protein prenylation is known as the ‘CAAX box’, a C-terminal motif featuring the prenylated cysteine, two aliphatic amino acids, and a variable amino acid (usually serine, methionine, cysteine, alanine, glutamine or leucine). This variable terminal residue of the CAAX box is a determinant of the choice between the two types of prenylation, e.g. if X is leucine and if the CAAX box has a proximal polybasic domain

enriched in lysine and arginine, the protein tends to be geranyl-geranylated (e.g., Rap, Rho, Rac)⁽¹⁸⁾. Prenylation is carried out by heterodimeric enzymes, either Farnesyl Transferase (FTase) or one of two Geranyl Geranyl Transferases (GGTase -I and -II), and used for recruitment of otherwise soluble proteins to the cytoplasmic leaflet of the endoplasmic reticulum (ER). Here, the Ras-converting enzyme (Rce1) proteolytically removes the –AAX terminal motif, followed by carboxymethylation of the exposed C-terminal cysteine by isoprenyl cysteine carboxy methyl transferase (ICMT)⁽¹⁸⁾. The complete processing pathway is required for the production of stable prenylated proteins.

It is believed that a C₂₀ geranyl-geranyl group is sufficiently hydrophobic to autonomously anchor a protein to membranes, whereas farnesyl is not⁽¹⁹⁾. Some geranyl-geranylated proteins – e.g. the small GTPases of the Rho family - are regulated by a similar mechanism to the “myristoyl switch”, except that the hydrophobic pocket that sequesters the lipid anchor exists in a separate protein, Rho GDP-dissociation inhibitor (Rho-GDI)⁽²⁰⁾. For Rho, burial of its lipid anchor in a hydrophobic pocket of Rho-GDI is sufficient to prevent its association with membranes, preventing interaction with membrane-resident GTP-exchange factors (GEFs) and subsequent activation. Farnesylated proteins require a second signal for stable membrane interaction, as is the case for the proto-oncogenes of the Ras family⁽²¹⁾. This two-signal paradigm allows reversible tuning of membrane association, which can then be used for regulation of subcellular localization⁽²²⁾.

Palmitoylation

Whereas the lipid modifications discussed thus far have well-defined sequence specificities that mark their target proteins and relatively narrow substrate groups, palmitoylation is both less specific and broader. Acyl biotin exchange and proteomic analyses have annotated hundreds, if not thousands of different palmitoylated proteins^(23–28), and despite this wealth of data, no obvious sequence motifs specifying palmitoylation have emerged. There are three variations of protein palmitoylation, S-, N- and O-palmitoylation, with S-palmitoylation being the most widely explored.

S-palmitoylation—S-acylation involves the addition of a fatty acid onto proteins via cysteine thioesterification. Although a number of fatty acids (including stearate, oleate, and even polyunsaturated fatty acids⁽²⁹⁾) can be incorporated by this mechanism, the most common is 16-carbon, saturated palmitic acid, thus S-acylation is often referred to as ‘palmitoylation’. Unlike other lipidations that exclusively modify otherwise soluble proteins, palmitoylation modifies both peripheral membrane proteins and transmembrane ones. Another distinguishing feature of palmitoylation is that it is reversible, thus serving as a mechanism to shuttle substrate proteins between the cytosol and the membranes⁽³⁰⁾, or between coexisting membrane domains⁽³⁾. Palmitoylation is often observed as a second lipid modification, imparting stable membrane attachment to proteins transiently interacting with membranes through myristoyl or prenyl groups; however, single palmitoylation and multiple palmitoylation of a single protein are also quite common.

S-palmitoylation is mediated by a family of cysteine-rich domain (CRD)-containing palmitoyl acetyl transferases (PAT) termed *DHHC* for a consensus Asp-His-His-Cys

sequence in their active site. This DHHC PAT motif is conserved from yeast to primates, the latter possessing 23 such enzymes with seemingly overlapping specificities^(31, 32). DHHCs are multipass membrane proteins having 4–6 transmembrane domains (TMD) and are broadly distributed in cellular membranes, including the endoplasmic reticulum (ER), Golgi and the plasma membrane (PM). Transfer of the palmitate from the donor (palmitoyl-CoA) to the protein proceeds via an acyl enzyme intermediate⁽³³⁾. Palmitoylation is also regulated by the oligomerization status of the DHHC enzymes. Specifically, DHHC2 and DHHC3 were found to exist in equilibrium between monomer and homo/heterodimeric states, with the monomers as the more active form⁽³⁴⁾. Perhaps because of the multiplicity of enzymes mediating palmitoylation, there is no single consensus motif to indicate a palmitoylation site, although such sites can be predicted using algorithms based on the context in which the modified cysteine residue is present^(35, 36), e.g. a cysteine in a transmembrane domain at the interface between the cytoplasm and the membrane. Depalmitoylation is even less understood, with three possible palmitoyl protein thioesterases (PPTs) described: the Acyl Protein Thioesterases (APT1 and 2) and Palmitoyl Protein Thioesterase (PPT1)^(37–39). The latter is a lysosomal lumen hydrolase that non-specifically de-acylates C14–18 acyl side-chains (myristate, palmitate, stearate, and oleate) *in vitro*. Thus, there are only two known cytoplasmic thioesterases compared to 23 palmitoyl transferases, suggesting that the former have broader specificity, while the latter regulate the dynamic palmitoylation of specific substrates⁽⁴⁰⁾.

The increased membrane affinity of lipidated proteins affects their localization and function. For example, dynamic cycles of palmitoylation via PATs and de-palmitoylation via thioesterases enable temporal cycles of membrane association and dissociation within the cell, which act as an organizing system for subcellular localization of peripheral proteins⁽³⁰⁾. A prominent example is the continuous acylation and de-acylation cycles of H-Ras and N-Ras that are required for the dynamic shuttling of these important oncogenes between cellular locations. According to a kinetic trapping model, prenylation of these proteins promotes transient interactions with membranes, allowing them to sample all cellular membranes. Palmitoylation then traps the protein in the membrane, thus fixing the location of the lipidated protein at the sub-cellular localization of its specific PAT, which in the case of Ras is the Golgi Apparatus, from which Ras is delivered to the PM by vesicular traffic^(22, 41). In concordance with this model, interfering with either palmitoylation or depalmitoylation causes Ras mislocalization⁽⁴²⁾ and perturbation of mitogenic activity⁽⁴³⁾.

N- and O-Palmitoylation—In addition to the cholesterol-mediated proteolytic processing described above, the secreted morphogens of the Hedgehog (Hh) family are modified with palmitate. However, distinct from S-acylation, the palmitic acid is attached to the N-terminus rather than a cysteine thiol group. This reaction is mediated by an enzyme called Hedgehog acetyl transferase (Hhat), a multipass membrane protein of the MBOAT family (membrane-bound O-acyl transferase), and is required for the formation of large soluble Hh oligomers and the resulting functionality of the Hedgehog proteins⁽⁴⁴⁾. Another MBOAT family member, Porcupine, is responsible for palmitoylation of several of the ubiquitous secreted morphogens of the Wnt family, though here the palmitoylated residue is a serine,

and thus the modification is an O-acylation⁽⁴⁵⁾. As for Hh, lipidation is necessary for protein function, as interference with palmitoylation also blocks Wnt secretion⁽⁴⁶⁾.

SMALL MOLECULE MODULATORS OF PROTEIN LIPIDATION

As described above, lipidation is an essential modification for the activity of many disease-related proteins. For example, src-family kinases (c-Src, Fyn, Lck, Lyk) implicated in a variety of cancers are myristoylated and often palmitoylated. Myristoylated HIV-gag is an attractive target in the treatment of HIV. Oncogenic Ras is always prenylated and often palmitoylated. Importantly, some of these lipidated disease targets (e.g. Hh) have no enzyme activity of their own, while the enzymatic activity of others (e.g. Ras) has proven intractable to pharmacological perturbations⁽⁴⁷⁾. Thus, their lipid modifications, and the enzymes associated with these, may be attractive options for inhibition of pathogenic activity via pharmacological agents⁽⁴⁸⁾. In the following section, we present a non-exhaustive review of several exemplary studies of targeting protein lipidations for interference with pathogenic protein function.

Inhibition of cholesterol synthesis by statins

Statins are a class of drugs that are widely used to lower circulating cholesterol levels via interference with cholesterol biosynthesis. Statin treatment has been shown to affect Hh signaling *in vitro*⁽⁴⁹⁾, with the potential for interfering with development Hh-associated cancers (e.g. basal cell carcinoma)⁽⁵⁰⁾. This effect is potentially related to the cholesteroylation of these secreted morphogens, though this has not been directly demonstrated.

Separately, although statins are highly effective and relatively specific at inhibiting cholesterol synthesis, pleiotropic effects have been reported. Statins inhibit the rate-limiting enzyme in the mevalonate pathway (3-hydroxy 3-methyl glutaryl CoA reductase), an essential intermediate for cholesterol synthesis, but also for the production of isoprenoids involved in prenylation. It is likely that some of the off-target effects of statins in cultured model systems and possibly *in vivo* are related to statins' interference with protein prenylation (discussed in detail in⁽⁵¹⁾). This effect was clearly demonstrated in *C. elegans*, which lack the branch of the mevalonate pathway leading to cholesterol synthesis, but nevertheless displaying profound developmental defects upon statin treatment, associated with the unfolded protein response⁽⁵²⁾.

Inhibition of GPI-anchoring

Arising as a false positive hit in a high-throughput screen for compounds targeting heat shock proteins in *Saccharomyces cerevisiae*, came the discovery of gepinacin, a selective antifungal agent. Making use of the genetic tractability of *S. cerevisiae*, gepinacin was found to target Gwt1, an acyltransferase essential for the biosynthesis of fungal GPI anchors, but not its functional mammalian ortholog PIG-W. The therapeutic implication of this inhibition were revealed in the less genetically tractable but more therapeutically relevant fungus *Candida albicans*: gepinacin impaired fungal filamentation and exposed cell-wall β -glucan, thereby promoting a macrophage-mediated immune response⁽⁵³⁾.

Inhibitors of myristoylation

Although several disease associated proteins - including src-family kinases, HIV-gag proteins, and the α subunits of trimeric G-proteins - are myristoylated by N-myristoyl transferase isoforms 1 and 2, this modification has not been widely targeted for inhibition because of the hundreds of other myristoylated protein substrates, many of which are essential for cell viability. However, the evolutionary divergence between the human, fungal and parasitic protozoan N-methyl transferases (NMT) has been exploited in the development of species-specific inhibitors against *Plasmodium falciparum*, *Trypanosoma brucei* and *Leishmania donovani* for treating malaria, sleeping sickness (trypanosomiasis), and leishmaniasis, respectively. High-throughput screening identified four series (aminoacylpyrrolidines, piperidinyldoles, thienopyrimidines, and bisphenyl derivatives) of Leishmania-selective inhibitors with considerable selectivity over all other NMTs tested^(54, 55). Co-crystallization with aminoacylpyrrolidine derivatives revealed the active diastereoisomer, facilitation structure-guided fusion with the piperidinyldoles, leading to increased potency⁴⁶.

Another example of targeting myristoylation for therapeutic purposes is by exploiting differences in NMT expression levels. Downregulation of the human NMT isoforms expression and activity was shown in HIV-1 infected T-cell lines. Chronically infected cells with downregulated NMTs showed increased sensitivity to synthetic NMT inhibitors (serinal derivatives) when compared to those with acute HIV infection. Thus, although NMT-targeted inhibitors may affect host cells, there may be a therapeutic window to specifically target HIV-infected cells, making human NMTs an attractive target in the treatment of HIV⁽⁵⁶⁾.

Prenylation inhibitors

Prenylation and its associated proteolytic processing are essential for the proper localization and function of many cellular proteins associated with oncogenesis, most notably the highly homologous small GTPases of the Ras family (H-Ras, N-Ras and the splice variants K-Ras 4A and B), which act as signal transducers for growth factor receptors at the cell surface. Ras was the first human oncogene discovered and is mutated in a significant number of cancers⁽⁵⁷⁾. Despite being one of the first, and still most sought after targets for anti-cancer drug discovery, an effective small molecule modulator of Ras activity remains elusive (though interference with downstream effectors, e.g. MAPK, have shown some clinical efficacy⁽⁴⁷⁾). Targeting Ras activity directly is difficult: (1) the very high (picomolar) affinity for its enzymatic substrate (GTP) prohibits competitive inhibition; (2) allosteric inhibition is challenging due to the absence of well-defined 'druggable' pockets. Thus, there has been great interest in pharmacological intervention of Ras processing (reviewed in ⁽⁵⁸⁾).

Ras interacts with both its activators and downstream effectors on the cytosolic surface of membranes, most often the plasma membrane. Thus it is no surprise that membrane anchorage is indispensable for Ras activation^(59, 60). The mechanisms of anchorage are somewhat isoform specific: all 4 Ras isoforms share the CAAX box that specifies prenylation and proteolytical processing, and all require a second signal for stable membrane attachment. For K-Ras, that signal is a polybasic stretch of C-terminal lysine residues; for N-

and H-Ras, it is a single or double palmitoylation, respectively^(21, 61). Because inhibition of Ras association with the membrane interferes with its mitogenic activity⁽⁴³⁾, targeting the lipid modifications that mediate this association is an attractive paradigm for inhibiting Ras hyperactivation. This possibility has been explored using prenylation inhibitors. Ras CAAX box peptidomimetics were designed to compete for the enzyme farnesyl transferase (FTase)⁽⁶²⁾. These peptides were cell-impermeable, so mixed CAAX peptidomimetics with “AA” replaced with benzodiazepine or aminomethylbenzoic acid (C-BZA-M/C-AMBA-M) were designed to increase membrane permeability. These were highly potent ($IC_{50} < 1$ nM) and blocked the attachment of farnesyl to Ras; however this effect was nonspecific, also affecting the modification of nuclear lamins, among other modified targets⁽⁶³⁾. Targeting the prenyl side of the lipidation reaction, farnesyl pyrophosphate (FPP) analogs⁽⁶⁴⁾, as well as bisubstrate analogs targeting both Ras and FPP have been explored with the aim of achieving target-specific inhibition of FTase⁽⁶⁵⁾. The most successful have been FTase inhibitors identified by high-throughput screening and optimized by structure-directed medicinal chemistry, e.g. Lonafarnib (SCH66336 IC_{50} 1.9 nM – competitive and 25,000 fold more specific to FTase compared to GGTase inhibition) and Tipifarnib (R115777, IC_{50} 7.9 nM), which have achieved some clinical success and are used in combination antineoplastic chemotherapies⁽⁶⁶⁾. Unfortunately, there remain some significant challenges for prenylation inhibitors as anti-oncogenic Ras therapies. FTase and geranyl geranyl transferase (GGTase) have some target specificity, but when one is inhibited, cross-prenylation can compensate⁽⁶⁷⁾. Although combinatorial therapies can simultaneously inhibit both sets of prenylation enzymes – for example, the pyrophosphate analog GGTI-2Z in combination with lovastatin⁽⁶⁸⁾, - the combined inhibition of both prenyl precursors leads to loss of function of the entire RAS superfamily, along with other targets of prenylation, whose functions are essential for the cell^(58, 69). Thus, while the individual treatments (e.g. FTase inhibitors alone) are not sufficiently effective, the combination therapies are untenably toxic⁽⁷⁰⁾. An exciting recent development was the identification and inhibition of the interaction between K-Ras and a protein called PDE δ , which appears to modulate the spatial organization of K-Ras by facilitating its diffusion in the cytoplasm, essentially acting as a K-ras GDI. The identified inhibitors bind to the prenyl binding pocket of PDE δ with nanomolar affinity, and thereby inhibit oncogenic signaling and suppress the K-Ras dependent proliferation of human ductal adenocarcinoma cells *in vitro* and *in vivo*⁽⁷¹⁾.

Hutchinson-Gilford progeria syndrome (HGPS) is a very rare, multisystem disorder, leading to premature ageing and death in children. The underlying molecular mechanism is a point mutation in the Lamin-A gene (LMNA), which alters the splicing of the pre-mRNA prelamin A, resulting in a 50 amino acid deletion mutant called progerin. The mutant protein progerin is farnesylated, carboxymethylated, and targeted to the nucleus, where it disturbs nuclear morphology⁽⁷²⁾. Since the farnesyltransferase inhibitor (FTIs) lonafarnib ameliorated disease phenotypes in pre-clinical studies, it was tested in a clinical trial involving 25 patients who were treated for a minimum of two years. Lonafarnib treatment improved the vascular stiffness, bone structure, and audiological status in some of the subjects and improved survival in all of them⁽⁷³⁾.

Modulators of palmitoylation

S-palmitoylation of Ras—In contrast to the non-specificity inherent to inhibition of prenylation, selective inhibition of Ras variants may be achieved through targeting their palmitoylation. This selectivity is potentially possible due to the multiplicity of known palmitoyl transferases and characterization of the one associated with Ras palmitoylation, the zDHHC9-GCP16 complex^(32, 74–76). Currently, only a single, non-specific inhibitor of protein S-palmitoylation is available, the palmitic acid analog 2-bromopalmitate (2-BP)⁽²⁹⁾. This inhibitor can covalently modify the active site of DHHC PATs *in vitro*⁽⁷⁷⁾, thus showing a ‘suicide inhibitor’ mechanism of action. Moreover, recent demonstrations of promiscuity prevent its clinical utility. The use of click-chemistry enabled 2-BP analogs identified hundreds of covalent targets, including not only the DHHC proteins, but also a broad variety of palmitoylated proteins, as well as some non-palmitoylated proteins^(77, 78). This promiscuity of 2-BP thus prevents its translational potential. Other compounds with reported inhibitory potential for Ras palmitoylation are 2-(2-hydroxy-5-nitro-benzylidene)-benzo[b]thiophen-3-one, a reversible non-specific inhibitor of DHHC PATs with potency comparable to that of 2-BP⁽⁷⁹⁾, and the less potent antibiotics cerulenin and tunicamycin^(80–82), all of which are unlikely to be sufficiently specific for clinical utility. Therefore, there remains a dire need and tremendous opportunity for selective, potent inhibitors of palmitoylation that can serve as both molecular tools to probe the physiology of Ras palmitoylation as well as starting points for anticancer drug discovery.

With the hypothesis that interfering with either direction of the palmitoylation/depalmitoylation process would affect Ras localization and function, a potent inhibitor of the putative depalmitoylating enzyme for H-Ras and N-Ras (APT1) was developed by a knowledge-based bioinformatics strategy called ‘protein structure similarity clustering’ (PSSC). This approach identified a set of enantiomers Palmostatin A-D as inhibitors of APT1. Palmostatin B exhibited greater affinity *in vitro* for APT1 and APT2 compared to other lipases tested (PLA1, PLA2, PLD and PLC β), and was demonstrated to interfere with the dynamic acylation of Ras^{(42),(83)}. Since APT1 has a wide range of substrates, structural characteristics of the substrates were used to guide development of more potent derivatives, resulting in Palmostatin M, a nanomolar inhibitor of APT1⁽⁸⁴⁾. However, inhibitory activity of the Palmostatin analogs against APT1 has yet to be directly demonstrated in cells or *in vivo*. Other inhibitors for palmitoyl protein thioesterases include the triazole urea derivative ML21, which reacts covalently *in vitro* with APT1 and 2 (LYPLA1 and 2).⁽⁸⁵⁾ Moreover, *in vivo* potency against the depalmitoylating enzymes has been demonstrated for reversible piperazine amide derivatives⁽⁸⁶⁾.

Alternative palmitoylation/acylation—Dysregulation of Wnt signaling through mutations in downstream signaling components (β -catenin), overexpression of Wnt ligands, or silencing of Wnt inhibitor genes occurs in multiple cancers incl. pancreatic, colon, and hepatocellular carcinoma⁽⁸⁷⁾. Wnt proteins are O-palmitoleated by porcupine (Porcn), an MBOAT family acyl transferase that modifies Ser 209 of Wnt 3a. Recently, a high-throughput, cell-based screen for Wnt secretion discovered a specific and potent PORCN inhibitor, LGK947. This compound inhibits Wnt signaling both *in vitro* and *in vivo*, and appears to be effective in mouse tumor models⁽⁸⁸⁾.

An exciting recent development for drug discovery of palmitoylation inhibitors comes from the discovery of a specific inhibitor of Shh palmitoylation. Using a target-based high throughput screen with a Shh mimetic peptide, 4 unique compounds were selected that displayed low micromolar inhibitory activity against the Shh palmitoylation enzyme Hhat. These compounds effectively blocked downstream signaling in cell lines, suggesting potential utility for Hh associated chemotherapy⁽⁸⁹⁾.

Finally, ghrelin is a growth hormone secretagogue and orexigenic (appetite stimulating) peptide that binds its receptor (GHS-R1a) when it is modified by an 8-carbon fatty acid, i.e. octanoylated. This modification is mediated by the unique enzyme Ghrelin O-acetyl transferase (GOAT), which has been identified as a promising therapeutic target for obesity and other metabolic disorders driven by impaired glucose homeostasis. Recently a bi-substrate analog GO-CoA-Tat peptide antagonist was developed to target the ternary complex between GOAT, octanoyl CoA and ghrelin. This tripartite macromolecule comprises the minimal amino acid sequence of the ghrelin peptide required for recognition by GOAT and an amino hexanoyl linker to a eleven amino acid sequence derived from HIV-tat at the C-terminus added to enhance its cell permeability. The compound inhibited the production of acylated ghrelin with an IC₅₀ of 5μM *in vitro*, also exhibiting selectivity for GOAT over other acyl transferases tested. Treatment with GO-CoA-Tat reduced the serum levels of acyl-ghrelin, reduced the weight gain in mice fed on a high fat diet ⁽⁹⁰⁾, and decreased plasma levels of aldosterone and corticosterone in rats ⁽⁹¹⁾, suggesting a bright future for this unique therapeutic.

CONCLUSIONS AND PERSPECTIVES

Because protein lipid modifications are widespread and often indispensable for protein localization and function, they potentially represent attractive targets for pharmacological intervention. This review discusses several examples in which targeting protein lipidation has been used as a viable strategy to obtain therapeutics of clinical value. However, several issues limit the utility of targeting protein lipidation. These include the lack of suitable, well-characterized high-throughput screening assays that target the lipid-modifying enzymes or the lipidation process. Since many of the lipid modifying enzymes are transmembrane proteins, they are difficult to crystallize and study in molecular detail, posing a further challenge to structure-guided drug discovery⁽⁴⁰⁾. This fact is evidenced by the observation that most successes described in this review were results of screens of diverse compound libraries, followed by structure-guided medicinal chemistry refinement, including the GPI anchor inhibitor gepinacin, myristoylation inhibitors, and prenylation inhibitors (e.g. lonafarnib). An example of a structure-guided success story are the palmostatins that inhibit Ras depalmitoylation.

Another challenge is that most lipid modifications are irreversible, and the modifying enzymes have a broad range of substrates. For example, humans have only three enzymes for prenylation, and only two for myristoylation, to serve hundreds of targets. Moreover, these enzymes are redundant in function, with inhibition of one evoking the activity of another (albeit with lower affinity for the target). An example is the failure of FTIs in inhibiting Ras, which were alternatively geranyl geranylated. A widely explored paradigm to

achieve substrate specificity when targeting enzymes that modify many substrates, is the development of bi-substrate analogs that incorporate the structural features of the modified protein in the design of inhibitors targeting the modifying enzyme. This will then target with the greater affinity, the enzyme-substrate intermediary complex, than the enzyme or the substrate itself. Examples for this include those developed against FTase, GGTase as well as GOAT. However combining peptides to the small molecules compromises their pharmacokinetics. Successful small molecules in such cases have also been developed by exploiting the evolutionary divergence of lipidation enzymes between humans and disease-causing pathogens. Examples are myristoylation inhibitors for Leishmaniasis and anti-fungals targeting GPI anchor synthesis.

Palmitoylation is a widely occurring protein modification, affecting hundreds if not thousands of mammalian proteins. Palmitoylation is unique among the lipid modifications in that it is mediated by a large number of enzymes, suggesting the possibility of specific perturbation of palmitoylated targets. It is also the only reversible modification, providing another point of vulnerability for pharmacological interference. Essential for development of specific pharmacological targeting are profiles of the palmitoylated proteome and enzyme-substrate specificities – efforts to characterize these are underway, but are far from complete. Thus far, the palmitoylation machinery appears largely redundant, both in terms of many enzymes modifying a specific substrate, and many targets for any given enzyme. This situation would not be ideal for drug development, as off-target side effects and lack of efficacy may result. Here, target-based/process-based approaches may likely yield specific hits through well-designed high throughput screening and counter-screening strategies.

Acknowledgments

This work was supported by Cancer Prevention and Research Institute of Texas (CPRIT) New Investigator Recruitment Grant (R1215).

References

1. Resh MD. Covalent lipid modifications of proteins. *Curr Biol.* 2013; 23:R431–435. [PubMed: 23701681]
2. Hang HC, Linder ME. Exploring protein lipidation with chemical biology. *Chem Rev.* 2011; 111:6341–6358. [PubMed: 21919527]
3. Levental I, Grzybek M, Simons K. Greasing their way: lipid modifications determine protein association with membrane rafts. *Biochemistry.* 2010; 49:6305–6316. [PubMed: 20583817]
4. Lewis PM, Dunn MP, McMahon JA, Logan M, Martin JF, St-Jacques B, McMahon AP. Cholesterol modification of sonic hedgehog is required for long-range signaling activity and effective modulation of signaling by Ptc1. *Cell.* 2001; 105:599–612. [PubMed: 11389830]
5. Porter JA, Young KE, Beachy PA. Cholesterol modification of hedgehog signaling proteins in animal development. *Science.* 1996; 274:255–259. [PubMed: 8824192]
6. Heal WP, Jovanovic B, Bessin S, Wright MH, Magee AI, Tate EW. Bioorthogonal chemical tagging of protein cholesterylation in living cells. *Chemical communications.* 2011; 47:4081–4083. [PubMed: 21221452]
7. Hulce JJ, Cognetta AB, Niphakis MJ, Tully SE, Cravatt BF. Proteome-wide mapping of cholesterol-interacting proteins in mammalian cells. *Nat Methods.* 2013; 10:259–264. [PubMed: 23396283]
8. Eisenhaber B, Bork P, Eisenhaber F. Post-translational GPI lipid anchor modification of proteins in kingdoms of life: analysis of protein sequence data from complete genomes. *Protein engineering.* 2001; 14:17–25. [PubMed: 11287675]

9. Magee AI. Lipid modification of proteins and its relevance to protein targeting. *J Cell Sci.* 1990; 97(Pt 4):581–584. [PubMed: 2077033]
10. Mayor S, Riezman H. Sorting GPI-anchored proteins. *Nature reviews Molecular cell biology.* 2004; 5:110–120. [PubMed: 15040444]
11. Chatterjee S, Mayor S. The GPI-anchor and protein sorting. *Cell Mol Life Sci.* 2001; 58:1969–1987. [PubMed: 11814051]
12. Muller A, Kloppel C, Smith-Valentine M, Van Houten J, Simon M. Selective and programmed cleavage of GPI-anchored proteins from the surface membrane by phospholipase C. *Biochimica et biophysica acta.* 2012; 1818:117–124. [PubMed: 22024023]
13. Low MG, Finean JB. Non-lytic release of acetylcholinesterase from erythrocytes by a phosphatidylinositol-specific phospholipase C. *FEBS Lett.* 1977; 82:143–146. [PubMed: 913568]
14. Low MG, Prasad AR. A phospholipase D specific for the phosphatidylinositol anchor of cell-surface proteins is abundant in plasma. *Proceedings of the National Academy of Sciences of the United States of America.* 1988; 85:980–984. [PubMed: 3422494]
15. Sharom FJ, Lehto MT. Glycosylphosphatidylinositol-anchored proteins: structure, function, and cleavage by phosphatidylinositol-specific phospholipase C. *Biochem Cell Biol.* 2002; 80:535–549. [PubMed: 12440695]
16. Chen CA, Manning DR. Regulation of G proteins by covalent modification. *Oncogene.* 2001; 20:1643–1652. [PubMed: 11313912]
17. Tang C, Loeliger E, Luncsford P, Kinde I, Beckett D, Summers MF. Entropic switch regulates myristate exposure in the HIV-1 matrix protein. *Proceedings of the National Academy of Sciences of the United States of America.* 2004; 101:517–522. [PubMed: 14699046]
18. Zhang FL, Casey PJ. Protein prenylation: molecular mechanisms and functional consequences. *Annu Rev Biochem.* 1996; 65:241–269. [PubMed: 8811180]
19. Bhatnagar RS, Gordon JI. Understanding covalent modifications of proteins by lipids: where cell biology and biophysics mingle. *Trends in cell biology.* 1997; 7:14–20. [PubMed: 17708893]
20. Nomanbhoy TK, Erickson JW, Cerione RA. Kinetics of Cdc42 membrane extraction by Rho-GDI monitored by real-time fluorescence resonance energy transfer. *Biochemistry.* 1999; 38:1744–1750. [PubMed: 10026253]
21. Hancock JF, Paterson H, Marshall CJ. A polybasic domain or palmitoylation is required in addition to the CAAX motif to localize p21ras to the plasma membrane. *Cell.* 1990; 63:133–139. [PubMed: 2208277]
22. Rocks O, Peyker A, Kahms M, Verveer PJ, Koerner C, Lumbierres M, Kuhlmann J, Waldmann H, Wittinghofer A, Bastiaens PI. An acylation cycle regulates localization and activity of palmitoylated Ras isoforms. *Science.* 2005; 307:1746–1752. [PubMed: 15705808]
23. Martin BR, Wang C, Adibekian A, Tully SE, Cravatt BF. Global profiling of dynamic protein palmitoylation. *Nature methods.* 2012; 9:84–89. [PubMed: 22056678]
24. Ivaldi C, Martin BR, Kieffer-Jaquinod S, Chapel A, Levade T, Garin J, Journet A. Proteomic analysis of S-acylated proteins in human B cells reveals palmitoylation of the immune regulators CD20 and CD23. *PLoS One.* 2012; 7:e37187. [PubMed: 22615937]
25. Martin BR, Cravatt BF. Large-scale profiling of protein palmitoylation in mammalian cells. *Nat Methods.* 2009; 6:135–138. [PubMed: 19137006]
26. Merrick BA, Dhungana S, Williams JG, Aloor JJ, Peddada S, Tomer KB, Fessler MB. Proteomic profiling of S-acylated macrophage proteins identifies a role for palmitoylation in mitochondrial targeting of phospholipid scramblase 3. *Mol Cell Proteomics.* 2011; 10:M110 006007. [PubMed: 21785166]
27. Ren W, Jhala US, Du K. Proteomic analysis of protein palmitoylation in adipocytes. *Adipocyte.* 2013; 2:17–28. [PubMed: 23599907]
28. Yount JS, Moltedo B, Yang YY, Charron G, Moran TM, Lopez CB, Hang HC. Palmitoylome profiling reveals S-palmitoylation-dependent antiviral activity of IFITM3. *Nat Chem Biol.* 2010; 6:610–614. [PubMed: 20601941]
29. Webb Y, Hermida-Matsumoto L, Resh MD. Inhibition of protein palmitoylation, raft localization, and T cell signaling by 2-bromopalmitate and polyunsaturated fatty acids. *J Biol Chem.* 2000; 275:261–270. [PubMed: 10617614]

30. Rocks O, Gerauer M, Vartak N, Koch S, Huang ZP, Pechlivanis M, Kuhlmann J, Brunsfeld L, Chandra A, Ellinger B, Waldmann H, Bastiaens PI. The palmitoylation machinery is a spatially organizing system for peripheral membrane proteins. *Cell*. 2010; 141:458–471. [PubMed: 20416930]
31. Ohno Y, Kashio A, Ogata R, Ishitomi A, Yamazaki Y, Kihara A. Analysis of substrate specificity of human DHHC protein acyltransferases using a yeast expression system. *Molecular biology of the cell*. 2012; 23:4543–4551. [PubMed: 23034182]
32. Greaves J, Chamberlain LH. DHHC palmitoyl transferases: substrate interactions and (patho)physiology. *Trends in biochemical sciences*. 2011; 36:245–253. [PubMed: 21388813]
33. Dietrich LE, Ungermann C. On the mechanism of protein palmitoylation. *EMBO Rep*. 2004; 5:1053–1057. [PubMed: 15520806]
34. Lai J, Linder ME. Oligomerization of DHHC protein S-acyltransferases. *J Biol Chem*. 2013; 288:22862–22870. [PubMed: 23793055]
35. Ren J, Wen L, Gao X, Jin C, Xue Y, Yao X. CSS-Palm 2.0: an updated software for palmitoylation sites prediction. *Protein engineering, design & selection: PEDS*. 2008; 21:639–644.
36. Kumari B, Kumar R, Kumar M. PalmPred: an SVM based palmitoylation prediction method using sequence profile information. *PloS one*. 2014; 9:e89246. [PubMed: 24586628]
37. Das AK, Dasgupta B, Bhattacharya R, Basu J. Purification and biochemical characterization of a protein-palmitoyl acyltransferase from human erythrocytes. *The Journal of biological chemistry*. 1997; 272:11021–11025. [PubMed: 9110994]
38. Soyombo AA, Hofmann SL. Molecular cloning and expression of palmitoyl-protein thioesterase 2 (PPT2), a homolog of lysosomal palmitoyl-protein thioesterase with a distinct substrate specificity. *The Journal of biological chemistry*. 1997; 272:27456–27463. [PubMed: 9341199]
39. Camp LA, Verkruse LA, Afendis SJ, Slaughter CA, Hofmann SL. Molecular cloning and expression of palmitoyl-protein thioesterase. *The Journal of biological chemistry*. 1994; 269:23212–23219. [PubMed: 7916016]
40. Chavda B, Arnott JA, Planey SL. Targeting protein palmitoylation: selective inhibitors and implications in disease. *Expert opinion on drug discovery*. 2014:1–15.
41. Goodwin JS, Drake KR, Rogers C, Wright L, Lippincott-Schwartz J, Philips MR, Kenworthy AK. Depalmitoylated Ras traffics to and from the Golgi complex via a nonvesicular pathway. *The Journal of cell biology*. 2005; 170:261–272. [PubMed: 16027222]
42. Dekker FJ, Rocks O, Vartak N, Menninger S, Hedberg C, Balamurugan R, Wetzel S, Renner S, Gerauer M, Scholermann B, Rusch M, Kramer JW, Rauh D, Coates GW, Brunsfeld L, Bastiaens PI, Waldmann H. Small-molecule inhibition of APT1 affects Ras localization and signaling. *Nat Chem Biol*. 2010; 6:449–456. [PubMed: 20418879]
43. Cuiffo B, Ren R. Palmitoylation of oncogenic NRAS is essential for leukemogenesis. *Blood*. 2010; 115:3598–3605. [PubMed: 20200357]
44. Chen MH, Li YJ, Kawakami T, Xu SM, Chuang PT. Palmitoylation is required for the production of a soluble multimeric Hedgehog protein complex and long-range signaling in vertebrates. *Genes Dev*. 2004; 18:641–659. [PubMed: 15075292]
45. Willert K, Brown JD, Danenberg E, Duncan AW, Weissman IL, Reya T, Yates JR 3rd, Nusse R. Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature*. 2003; 423:448–452. [PubMed: 12717451]
46. Gao X, Arenas-Ramirez N, Scales SJ, Hannoush RN. Membrane targeting of palmitoylated Wnt and Hedgehog revealed by chemical probes. *FEBS letters*. 2011; 585:2501–2506. [PubMed: 21740904]
47. Cox AD, Fesik SW, Kimmelman AC, Luo J, Der CJ. Drugging the undruggable RAS: Mission possible? *Nature reviews Drug discovery*. 2014; 13:828–851. [PubMed: 25323927]
48. Resh MD. Targeting protein lipidation in disease. *Trends Mol Med*. 2012; 18:206–214. [PubMed: 22342806]
49. Cooper MK, Wassif CA, Krakowiak PA, Taipale J, Gong R, Kelley RI, Porter FD, Beachy PA. A defective response to Hedgehog signaling in disorders of cholesterol biosynthesis. *Nature genetics*. 2003; 33:508–513. [PubMed: 12652302]

50. Tang JY, So PL, Epstein EH Jr. Novel Hedgehog pathway targets against basal cell carcinoma. *Toxicology and applied pharmacology*. 2007; 224:257–264. [PubMed: 17276471]
51. Gazzerro P, Proto MC, Gangemi G, Malfitano AM, Ciaglia E, Pisanti S, Santoro A, Laezza C, Bifulco M. Pharmacological actions of statins: a critical appraisal in the management of cancer. *Pharmacological reviews*. 2012; 64:102–146. [PubMed: 22106090]
52. Morck C, Olsen L, Kurth C, Persson A, Storm NJ, Svensson E, Jansson JO, Hellqvist M, Enejder A, Faergeman NJ, Pilon M. Statins inhibit protein lipidation and induce the unfolded protein response in the non-sterol producing nematode *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:18285–18290. [PubMed: 19826081]
53. McLellan CA, Whitesell L, King OD, Lancaster AK, Mazitschek R, Lindquist S. Inhibiting GPI anchor biosynthesis in fungi stresses the endoplasmic reticulum and enhances immunogenicity. *ACS chemical biology*. 2012; 7:1520–1528. [PubMed: 22724584]
54. Hutton JA, Goncalves V, Brannigan JA, Paape D, Wright MH, Waugh TM, Roberts SM, Bell AS, Wilkinson AJ, Smith DF, Leatherbarrow RJ, Tate EW. Structure-based design of potent and selective *Leishmania* N-myristoyltransferase inhibitors. *Journal of medicinal chemistry*. 2014; 57:8664–8670. [PubMed: 25238611]
55. Bell AS, Mills JE, Williams GP, Brannigan JA, Wilkinson AJ, Parkinson T, Leatherbarrow RJ, Tate EW, Holder AA, Smith DF. Selective inhibitors of protozoan protein N-myristoyltransferases as starting points for tropical disease medicinal chemistry programs. *PLoS neglected tropical diseases*. 2012; 6:e1625. [PubMed: 22545171]
56. Takamune N, Hamada H, Misumi S, Shoji S. Novel strategy for anti-HIV-1 action: selective cytotoxic effect of N-myristoyltransferase inhibitor on HIV-1-infected cells. *FEBS letters*. 2002; 527:138–142. [PubMed: 12220649]
57. Pylyayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: weaving a tumorigenic web. *Nature reviews Cancer*. 2011; 11:761–774. [PubMed: 21993244]
58. Gysin S, Salt M, Young A, McCormick F. Therapeutic strategies for targeting ras proteins. *Genes & cancer*. 2011; 2:359–372. [PubMed: 21779505]
59. von Lintig FC, Dreilinger AD, Varki NM, Wallace AM, Casteel DE, Boss GR. Ras activation in human breast cancer. *Breast cancer research and treatment*. 2000; 62:51–62. [PubMed: 10989985]
60. Meder D, Simons K. Cell biology. Ras on the roundabout, *Science*. 2005; 307:1731–1733. [PubMed: 15774748]
61. Song SP, Hennig A, Schubert K, Markwart R, Schmidt P, Prior IA, Bohmer FD, Rubio I. Ras palmitoylation is necessary for N-Ras activation and signal propagation in growth factor signalling. *The Biochemical journal*. 2013
62. Reiss Y, Goldstein JL, Seabra MC, Casey PJ, Brown MS. Inhibition of purified p21ras farnesyl:protein transferase by Cys-AAX tetrapeptides. *Cell*. 1990; 62:81–88. [PubMed: 2194674]
63. James GL, Goldstein JL, Brown MS, Rawson TE, Somers TC, McDowell RS, Crowley CW, Lucas BK, Levinson AD, Marsters JC Jr. Benzodiazepine peptidomimetics: potent inhibitors of Ras farnesylation in animal cells. *Science*. 1993; 260:1937–1942. [PubMed: 8316834]
64. Patel DV, Schmidt RJ, Biller SA, Gordon EM, Robinson SS, Manne V. Farnesyl diphosphate-based inhibitors of Ras farnesyl protein transferase. *Journal of medicinal chemistry*. 1995; 38:2906–2921. [PubMed: 7636851]
65. Lannuzel M, Lamothe M, Schambel P, Etievant C, Hill B, Perez M. From pure FPP to mixed FPP and CAAX competitive inhibitors of farnesyl protein transferase. *Bioorganic & medicinal chemistry letters*. 2003; 13:1459–1462. [PubMed: 12668012]
66. End DW, Smets G, Todd AV, Applegate TL, Fuery CJ, Angibaud P, Venet M, Sanz G, Poignet H, Skrzat S, Devine A, Wouters W, Bowden C. Characterization of the antitumor effects of the selective farnesyl protein transferase inhibitor R115777 in vivo and in vitro. *Cancer research*. 2001; 61:131–137. [PubMed: 11196150]
67. Fiordalisi JJ, Johnson RL 2nd, Weinbaum CA, Sakabe K, Chen Z, Casey PJ, Cox AD. High affinity for farnesyltransferase and alternative prenylation contribute individually to K-Ras4B resistance to farnesyltransferase inhibitors. *The Journal of biological chemistry*. 2003; 278:41718–41727. [PubMed: 12882980]

68. Sane KM, Mynderse M, Lalonde DT, Dean IS, Wojtkowiak JW, Fouad F, Borch RF, Reiniers JJ Jr, Gibbs RA, Mattingly RR. A novel geranylgeranyl transferase inhibitor in combination with lovastatin inhibits proliferation and induces autophagy in STS-26T MPNST cells. *The Journal of pharmacology and experimental therapeutics*. 2010; 333:23–33. [PubMed: 20086055]
69. Konstantinopoulos PA, Karamouzis MV, Papavassiliou AG. Post-translational modifications and regulation of the RAS superfamily of GTPases as anticancer targets. *Nature reviews Drug discovery*. 2007; 6:541–555. [PubMed: 17585331]
70. Lobell RB, Omer CA, Abrams MT, Bhimnathwala HG, Brucker MJ, Buser CA, Davide JP, de Solms SJ, Dinsmore CJ, Ellis-Hutchings MS, Kral AM, Liu D, Lumma WC, Machotka SV, Rands E, Williams TM, Graham SL, Hartman GD, Oliff AI, Heimbrook DC, Kohl NE. Evaluation of farnesyl:protein transferase and geranylgeranyl:protein transferase inhibitor combinations in preclinical models. *Cancer research*. 2001; 61:8758–8768. [PubMed: 11751396]
71. Zimmermann G, Papke B, Ismail S, Vartak N, Chandra A, Hoffmann M, Hahn SA, Triola G, Wittinghofer A, Bastiaens PI, Waldmann H. Small molecule inhibition of the KRAS-PDEdelta interaction impairs oncogenic KRAS signalling. *Nature*. 2013; 497:638–642. [PubMed: 23698361]
72. Yang SH, Qiao X, Fong LG, Young SG. Treatment with a farnesyltransferase inhibitor improves survival in mice with a Hutchinson-Gilford progeria syndrome mutation. *Biochimica et biophysica acta*. 2008; 1781:36–39. [PubMed: 18082640]
73. Gordon LB, Kleinman ME, Miller DT, Neuberg DS, Giobbie-Hurder A, Gerhard-Herman M, Smoot LB, Gordon CM, Cleveland R, Snyder BD, Fligor B, Bishop WR, Statkevich P, Regen A, Sonis A, Riley S, Ploski C, Correia A, Quinn N, Ullrich NJ, Nazarian A, Liang MG, Huh SY, Schwartzman A, Kieran MW. Clinical trial of a farnesyltransferase inhibitor in children with Hutchinson-Gilford progeria syndrome. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109:16666–16671. [PubMed: 23012407]
74. Swarthout JT, Lobo S, Farh L, Croke MR, Greentree WK, Deschenes RJ, Linder ME. DHHC9 and GCP16 constitute a human protein fatty acyltransferase with specificity for H- and N-Ras. *The Journal of biological chemistry*. 2005; 280:31141–31148. [PubMed: 16000296]
75. Mitchell DA, Vasudevan A, Linder ME, Deschenes RJ. Protein palmitoylation by a family of DHHC protein S-acyltransferases. *J Lipid Res*. 2006; 47:1118–1127. [PubMed: 16582420]
76. Salaun C, Greaves J, Chamberlain LH. The intracellular dynamic of protein palmitoylation. *The Journal of cell biology*. 2010; 191:1229–1238. [PubMed: 21187327]
77. Zheng B, DeRan M, Li X, Liao X, Fukata M, Wu X. 2-Bromopalmitate analogues as activity-based probes to explore palmitoyl acyltransferases. *J Am Chem Soc*. 2013; 135:7082–7085. [PubMed: 23631516]
78. Davda D, El Azzouny MA, Tom CT, Hernandez JL, Majmudar JD, Kennedy RT, Martin BR. Profiling targets of the irreversible palmitoylation inhibitor 2-bromopalmitate. *ACS chemical biology*. 2013; 8:1912–1917. [PubMed: 23844586]
79. Jennings BC, Nadolski MJ, Ling Y, Baker MB, Harrison ML, Deschenes RJ, Linder ME. 2-Bromopalmitate and 2-(2-hydroxy-5-nitro-benzylidene)-benzo[b]thiophen-3-one inhibit DHHC-mediated palmitoylation in vitro. *J Lipid Res*. 2009; 50:233–242. [PubMed: 18827284]
80. Omura S. The antibiotic cerulenin, a novel tool for biochemistry as an inhibitor of fatty acid synthesis. *Bacteriological reviews*. 1976; 40:681–697. [PubMed: 791237]
81. Lawrence DS, Zilfou JT, Smith CD. Structure-activity studies of cerulenin analogues as protein palmitoylation inhibitors. *Journal of medicinal chemistry*. 1999; 42:4932–4941. [PubMed: 10585203]
82. De Vos ML, Lawrence DS, Smith CD. Cellular pharmacology of cerulenin analogs that inhibit protein palmitoylation. *Biochemical pharmacology*. 2001; 62:985–995. [PubMed: 11597568]
83. Dekker FJ, Hedberg C. Small molecule inhibition of protein depalmitoylation as a new approach towards downregulation of oncogenic Ras signalling. *Bioorganic & medicinal chemistry*. 2011; 19:1376–1380. [PubMed: 21129981]
84. Hedberg C, Dekker FJ, Rusch M, Renner S, Wetzel S, Vartak N, Gerding-Reimers C, Bon RS, Bastiaens PI, Waldmann H. Development of highly potent inhibitors of the Ras-targeting human acyl protein thioesterases based on substrate similarity design. *Angewandte Chemie*. 2011; 50:9832–9837. [PubMed: 21905185]

85. Adibekian, A.; Martin, BR.; Speers, AE.; Brown, SJ.; Spicer, T.; Fernandez-Vega, V.; Ferguson, J.; Cravatt, BF.; Hodder, P.; Rosen, H. Probe Reports from the NIH Molecular Libraries Program. Bethesda (MD): 2010. Optimization and characterization of a triazole urea dual inhibitor for lysophospholipase 1 (LYPLA1) and lysophospholipase 2 (LYPLA2).
86. Adibekian A, Martin BR, Chang JW, Hsu KL, Tsuboi K, Bachovchin DA, Speers AE, Brown SJ, Spicer T, Fernandez-Vega V, Ferguson J, Hodder PS, Rosen H, Cravatt BF. Confirming target engagement for reversible inhibitors in vivo by kinetically tuned activity-based probes. *Journal of the American Chemical Society*. 2012; 134:10345–10348. [PubMed: 22690931]
87. Polakis P. Wnt signaling in cancer. *Cold Spring Harbor perspectives in biology*. 2012; 4
88. Liu J, Pan S, Hsieh MH, Ng N, Sun F, Wang T, Kasibhatla S, Schuller AG, Li AG, Cheng D, Li J, Tompkins C, Pferdekamper A, Steffy A, Cheng J, Kowal C, Phung V, Guo G, Wang Y, Graham MP, Flynn S, Brenner JC, Li C, Villarroel MC, Schultz PG, Wu X, McNamara P, Sellers WR, Petruzzelli L, Boral AL, Seidel HM, McLaughlin ME, Che J, Carey TE, Vanasse G, Harris JL. Targeting Wnt-driven cancer through the inhibition of Porcupine by LGK974. *Proceedings of the National Academy of Sciences of the United States of America*. 2013; 110:20224–20229. [PubMed: 24277854]
89. Petrova E, Rios-Esteves J, Ouerfelli O, Glickman JF, Resh MD. Inhibitors of Hedgehog acyltransferase block Sonic Hedgehog signaling. *Nat Chem Biol*. 2013; 9:247–249. [PubMed: 23416332]
90. Barnett BP, Hwang Y, Taylor MS, Kirchner H, Pfluger PT, Bernard V, Lin YY, Bowers EM, Mukherjee C, Song WJ, Longo PA, Leahy DJ, Hussain MA, Tschop MH, Boeke JD, Cole PA. Glucose and weight control in mice with a designed ghrelin O-acyltransferase inhibitor. *Science*. 2010; 330:1689–1692. [PubMed: 21097901]
91. Rucinski M, Ziolkowska A, Szyszka M, Hochol A, Malendowicz LK. Evidence suggesting that ghrelin O-acyl transferase inhibitor acts at the hypothalamus to inhibit hypothalamo-pituitary-adrenocortical axis function in the rat. *Peptides*. 2012; 35:149–159. [PubMed: 22543218]

Figure 1. Protein Lipidation and its Pharmacological Inhibition

(A) N-Palmitoylation of Hedgehog (Hh), which is also cholesterylated, can be inhibited by targeting the enzyme Hedgehog acetyl transferase (HhAT) with RUSKI-41. (B) Myristate can reversibly target proteins to membranes via a “myristoyl switch”. This process can be targeted in pathogenic parasites by inhibiting their N-myristoyl transferase (NMT), e.g. by LGK947. (C) S-palmitoylation is a widespread, reversible lipidation that recruits proteins (e.g. the oncogenes N-/H-ras) to membranes and ordered membrane subdomains known as rafts. Palmitoylation is the only reversible lipid modification: the forward reaction is catalyzed by a family of DHHC palmitoyl transferases (PATs) while depalmitoylation is mediated by one of three known protein thioesterases (APT1/2 and PPT1). PATs are inhibited non-specifically by 2-bromopalmitate (2-BP) while depalmitoylation can be affected by palmostatins. (D) Geranyl-geranylation of the small GTPase Rho is catalyzed by geranyl-geranyl transferases, which are inhibited by GGTI-2Z.

Table 1

Summary of Lipid Modifications of Proteins

Lipid Modification	Modifying Group	Attachment/ Consensus motif	Linkage	Modification type	Modifying enzyme(s)	Stability	Examples
N- Myristoylation	C14 Myristate	N-terminal Glycine MGXXXS/T	Amide	Co- translational	N-myristoyl transferase (NMT1 & 2)	stable	c-Src, HIV- gag
Prenylation	Isoprenoid C15 (Farnesyl) pyrophosphate	C-terminal Cysteine CAAX box	Thioether	Co- translational	Farnesyl transferase (FTase)	stable	HRas, NRas, K-Ras, Cdc42, CENP-E, CENP-F, RhoB, Rhe
	C20 – Geranyl geranyl pyrophosphate	CAAL CC/CXC	Thioether	Co- translational	Geranyl geranyl transferase (GGTase I & II)	stable	Rab, Rho GTPases
S-acylation	Palmitate (C16)	Cysteine	Thioester	Post- translational	Palmitoyl transferases (PATs) Acyl protein thioesterases (APT's)	labile	H-Ras, N- Ras, LAT, Fyn, Lck
N- Palmitoylation	Palmitate (C16) Palmitoleate	Cysteine Serine	Amide oxyester	Post- translational	MBOATs HHat Porcin		Hedgehog, Wnt3a
Glycophosphatidyl inositol (GPI)	Phosphatidyl inositol		Amide	Co- translational	Many enzymes Phospholipase C	labile	acetylcholine esterase Placental alkaline phosphatase

Table 2

Summary of Small Molecule Modulators of Protein Lipidation

Structure	Primary target	IC ₅₀ (μM)	Mechanism
<p>Lovastatin</p>	HMG-CoA Reductase	0.003 – 0.011	Interferes with cholesterol and isoprenoid biosynthesis by inhibiting the rate-limiting enzyme in the mevalonate pathway ⁽⁶²⁾
<p>Gepinacin</p>	Gwt1 (Fungal acyltransferase)	5–10	Inhibits a critical acyltransferase in the biosynthetic pathway of fungal GPI anchors ⁽⁵⁵⁾
<p>PF-03402623</p>	N-Myristoyl transferase (<i>Leishmania donovani</i>)	0.093	Species-selective inhibition of <i>Leishmania donovani</i> NMT with demonstrated selectivity over human and plasmodium species ^(54, 55)

Structure	Primary target	IC ₅₀ (μM)	Mechanism
<p>Lonafarnib</p>	Farnesyl transferase	0.002	Blocks the post-translational attachment of the prenyl moiety to C-terminal cysteine residue of prenylated proteins by inhibiting the enzyme farnesyl transferase ⁽⁶⁶⁾
<p>2-Bromopalmitate</p>	over 450 targets that include both palmitoylated and non-palmitoylated proteins	10 – 15	A “suicide inhibitor” with promiscuous effects on over 450 targets that include both palmitoylated and non-palmitoylated proteins ⁽⁷⁸⁾
<p>LGK947</p>	PORCN	0.0004	Disregulates Wnt signaling, by interfering with PORCN, an MBOAT family O-acetyl transferase, responsible for its palmitoleation ⁽⁸⁸⁾

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Structure	Primary target	IC ₅₀ (μM)	Mechanism
<div>RU-SKI 41</div> <div> </div>	Hhat	0.18	Inhibits the palmitoylation of Shh by targeting its palmitoylating enzyme Hhat, an MBOAT family O-acetyl transferase ⁽⁸⁹⁾
GO-CoA-Tat	GOAT	5	Bi-substrate (octanoyl CoA and ghrelin) analog peptide that selectively inhibits the octanoylation of ghrelin by GOAT ⁽⁹⁰⁾