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The Bis(monoacylglycero)-phosphate Hypothesis: From Lysosomal Function to Therapeutic Avenues

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Keywords

bis(monoacylglycero)phosphate, lysobisphosphatidic acid, neurodegeneration, lipid, metabolism, lysosome

Abstract

Lysosomes catabolize and recycle lipids and other biological molecules to maintain cellular homeostasis in diverse nutrient environments. Lysosomal lipid catabolism relies on the stimulatory activity of bis(monoacylglycero)phosphate (BMP), an enigmatic lipid whose levels are altered across myriad lysosome-associated diseases. Here, we review the discovery of BMP over half a century ago and its structural properties that facilitate the activation of lipid hydrolases and recruitment of their coactivators. We further discuss the current, yet incomplete, understanding of BMP catabolism and anabolism. To conclude, we discuss its role in lysosome-associated diseases and the potential for modulating its levels by pharmacologically activating and inhibiting the BMP synthase to therapeutically target lysosomal storage disorders, drug-induced phospholipidosis, Alzheimer's disease, Parkinson's disease, frontotemporal dementia, cancer, and viral infection.

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INTRODUCTION

Late endosomes and lysosomes (LE/LYs) are membrane-bound, acidic organelles within the cytoplasm of eukaryotic cells; they sequester proteases, lipases, and nucleases and function to degrade diverse biological macromolecules, such as proteins, glycans, nucleic acids, and lipids, that are delivered to that compartment from the extracellular space by endocytosis or from within cells via autophagy. Inside LE/LYs, lipids are degraded, at least in part, on the surface of structures referred to as intralysosomal vesicles (ILVs), which are rich in a lipid called bis(monoacylglycerol)phosphate (BMP). BMP potently stimulates lysosomal lipid catabolism, and its dyshomeostasis is a signature of several age-related diseases. Still, the role of BMP in disease has been elusive, due to an incomplete understanding of its origin, roles, catabolism, and anabolism, in addition to a lack of selective tools for its perturbation in well-established

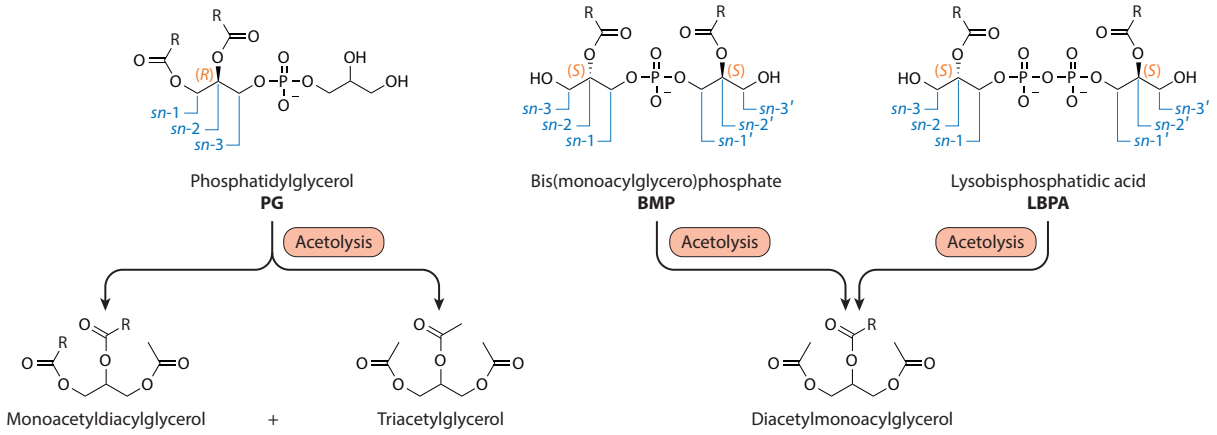

Figure 1

Acetolysis of BMP, LBPA, and PG. Acetylation of nonacylated, glyceryl alcohols indicates the number of unique glycerols and their acylation states in glycerophospholipids. Unlike PG, acetolysis of BMP and LBPA exclusively forms diacetylmonoacylglycerol and provides chemical evidence for their symmetric structures. Abbreviations: BMP, bis(monoacylglycero)phosphate; LBPA, lysobisphosphatidic acid; PG, phosphatidylglycerol.

disease models. Nonetheless, BMP's essentiality for LE/LY function and its association with diverse diseases point to the importance of studying this unusual lipid. Here, we review current knowledge of BMP metabolism in mammalian cells, identifying gaps and discussing, based on the available literature, how BMP modulation might cure intractable lysosome-associated diseases.

DISCOVERY AND LOCALIZATION OF BIS(MONOACYLGLYCERO)PHOSPHATE

Working with lung lipid extracts from pigs, Body & Gray (1) first described the existence of a glycerophospholipid with unique chromatographic properties yet chemical similarity to phosphatidylglycerol (PG) in 1967. Alkaline hydrolysis of this unknown lipid and PG both yielded the water-soluble product glycerophosphoglycerol (1). However, their lack of comigration on a thin-layer silica gel plate prodded the group to acetolyze the lipids and analyze their products. As expected, PG acetolysis yielded two distinct products, monoacetyldiacylglycerol and triacetylglycerol, indicative of its phosphodiester asymmetry, but acetolysis of the unknown lipid produced only one product, diacetylmonoacylglycerol, suggesting a symmetrical lipid species (**Figure 1**). Given this information, the group erroneously concluded that the identity of the unknown lipid was lysobisphosphatidic acid, as it was consistent with their acetolysis studies (1) (**Figure 1**). Soon after, studies unmasked the novel lipid as BMP instead, and biochemists quickly sought to elucidate its subcellular distribution (2–6). Although subcellular fractionation of rat liver homogenates and isolation of LE/LY compartments could strongly enrich for BMP, the precise subcellular localization of BMP was not known until 1998, when Kobayashi et al. (7, 8) developed an anti-BMP antibody and used immunofluorescence to reveal that BMP resides exclusively within LE/LYs.

ROLES OF BMP IN LATE ENDOSOME AND LYSOSOME LIPID METABOLISM

The unusual structure of BMP facilitates its function to stimulate LE/LY lipid metabolism and can be characterized by its positions of esterification, exposed negative charge, fatty acid

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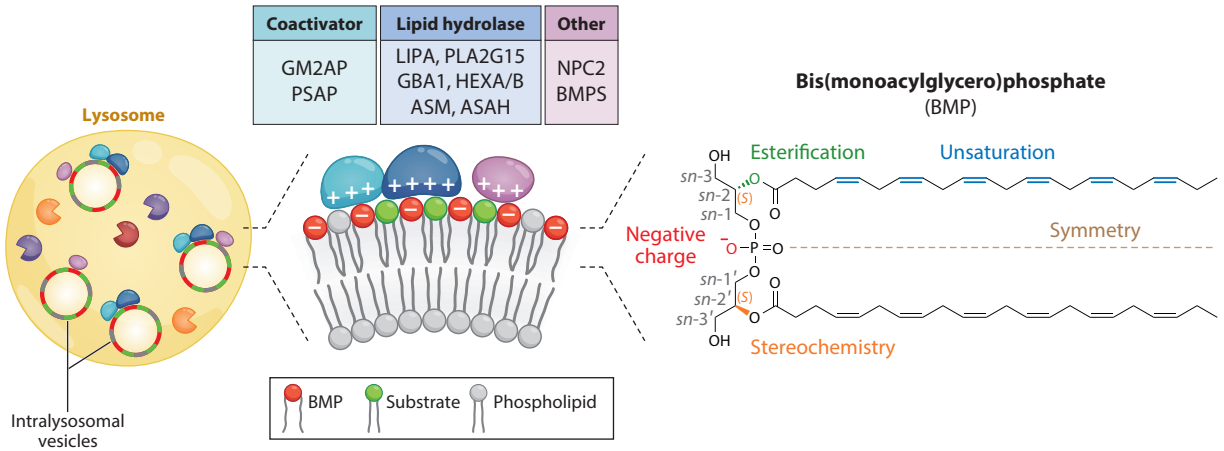

Figure 2

Structure and function of BMP. Lysosomal lipid catabolism occurs on BMP-laden intralysosomal vesicles (ILVs). Cationic hydrolases, coactivator proteins, and other lipid-related proteins associate with ILVs through electrostatic interactions with BMP whose negative charge, position of esterification, unsaturation, stereochemistry, and symmetry help facilitate its function to stimulate lipid catabolism. Abbreviations: ASAH, acid ceramidase; ASM, acid sphingomyelinase; BMP, bis(monoacylglycero)phosphate; BMPS, BMP synthase; GBA1, glucocerebrosidase 1; GM2AP, GM2 activator protein; HEXA/B, hexosaminidase A/B; LIPA, acid lipase; NPC2, Niemann-Pick disease type C intracellular cholesterol transporter 2; PLA2G15, phospholipase A2 group 15; PSAP, prosaposin.

composition, and stereoconfiguration (**Figure 2**). The following subsections describe how these structural features contribute to its role in ILV biogenesis, sphingolipid and phospholipid catabolism, and cholesterol homeostasis.

Esterification

Unlike other glycerophospholipids, BMP is a symmetrical molecule. Its fatty acids are esterified on separate glycerols. However, the exact esterification position on each glycerol is debated, as endogenous BMP is monoacylated on either its *sn*-3 and *sn*-3′ carbons or its *sn*-2 and *sn*-2′ carbons (9, 10). While the 3,3′-BMP isoform is more thermodynamically stable, the 2,2′-BMP isoform appears paradoxically to be more abundant (9–11). This represents a paradox because it is well established that acidic pH favors acyl migration from secondary alcohols to primary alcohols (12). Thus, the mechanism by which LE/LYs maintain BMP in its 2,2′-esterification state is not known. The preference of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) for secondary over primary positions in lysophospholipids may explain this phenomenon. Alternatively, maintenance of the 2,2′-BMP isoform may involve a yet-to-be-identified biological machinery.

Previous work has shown that 2,2′-BMP represents the biologically active isoform (11, 13, 14). One proposed role for BMP is in the formation of the intralumenal vesicles that are found within lysosomes. In an elegant reconstitution experiment, Matsuo et al. (13) generated liposomes containing 2,2′-BMP, 3,3′-BMP, or no BMP, subjected them to either acidic or neutral pH, and monitored the liposomes for the generation of luminal vesicles by fluorescence microscopy and electron microscopy. The formation of multivesicular liposomes required both acidic pH and BMP. Interestingly, however, multivesicular liposome formation occurred dramatically in the presence of 2,2′-BMP; 3,3′-BMP was approximately fivefold less active, supporting the conclusion that 2,2′-BMP is required for the formation of ILVs in cells (13). The same group later found

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that 2,2′-BMP liposome supplementation promotes cholesterol homeostasis in disease models of LE/LY cholesterol accumulation; 3,3′-BMP had little to no effect in this context (14).

Future studies should investigate the mechanism(s) by which 2,2′-BMP exhibits differential biology from 3,3′-BMP. Informed by physicochemical predictions in silico, one group has speculated that the structure of 2,2′-BMP promotes its lipid deformative and fusogenic properties (15). Although acyl migration makes chemical synthesis and isolation of 2,2′-BMP challenging (16, 17), measurement of these isoforms in biological samples using modern methods based on liquid chromatography–tandem mass spectrometry may be more tractable, as the isoforms exhibit slightly different chromatographic properties (9, 10). Common lipid extraction methods, however, need to be modified to maintain the distribution of BMP isoforms and may require the addition of alkaline agents, such as boric acid.

Negative Charge

The negative charge of BMP is indispensable for its function in enhancing LE/LY lipid metabolism. While several glycerophospholipids, such as PG, phosphatidylserine, and phosphatidylinositol, can imbue ILVs with a negative surface charge (18–20), BMP comprises as much as 77% of the total ILV lipid content (11). On the surface of ILVs, BMP attracts cationic lipid hydrolases, coactivators, and other soluble LE/LY proteins to degrade and traffic substrates to maintain LE/LY lipid homeostasis (**Figure 2**).

Degradation of sphingomyelin-containing vesicles by acid sphingomyelinase (ASM) in vitro is stimulated by addition of BMP, and enrichment of liposomes with BMP further enhances sphingolipid activator protein C–mediated activation of ASM activity (21). Similarly, BMP has been shown to activate glucocerebrosidase (GBA), GM2 activator protein (GM2AP), and hexosaminidase A (HEXA) activities in vitro (20, 22). Moreover, Abe & Shayman (18) reported that lysosomal phospholipase A2 (LPLA2) activity with phospholipid substrates, such as phosphatidylcholine, is also stimulated by BMP.

The acidic pH dependency of interactions between soluble LE/LY proteins and BMP-enriched liposomes supports a model in which protein–lipid electrostatic interactions contribute to BMP activation of lipid metabolism on ILVs (23). Future work is needed to characterize the precise mechanisms by which BMP activates these diverse lysosomal enzymes.

Fatty Acid Composition

Dioleoyl (18:1/18:1) and didocosahexaenoyl (22:6/22:6) constitute forms of BMP found in biological samples, and importantly, the acyl composition of BMP varies across tissues, diseases, and physiological conditions (9, 24, 25). Fatty acid supplementation experiments in THP-1 macrophages, rabbit pulmonary macrophages, patient fibroblasts, and rat uterine stromal cells revealed the selective incorporation of docosahexaenoic acid (DHA) into BMP (9, 24, 26), supporting the notion that this may be a common phenomenon in BMP metabolism. However, the exact fatty acid preference is cell-type dependent, as various compositions are found across different cell types (24). It has been suggested that unsaturated fatty acids, such as oleic acid and DHA, increase membrane fluidity and/or fusion and produce discontinuities that enable the efficient extraction of lipid substrates (27).

How cells selectively incorporate particular fatty acids into BMP is a mystery. Furthermore, how cells protect PUFA-BMPs from oxidative damage is not known. PUFAs are prone to deleterious lipid peroxidation, and Bouvier et al. (28) reported that BMP (22:6/22:6) is significantly degraded when exposed to oxidative conditions in RAW macrophages, while BMP (18:1/18:1) was unaffected. Treatment with vitamin E, an antioxidant, rescued BMP (22:6/22:6) degradation and

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increased its levels in these cells (28). In a more recent study, treatment of bone marrow–derived macrophages with oxidized low-density lipoprotein (LDL) decreased PUFA-BMPs (23). Such lability to oxidation has led some researchers to speculate that BMP may also function as a suicide antioxidant that protects nearby lipids from oxidative damage (28). Given these data, cells may regulate the distribution of MUFA- and PUFA-BMPs in response to LE/LY lipid metabolism demands, as the former is less potent in stimulating lipid catabolism yet more stable (27, 28). Indeed, the levels and compositions of BMP have been shown to respond to nutrient availability in cells (10, 29). Future work should explore the mechanisms by which cells establish and maintain MUFA- and PUFA-BMPs and their differential roles in LE/LY lipid metabolism.

Stereoconfiguration

The stereoconfiguration of BMP is unusual (**Figure 2**). All glycerophospholipids are optically active due to chirality at the *sn*-2 position, and unlike other glycerophospholipids, PG and BMP contain an additional chiral center at the *sn*-2′ position. While PG and BMP share an identical S configuration at the *sn*-2′ position, BMP is the only glycerophospholipid that possesses an S configuration at the *sn*-2 position (**Figure 2**) (25, 30). This stereoinversion results from its unique, phosphoester-bound *sn*-1 alcohol, as opposed to an *sn*-3 alcohol; it was discovered upon treatment of BMP alkaline hydrolysates with a stereospecific glycerol-3-phosphate dehydrogenase that could not detect glycerol-3-phosphate (25). A later study confirmed release of glycerol-1-phosphate instead following alkaline hydrolysis (31).

Contrary to popular claims, the purpose of BMP's unusual *sn*-1:*sn*-1′ stereoconfiguration is unclear. BMP diastereomers may not exhibit differential activation of lipid enzymes, coactivators, and trafficking proteins, as *sn*-1:*sn*-1′, *sn*-3:*sn*-1′, and *sn*-3:*sn*-3′ BMPs activate NPC2-mediated cholesterol transfer activity in vitro with similar efficacy (32). Furthermore, although it is speculated that BMP's stereoconfiguration confers resistance toward degradation, this claim has not been rigorously tested in a single experiment with different BMP diastereomers (31, 33–36). Therefore, the role of stereochemistry in BMP function remains enigmatic and presents an exciting avenue for future work.

The unique stereochemistry of BMP may help it evade immunorecognition by endogenous antibodies. Several studies demonstrate that bacteria can synthesize BMP, and the stereoconfiguration of bacterial BMP is unclear (37–39). If bacterial BMP possesses the common *sn*-3:*sn*-1′ stereoconfiguration, stereoinversion of eukaryotic BMP may distinguish self-BMP antigens from nonself-BMP antigens. Interestingly, recent work on antiphospholipid syndrome, an autoimmune disease caused by antiphospholipid antibodies that drives abnormal blood clotting and pregnancy complications, lends credence to this hypothesis (40).

It has long been established that antiphospholipid antibodies recognize BMP (7), but Müller-Calleja et al. (40) found that the proinflammatory action of antiphospholipid antibodies exclusively depends on *sn*-3:*sn*-1′ BMP. Specifically, the presentation of BMP on the endothelial protein C receptor is required to sustain antiphospholipid antibody production by B lymphocytes and drive tissue factor and tumor necrosis factor (TNF) release from monocytes. The authors found that *sn*-3:*sn*-1′ BMP supplementation induces monocytic TNF production and antiphospholipid antibody signaling in embryonic trophoblasts, and importantly, *sn*-1:*sn*-1′ BMP supplementation had no effect (40). These data provide the first evidence of differential biology between BMP diastereomers and potentially explain why such stereochemistry has necessarily been evolutionarily conserved. The development of modern methods, such as chiral high-performance liquid chromatography–coupled mass spectrometry or nuclear magnetic resonance, to measure BMP diastereomers in complex biological samples will inform this hypothesis about the function of BMP's unusual stereoconfiguration.

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THE CATABOLISM OF BMP

Early studies presented competing claims regarding the enzymatic activity responsible for BMP degradation (41, 42). Matsuzawa & Hostetler (41) described an acid phosphodiesterase activity responsible for BMP hydrolysis to monoacylglycerol. In their experiments, incubation of radiolabeled BMP with hepatic lysosomal protein extract resulted in a time-dependent decrease in BMP and concomitant increase in monoacylglycerol. However, the authors did not observe a concomitant increase in lysophosphatidic acid, and their procedure was not ideal for ensuring extraction of lysophospholipids from the aqueous phase (41). In contrast, Huterer & Wherrett (42) described an acid deacylase in both lysosomal and microsomal protein extracts that can degrade BMP into lysophosphatidylglycerol and fatty acid. Although these studies represent only the investigations into BMP catabolism using protein extracts, subsequent studies with specific enzymes lend credence to this latter model of BMP deacylation.

To date, numerous enzymes with deacylation activity toward BMP have been identified, refuting claims of BMP's resistance to degradation. Pancreatic lipase–related protein-2, lysosomal PLA2, and α/β-hydrolase domain-containing 6 and 12 (ABHD6 and ABDH12) all display deacylase activity toward BMP with variable catalytic efficiencies (43–46). Still, these enzymes have no validated lipase activity against BMP in cells, and genetic knockout of ABHD6 in mice did not affect tissue levels of BMP, although it was found to increase plasma levels (47). Therefore, the bona fide BMP hydrolase remains to be definitively identified. Of note, the activity of ABHD6 and lysosomal PLA2 toward *sn*-1:*sn*-1′ BMP further calls into question commonly held ideas about the protective function of BMP's stereoconfiguration against degradation (45, 46).

Encouragingly, Kobayashi et al.'s (11) work characterizing BMP ester isoforms in late endosomal membrane domains provides a clear research direction. Taking advantage of acyl migration during silica column chromatography, BMP was isolated from BHK cells and tested for lability toward *Rhizopus arrhizus* lipase before and after chromatographic purification. 2,2′-BMP is assumed present only before chromatography. Treatment of purified 3,3′-BMP with lipase resulted in complete deacylation; however, nonpurified 2,2′-BMP was fully resistant to degradation (11).

Provided their assumption is true and that no other lipid component is confounding, these data suggest that future investigations into the machinery responsible for BMP catabolism should use the major 2,2′-BMP isoform as substrate, as any other isoform may not reflect relevant catabolic biochemistry. These investigations should employ unbiased approaches to obtain a comprehensive understanding of BMP catabolism, as the machinery may not be uniquely lysosomal. Activity-based probes are a compelling example of such approaches, as these tools holistically trap cognate enzymes for subsequent isolation and identification by modern proteomic methods (48). In this context, suicide 2,2′-BMP activity–based probes can be engineered to identify the elusive BMP hydrolase(s).

THE ANABOLISM OF BMP

The site, machinery, and mechanism responsible for BMP anabolism are now established (**Figure 3**). Working with hepatic lysosomal protein extracts, Poorthuis & Hostetler (49) first demonstrated that lysosomes possess the capacity to synthesize BMP. They found that PG and lysophosphatidylglycerol serve as direct precursors, which was later validated by several groups, and no high-energy intermediates or cofactors are required. Later studies expanded on these findings and provided evidence that a lysosomal transacylase is responsible for BMP synthesis (50–53). Waite's group (52) demonstrated that this transacylase is distinct from phospholipases, as the two activities were separable by size-exclusion chromatography. They further discovered that lysophosphatidylglycerol alone is sufficient for BMP synthesis in a base-exchange reaction

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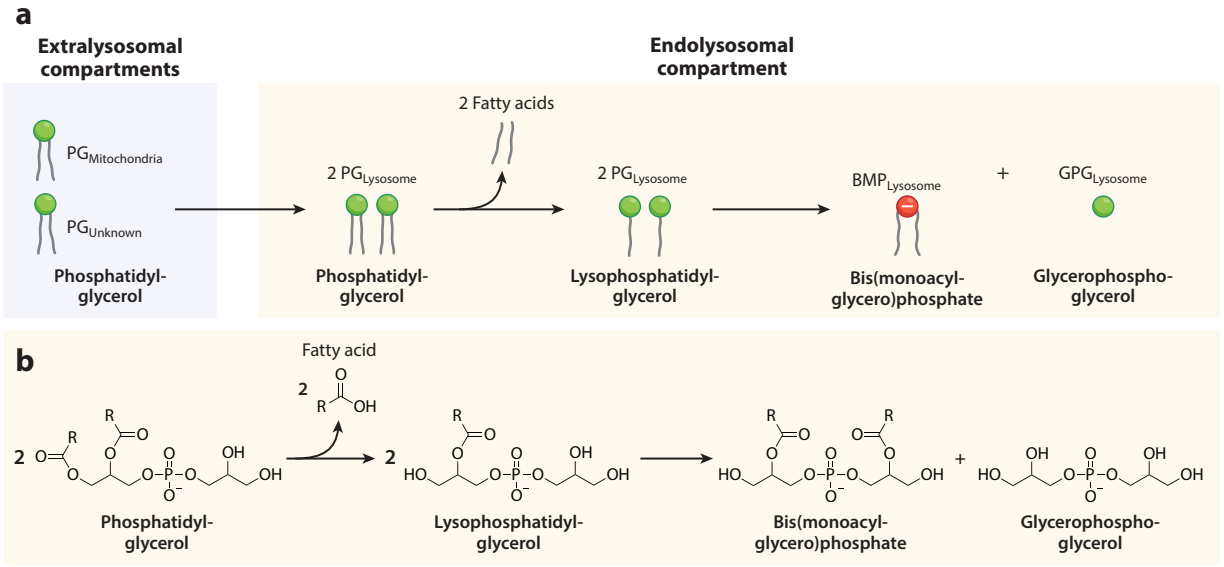

Figure 3

(*a*) BMPS-mediated synthesis of BMP in endolysosomes. Lysosomal phospholipases hydrolyze PG from mitochondria and/or other unknown compartments to produce LPG. In a base-exchange reaction, BMPS deacylates the first LPG, producing GPG, and transfers the acyl chain to a second LPG molecule to form BMP. (*b*) Chemical structures of products and substrates. Abbreviations: BMP, bis(monoacylglycero)phosphate; BMPS, BMP synthase; GPG, glycerophosphoglycerol; LPG, lysophosphatidylglycerol; PG, phosphatidylglycerol.

between two lysophosphatidylglycerol molecules to release glycerophosphoglycerol and BMP (53) (**Figure 3**).

The gene encoding this lysophosphatidylglycerol transacylase remained unknown for several decades until our group serendipitously discovered its identity (54). In seemingly unrelated work, we sought to determine the function of the Batten disease gene product CLN5. We analyzed the lipidome of lysosomes isolated from CLN5-deficient HEK293T cells, given hints that Batten disease may interfere with LE/LY lipid catabolism. This experiment revealed a massive accumulation of lysophosphatidylglycerol and deficiency in BMP inside lysosomes. A study describing CLN5 thioesterase activity against a chemical probe with striking structural similarity to lysophosphatidylglycerol and identification of extensive amphipathic grooves in its predicted structure led us to test whether CLN5 may in fact be the storied lysophosphatidylglycerol transacylase (55). Consistent with Waite and colleagues' (52) work, incubation of CLN5 with lysophosphatidylglycerol alone at acidic pH was sufficient to generate BMP, and CLN5 is now referred to as BMP synthase (BMPS) (54). As discussed in the section titled BMP Hypothesis for the Treatment of Lysosome-Associated Diseases, the identification of BMPS provides the opportunity to investigate the role of BMP in cell biology and disease using genetic and pharmacological tools. However, several questions remain regarding BMP anabolism.

Little is known about the origin of lysosomal PG. Normally, lysosomes contain little to no PG, and the relatively large amount of BMP in lysosomes suggests that PG is likely to come from extralysosomal sources (**Figure 3**), as first posited by Poorthuis & Hostetler (49). These authors suggested that PG may be derived from contacts between lysosomes and PG-containing membranes (49). It is attractive to speculate that mitochondria donate PG to lysosomes via membrane contact sites, as the majority of intracellular PG is found within mitochondria and a wealth of

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studies describe mechanisms that may deliver mitochondrial PG to lysosomes, such as mitophagy and lipid transfer at mitochondria–lysosome membrane contact sites (56). This model raises the possibility that mitochondria may regulate lysosomal BMP in response to the cell's nutrient needs and energy status.

A study elucidating the role of SERAC1, a mitochondrial phospholipid remodeling enzyme, provides compelling support for a role for mitochondria in contributing PG for BMP synthesis (57). SERAC1 deficiency, the cause of MEGDEL syndrome, results in the conversion of mitochondrial PG from PG (18:0/18:1) to PG (16:0/18:1). Predictably, this redistribution leads to a deficiency in cardiolipin, a mitochondrion-specific lipid derived from intramitochondrial PG metabolism. Unexpectedly, however, SERAC1-deficient cells are also deficient in various BMP species, leading to the conclusion that remodeled mitochondrial PG somehow feeds into BMP anabolism (57). Similarly, another study demonstrated that knockout of the mitochondria-localized phosphatidylglycerol-3-phosphate synthase reduces BMP levels (58). Future studies should investigate the potential contributions of mitochondria to BMP biosynthesis.

As discussed earlier, BMP has a unique stereoconfiguration, and it is unknown how such stereochemistry is generated (25). A glycerol reorientation of BMP or its precursors may be required, given an early study demonstrating that an oxidation/reduction mechanism is not responsible for the *sn*-2 carbon stereoinversion (59). Instead, that study favors a transient cyclic phosphate intermediate that stereospecifically hydrolyzes to form the *sn*-1 phosphoester bond (59). Alternatively, a yet-to-be-discovered lysosomal phospholipase D (PLD) activity toward *sn*-3:*sn*-1′ lysophosphatidylglycerol to release *sn*-1:*sn*-1′ lysophosphatidylglycerol in a base-exchange reaction may be responsible. In this model, either the PLD stereospecifically releases *sn*-1:*sn*-1′ lysophosphatidylglycerol/BMP or BMPS may preferentially utilize *sn*-1:*sn*-1′ lysophosphatidylglycerol. The identification of this lysophosphatidylglycerol isomerase requires previously discussed methods to resolve lysophosphatidylglycerol and/or BMP diastereomers.

It is also necessary to highlight a nebulous, alternative pathway for BMP synthesis (**Figure 4**). Another unusual, symmetric glycerophospholipid known as bis(diacylglycero)phosphate has been found in human fibroblasts, and van Blitterswijk & Hilkmann (60) describe a potential transphosphatidylation mechanism mediated by PLD between phosphatidylcholine and diacylglycerol to produce the tetra-acylated BMP variant. While using radiolabeled phosphorus and stimulants, such as fetal calf serum, bradykinin, and phorbol ester, to induce production of labeled bis(diacylglycero)phosphate, they also observed the concomitant production of hemi-BMP and BMP (60). This phenomenon was observed in a different study in which incubation of BHK cells in serum-free media for several days led to an increase in bis(diacylglycero)phosphate, hemi-BMP, and BMP; however, the concentration of phosphatidylcholine decreased dramatically (29). These findings support the potential existence of an alternative, inducible BMP synthetic pathway under conditions of cell starvation, since at least at a basal state, deletion of BMPS led to a global depletion of BMP in cells (54). In this alternative pathway, BMP synthesis results from sequential deacylation of bis(diacylglycero)phosphate to hemi-BMP to BMP, and PC and DAG serve as direct precursors. Future work is required to validate the existence of inducible bis(diacylglycero)phosphate in biological samples.

BMP HYPOTHESIS FOR THE TREATMENT OF LYSOSOME-ASSOCIATED DISEASES

While there is much to be discovered regarding the functions and metabolism of BMP, the existing literature suggests that the selective modulation of BMP in disease using genetic, recombinant, and pharmacological tools can have therapeutic impact across a wide range of human disorders, and the discovery of BMPS might facilitate such efforts. The following sections discuss the role

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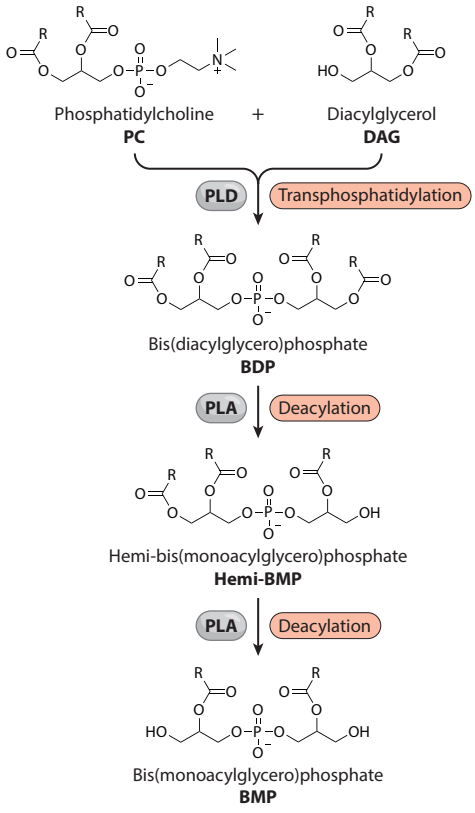

Figure 4

Alternative pathway for BMP synthesis. Studies hint at the existence of an inducible, alternative pathway for BMP synthesis. PLD mediates a transphosphatidylation reaction between PC and DAG to produce BDP. Sequential PLA-mediated deacylations produce BMP. Abbreviations: BDP, bis(diacylglycerol)phosphate; BMP, bis(monoacylglycero)phosphate; DAG, diacylglycerol; PC, phosphatidylcholine; PLA, phospholipase A; PLD, phospholipase D.

of LE/LY lipid metabolism and BMP in a plethora of human conditions including neurodegeneration, atherosclerotic cardiovascular disease, cancer, drug-induced phospholipidosis, and viral infection and evaluate the evidence for the potential use of BMP modulators such as BMPS activation and/or inhibition as a therapeutic approach (**Figure 5**).

MODULATION OF BMP LEVELS IN LYSOSOMAL STORAGE DISORDERS

Niemann-Pick Disease Type C1

The abundances of BMP and cholesterol are intimately linked within cells. Shortly after the discovery of BMP, Rouser et al. (61) described its striking accumulation in Niemann-Pick disease, a lysosomal cholesterol storage disorder. Seminal studies later found that antibody-mediated inhibition of BMP function sufficiently drives LE/LY cholesterol accumulation in healthy cells and supplementation of human Niemann-Pick disease type C (NPC) fibroblasts with BMP-containing liposomes potently rescues cholesterol storage (8, 14). These experiments support the claim that BMP controls LE/LY cholesterol levels, and later work provided additional mechanistic insight.

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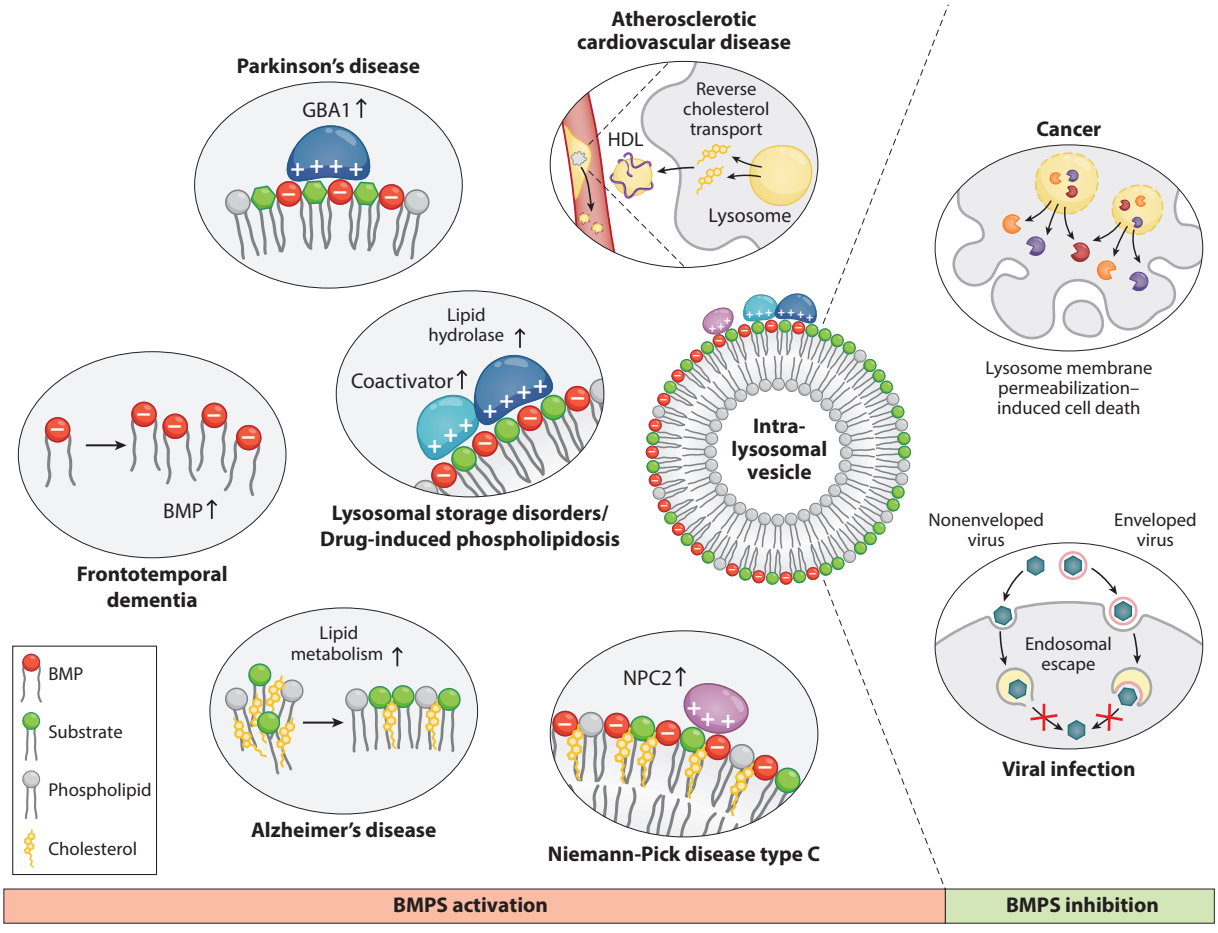

Figure 5

Therapeutic hypotheses for BMPS activation and inhibition in lysosome-associated diseases. The activation and inhibition of BMPS can treat myriad lysosome-associated diseases. BMPS activation may normalize lysosomal lipid metabolism by promoting the activities of soluble lysosomal proteins deficient and/or dysfunctional in lysosomal storage disorders, drug-induced phospholipidosis, Parkinson's disease, Alzheimer's disease, and frontotemporal dementia. BMPS inhibition may drive lysosome membrane permeabilization–induced cell death in cancer and interfere with the escape of viruses from endosomes. Abbreviations: BMP, bis(monoacylglycero)phosphate; BMPS, BMP synthase; GBA1, glucocerebrosidase; HDL, high-density lipoprotein; NPC2, Niemann-Pick disease type C intracellular cholesterol transporter 2.

Loss-of-function mutations in NPC1, a transmembrane lysosomal cholesterol exporter, and NPC2, a soluble lysosomal cholesterol trafficker, cause NPC. BMP enhances the ability of the cholesterol-binding NPC2 protein to harvest cholesterol from ILVs and pass it to the lumenal domain of NPC1 protein for LE/LY export (62, 63). This stimulatory activity depends on direct interactions between NPC2 and BMP on ILVs because NPC2 mutants that are unable to bind BMP fail to rescue cholesterol accumulation in NPC1-deficient patient fibroblasts (64). The interaction with BMP occurs on the NPC2 hydrophobic knob domain, which is evolutionarily conserved in eukaryotes, and the stereoconfiguration of BMP did not impact activation of NPC2 cholesterol transfer activity (64). Consistent with these findings, supplementation of NPC1-deficient cells with PG, a precursor for BMP, and recombinant NPC2 protein dramatically lowered cholesterol accumulation. This effect was attenuated in cells treated with mutant NPC2 protein that weakly

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bound BMP (64). In addition to catalyzing cholesterol transfer to NPC2 and NPC1 proteins, excess BMP may also trigger extracellular vesicle shedding or activated release of cholesterol-laden exosomes (65, 66).

Based on these observations, BMPS activation may be a highly efficacious means to rescue cholesterol accumulation in NPC1 disease. Indeed, intracerebroventricular injection of PG liposomes to raise BMP levels in NPC1 knockout mice rescues cholesterol storage, Purkinje somatic atrophy, and autophagic dysfunction (67). Therefore, pharmacological BMPS activators are expected to cure NPC1 disease with superior pharmacokinetics and tolerability to cyclodextrins, which do not cross the blood–brain barrier and display ototoxicity (68).

Other Lipid-Related Lysosomal Storage Disorders

When Rouser et al. (61) discovered the accumulation of BMP in Niemann-Pick disease, they also noted its dyshomeostasis in Gaucher's disease (caused by an inability to degrade glucosylceramide), Tay-Sachs disease (caused by an inability to degrade GM2 ganglioside), and metachromatic leukodystrophy (caused by an inability to degrade sulfated glycosphingolipids). A comprehensive study also observed BMP accumulation in these diseases along with other lipid-related lysosomal storage disorders (LSDs), such as Fabry disease (caused by an inability to degrade globotriaosylceramide), GM1 gangliosidosis (caused by an inability to degrade GM1 ganglioside), Krabbe disease (caused by an inability to degrade galactosylceramide), Niemann-Pick disease type A/B (caused by an inability to degrade sphingomyelin), and Sandhoff disease (caused by an inability to degrade GM2 ganglioside) (69). While the effect of BMP in these diseases is poorly understood, BMP's function in stimulating LE/LY metabolism and its ameliorative role in NPC1 disease suggest that pharmacological BMPS activators may be disease modifying as well.

Neuronal Ceroid Lipofuscinosis (Batten Disease)

Neuronal ceroid lipofuscinosis (Batten disease) is caused by biallelic loss of function of a heterogenous group of thirteen genes, resulting in childhood neurodegeneration characterized by the accumulation of lipofuscin in lysosomes (70). The heterogeneity of Batten disease gene product functions, which include a depalmitoylase, a peptidase/protease, a metabolite transporter, and a protein trafficker, has obscured the molecular etiology of this condition; however, the recent identification of CLN5 as BMPS strongly suggests that at least a subset of Batten disease forms are BMP deficiency disorders. We reason that loss of Batten disease gene products results in a depletion of BMP and/or disruption in its function, in contrast with other LSDs that are characterized by BMP accumulation.

For example, loss-of-function mutations in *CLN3*, which cause the most common form of Batten disease, deplete BMP from patient brain lipid extracts (71). Consistently, we observed a depletion of BMP and accumulation of lysophosphatidylglycerol in a *Cln3*$^{-/-}$ murine model (72). Considering that CLN3 and BMPS directly interact, CLN3 loss may disrupt the function of BMPS through a yet-to-be-elucidated mechanism, and such a paucity of BMP has also been described in CLN11 (Progranulin) knockout cells (23, 73, 74). Furthermore, CLN6, CLN7, CLN8, and CLN14 have been shown to modulate the levels of BMPS within the lysosome, and direct interactions between BMPS and other Batten disease gene products have been observed through in vitro pulldown assays (73, 75–79). Of note, genetic depletion of cathepsin D, whose loss causes another form of Batten disease, led to an increase in BMP levels in mouse brains (80), suggesting a complex relationship between Batten disease pathology and BMP. While further work is needed to understand how each CLN may impact BMPS function or BMP directly, a therapeutic rationale for activating BMPS in at least a subset of Batten disease cases is plausible.

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MODULATION OF BMP LEVELS IN AGE-RELATED NEURODEGENERATIVE DISEASES

Several genetic studies link lysosomal dysfunction to major age-related neurodegenerative diseases such as frontotemporal dementia (FTD), Alzheimer's disease (AD), and Parkinson's disease (PD), all of which exhibit BMP dyshomeostasis (81). The following subsections discuss the evidence for the potential therapeutic efficacy of activating BMP synthesis in these diseases.

Frontotemporal Dementia

Progranulin (PGRN/CLN11) haploinsufficiency is the genetic cause of 10–15% of FTD cases, and as described earlier, biallelic loss-of-function mutations in PGRN/CLN11 result in Batten disease (82). While FTD is an age-related, complex neurodegenerative disease characterized by marked cerebral atrophy and cortical neuron loss, often before the age of 65, it shares several pathological features with Batten disease, such as microgliosis and lipofuscinosis, suggesting a common etiology (82).

Notably, PGRN/CLN11-deficient murine and human brains exhibit a global, ~50% reduction in BMP, with metabolic anomalies consistent with lack of BMP function (23, 74). For example, PGRN/CLN11 knockout cells accumulate glucosylceramide, glucosylsphingosine, and gangliosides within LE/LYs and have fewer ILV-associated proteins, such as LAPTM4A, LAPTM4B, CD9, and CD81 (23, 74). Consistent with BMP's role in stimulating glycosphingolipid hydrolases and coactivators and ILV biogenesis, BMP supplementation rescues glycosphingolipid accumulation in PGRN/CLN11-deficient cells, and exogenous PGRN/CLN11 corrects LAPTM4B deficiency (23, 74). Intriguingly, BMP supplementation also enhances proteolytic degradation, indicative of a global restoration of lysosome homeostasis. While it is promising that sporadic FTD patients also show a decrease in BMP (22:6/22:6), it is unclear if the normalization of aberrant lysosomal function alone can cure FTD (74). Still, the established link between FTD and LE/LY lipid dysregulation argues that pharmacological BMPS activation may modify disease progression (23, 82).

Alzheimer's Disease

Dysregulated lipid metabolism is thought to contribute to AD, an age-related neurodegenerative disease with pathognomonic deposition of amyloid beta (Aβ) plaques and formation of neurofibrillary tangles (83). Often overlooked, the deposition of lipid saccules in microglia is an additional pathological feature, and apolipoprotein E4 (APOE4), the strongest risk factor for AD, impairs myelination in oligodendrocytes due to cholesterol dyshomeostasis (83–85). AD and NPC1 disease share cholesterol dyshomeostasis, Aβ plaques, neurofibrillary tangles, and lysosomal dysfunction, and cholesterol-chelating cyclodextrins are therapeutically viable, improving axonal myelination, learning, and memory formation (84, 86). Given recent associations of BMPS with AD and the temporal lobe–confined accumulation of BMP in an AD mouse model, activation of BMPS may ameliorate aberrant lipid metabolism in AD and synergize with current Aβ-clearing modalities (87–90). Consistent with this hypothesis, DHA supplementation is protective against AD; however, the exact mechanism of protection is unknown (91). Because exogenous DHA preferentially incorporates into BMP, one might speculate that DHA and BMP are therapeutically linked, though further investigation is needed.

Parkinson's Disease

Mutations in several lysosome and lysosome-associated genes, such as *LRRK2*, *GBA1*, *ATP13A2*/CLN12, and *VPS35*, are risk factors for PD, and alterations in BMP are used to

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monitor PD lysosomal dysfunction (92, 93). For example, higher urine levels of BMP were found in G2019S, R1441G, and R1444C *LRRK2* carriers and D650N *VPS35* carriers, and LRRK2 inhibitors normalize urinary BMP accumulation in the clinic (93–95). A recent study reported that LRRK2 hyperactivation drives the secretion of BMP- and glycosphingolipid-rich ILVs and BMP accumulates within lysosomes as a compensatory response to impaired GBA clearance of glycosphingolipid storage material (96). These data are reminiscent of lipid metabolism alterations observed in lipid-related LSDs (69, 81). Furthermore, another study found that loss-of-function mutations in GBA drive α-synuclein accumulation and neurotoxicity in neurons, providing a therapeutic rationale for current efforts to increase GBA function in PD (97). As discussed earlier, BMP stimulates GBA activity, thus BMPS activation may benefit PD patients.

MODULATION OF BMP LEVELS IN ATHEROSCLEROTIC CARDIOVASCULAR DISEASE

Atherosclerosis represents a multisystemic disease of cholesterol dysregulation and should be considered an acquired LSD, as excellently reviewed by Colin et al. (99). LDL particles transport dietary cholesterol throughout the vasculature for cellular uptake. In porous arteries, LDL cholesterol deposits oxidize to form oxidized LDL cholesterol and trigger a proinflammatory cascade driven by proliferating macrophages and macrophage-like cells that attempt to clear oxidized LDL cholesterol through LE/LY cholesterol metabolism (99).

Under normal conditions, LE/LYs degrade cholesterol esters via lysosomal acid lipase (100). Through the action of NPC2 and NPC1, free cholesterol is exported from the lysosome and transported to the plasma membrane for removal on high-density lipoprotein (HDL) particles (reverse cholesterol transport) or stored in lipid droplets in the form of cholesterol esters (62). However, when cellular cholesterol influx is overwhelming, macrophages undergo apoptosis, leaving large amounts of cellular debris. Here, the atherogenic cascade begins, as more macrophages extravasate, ineffectively clear cellular debris and oxidized LDL, and drive LE/LY cholesterol accumulation similar to that observed in NPC1 disease and Wolman disease (lysosomal cholesterol ester storage) (98, 99). As the lipid-laden (foamy) macrophages die, they form a necrotic plaque that is initially contained but eventually ruptures, enters the bloodstream, and causes myocardial infarction and stroke (101).

Given the etiology of atherosclerotic cardiovascular disease, most treatment modalities focus on lowering LDL and do so successfully. For example, statins inhibit cholesterol synthesis, Ezetimibe reduces gastrointestinal cholesterol absorption, and PCSK9 inhibitors promote hepatic clearance of LDL through the protection of LDL receptors from proteolytic degradation (102). However, atherosclerotic cardiovascular disease is still the leading cause of death worldwide, necessitating the further development of disease-modifying treatments. Interestingly, no treatment for atherosclerotic cardiovascular disease targets cholesterol within the atherosclerotic plaque, and in fact, all treatment modalities converge on reducing cellular cholesterol input (102).

Because BMP fundamentally modulates LE/LY cholesterol content and myeloid-derived cells like macrophages exhibit the highest expression of BMPS (78), pharmacological activators of BMPS offer an unprecedented opportunity to target cholesterol clearance within the atheroma itself and synergize with current treatments. Consistent with this hypothesis, foamy macrophages form in both NPC1 disease and atherosclerosis, and unsurprisingly, cyclodextrins are also efficacious in atherosclerosis (98). Through reverse cholesterol transport, cyclodextrins decrease atherosclerotic plaques in a murine atherosclerosis model and reduce cellular cholesterol content in macrophage-like vascular smooth muscle cells (103, 104). Arnal-Levron et al. (105)

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demonstrated the antiatherogenic properties of BMP directly, as BMP substrate supplementation in RAW macrophages potently lowers proapoptotic cholesterol oxidation products, such as 7-ketocholesterol, and protects macrophages from oxidized LDL-induced cell death. Contrarily, Luquain-Costaz et al. (106) find that BMP accumulation in atheromatous macrophages reduces reverse cholesterol transport machinery and promotes cholesterol dyshomeostasis. Given this conflicting literature, further investigation of the role of BMP in atherosclerotic cardiovascular disease is warranted.

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MODULATION OF BMP LEVELS IN DRUG-INDUCED PHOSPHOLIPIDOSIS

Drug-induced phospholipidosis, an acquired LSD, is a clinical side effect of chronic treatment with cationic amphiphilic drugs (36, 107), which contain a weakly basic tertiary amine and a hydrophobic core. Well-known cationic amphiphilic drugs with diverse scaffolds, such as chloroquine (antimalarial), amiodarone (antiarrhythmic), and chlorpromazine (antipsychotic), all result in drug-induced phospholipidosis, and the only intervention is cationic amphiphilic drug withdrawal, interfering with treatment of the underlying disease (107).

An early report first associated drug-induced phospholipidosis with a rise in BMP. In what they term foam-cell syndrome, patients treated with the cationic amphiphilic drug diethylaminoethoxyhexestrol exhibited a marked hepatic accumulation of phospholipids and cholesterol with a concomitant rise in BMP (108). The authors noted the striking resemblance to NPC disease, and several studies have generalized these findings to myriad cationic amphiphilic drugs (108–111). As mentioned earlier, BMP stimulates LPLA2 activity, and BMPS-deficient cells have impaired phospholipid catabolism (54). Interestingly, Abe & Shayman (18, 36) found that amiodarone inhibits LPLA2 activity, and they posited that amiodarone and other cationic amphiphilic drugs incorporate into BMP-laden ILVs, neutralize the negative surface charge, and thus reduce lipid enzyme activity. Consistent with this model, lysophosphatidylglycerol incorporation into BMP-containing liposomes stimulates BMPS activity, and our group has found that amiodarone inhibition of BMPS activity is greatly enhanced in the presence of lysophosphatidylglycerol- and BMP-containing liposomes (54). These data may explain the attenuated rise in BMP in response to cationic amphiphilic drugs, compared with high-fat diets where BMPS activity is not known to be partially inhibited (112).

Taken altogether, pharmacological activation of BMPS may mitigate drug-induced phospholipidosis. Indeed, a key experiment in amiodarone-treated COS7 cells demonstrated that BMP substrate supplementation completely rescues amiodarone-induced cytotoxicity (113). Such activators would enable patients to receive critical cationic amphiphilic drug treatments for longer durations without debilitating side effects.

REDUCING BMP LEVELS IN DISEASE

Up to this point, we have discussed indications for BMPS activation. Considering the essentiality of BMP for LE/LY homeostasis and function, reducing BMP levels through means such as pharmacological BMPS inhibition may have therapeutic disease relevance as well (**Figure 5**).

Cancer

Lysosome membrane permeabilization–induced cell death results from a loss of integrity of the lysosome limiting membrane and cytoplasmic release of acidic hydrolases and proteases, which degrade cytosolic components and trigger both programmed and necrotic cell death (114, 115). Agents, such as lysosomotropic detergents, cationic amphiphilic drugs, reactive oxygen species,

and bacterial products, can induce lysosome breakage, and membrane stabilization is required to limit cytotoxic cascades (114–116). One poorly elucidated mechanism relies on Hsp70 and BMP to inhibit lysosome membrane permeabilization–induced cell death. Like soluble lysosomal hydrolases, cationic Hsp70 interacts with anionic, BMP-rich ILVs in an acidic pH–dependent manner, and this interaction facilitates the recruitment and activation of ASM on ILVs, which in turn stabilizes lysosomes (117, 118). Because BMPS deficiency results in both BMP depletion and the accumulation of detergent-like lysophosphatidylglycerol within a small compartmental volume, pharmacological BMPS inhibition should induce lysosome membrane permeabilization–induced cell death. Notably, this effect would be against a background of marked lipidosis and nutrient deprivation due to pharmacologically induced BMP deficiency.

Interestingly, several cationic amphiphilic drugs known to inhibit BMP function (119) exhibit efficacy in treating cancer, and a recent case study of a woman with BMPS heterozygosity provides compelling support for BMPS inhibitors in cancer. Von Hippel Lindau (VHL) mutations increase the likelihood of tumorigenesis with complete penetrance by age 60, yet unprecedently, a VHL syndrome patient continues to live at age 72 (120). A monoallelic BMPS mutation was discovered that somehow confers resistance to VHL syndrome, as the patient's child who was a VHL carrier without this BMPS mutation developed pheochromocytomas by age 34. By silencing BMPS in primary *VHL*$^{-/-}$ hemangioblastoma and clear cell renal cell carcinoma cells, BMPS loss of function reduced cell viability (120). This study supports the therapeutic potential of BMPS inhibition in VHL-driven cancers. In glioblastoma, high expression of BMPS correlates with poorer prognoses, and knockdown of BMPS in U251 and U87MG glioblastoma cell lines inhibits proliferation, migration, and invasion with promotion of apoptosis (121). Furthermore, breast cancer cells preferentially incorporate DHA into BMP (122). Given these studies, future work should further characterize the efficacy of BMPS inhibition in these and other cancers.

Viral Infection

Most viruses require endocytic internalization for access to replicative machinery and subsequent endosomal escape (123). Interestingly, many studies demonstrate that BMP plays an essential role in viral cell entry (**Figure 5**). For example, the nonenveloped bluetongue virus (BTV) virulence factor VP5 requires BMP to form endosomal membrane pores. Additionally, BTV-1 entry is attenuated in HeLa cells treated with an anti-BMP antibody (124). This dependency on BMP for endosomal escape extends to enveloped viruses, as a recent study found that BMP also promotes the formation and enlargement of Lassa virus pores in an acidic pH–dependent manner (125). Given the fusogenic properties of BMP at acidic pH and its high abundance within endosomes, it is unsurprising that other viruses, such as vesicular stomatitis virus, dengue virus, flaviviruses, phleboviruses, and influenza virus, have also been shown to rely on BMP for fusion (126–133), and as such, BMP may be a bona fide host virulence factor.

Consistent with this theory, a recent study discovered that drug-induced phospholipidosis is a shared mechanism underlying the antiviral activity of all 23 cationic amphiphilic drugs tested against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (134). These authors additionally confirmed drug-induced phospholipidosis antiviral activity against other viruses in vitro, such as dengue virus, Ebola virus, hepatitis C, and Marburg virus, but did not observe cationic amphiphilic drug efficacy against SARS-CoV-2 infection in vivo, likely owing to the use of concentrations well below that required for phospholipidosis induction (134). The study did not determine the mechanism of action for drug-induced phospholipidosis antiviral activity, and given earlier discussions, it is compelling to speculate that inhibition of BMP function is responsible for such broad activity. Thus, the targeted inhibition of BMPS to induce

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drug-induced phospholipidosis robustly represents, at minimum, an untapped strategy to treat viral infection.

CONCLUDING REMARKS

Although there is much to elucidate regarding BMP function, catabolism, and biosynthesis, the modulation of BMP levels in lysosome-associated diseases using selective biological tools, including those that target BMPS, has great therapeutic potential. These genetic, pharmacological, and recombinant tools will be indispensable for testing the hypotheses described here and elsewhere. In addition, we believe that the BMPS-deficient mouse, which represents a model for advanced aging, will be instrumental in testing the broad importance of this unusual lipid molecule in health and disease (135).

DISCLOSURE STATEMENT

Stanford University filed a patent on behalf of the authors for the development of activators and inhibitors of the bis(monoacylglycero)phosphate synthase. M.A.-R. is a scientific advisory board member of Lycia Therapeutics.

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