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REVIEW

Bridging the molecular and biological functions of the oxysterol-binding protein family

Antonietta Pietrangelo[1] · **Neale D. Ridgway**[1]

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Abstract

Oxysterol-binding protein (OSBP) and OSBP-related proteins (ORPs) constitute a large eukaryotic gene family that transports and regulates the metabolism of sterols and phospholipids. The original classification of the family based on oxysterol-binding activity belies the complex dual lipid-binding specificity of the conserved OSBP homology domain (OHD). Additional protein- and membrane-interacting modules mediate the targeting of select OSBP/ORPs to membrane contact sites between organelles, thus positioning the OHD between opposing membranes for lipid transfer and metabolic regulation. This unique subcellular location, coupled with diverse ligand preferences and tissue distribution, has identified OSBP/ORPs as key arbiters of membrane composition and function. Here, we will review how molecular models of OSBP/ORP-mediated intracellular lipid transport and regulation at membrane contact sites relate to their emerging roles in cellular and organismal functions.

Keywords Oxysterol-binding proteins · Membrane contact sites · Intracellular lipid transport · Cancer · Dyslipidemia · Metabolism · Viral replication

Abbreviations

ALS	Amyotrophic lateral sclerosis
ABC	ATP binding cassette
CARTS	Carriers of the *trans*-Golgi network to the cell surface
CERT	Ceramide transfer protein
ER	Endoplasmic reticulum
FFAT	Two phenylalanines in an acidic tract
HDL	High density lipoprotein
25OH	25-Hydroxycholesterol
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
LDL	Low density lipoprotein
LEL	Late endosomes–lysosomes
LD	Lipid droplet
LRH-1	Liver receptor homolog-1
LXR	Liver X receptor
LTP	Lipid transfer protein
MCS	Membrane contact site
mTOR	Mammalian target of rapamycin
NPC	Niemann–Pick type C
OSBP	Oxysterol-binding protein
ORP	OSBP-related protein
OHD	OSBP homology domain
Osh	Oxysterol-binding protein homolog
PM	Plasma membrane
PI4K	Phosphatidylinositol-4-kinase
PI	Phosphatidylinositol
PI(4)P	Phosphatidylinositol 4-phosphate
PIPs	Phosphatidylinositol phosphates
PLC	Phospholipase C
PS	Phosphatidylserine
RILP	Rab7-interacting lysosomal protein
SM	Sphingomyelin
SNP	Single nucleotide polymorphisms
SREBP	Sterol regulatory element binding protein
TGN	*Trans*-Golgi network
VAP	Vesicle-associated membrane protein-associated protein

✉ Neale D. Ridgway
nridgway@dal.ca

[1] Atlantic Research Center, C306 CRC Bldg, Department of Pediatrics, and Biochemistry and Molecular Biology, Dalhousie University, 5849 University Av., Halifax, NS B3H4R2, Canada

Introduction

Intracellular lipid transport creates and maintains the unique lipid composition of membranes that define organelle function and regulate communication with the external environment. While vesicular transport and membrane fusion are responsible for the bulk transfer of lipids and sterols, the specific and localized movement of individual lipid species between membranes is mediated by lipid transfer proteins (LTPs) [1]. While LTP gene families are numerous and diverse in structure, they share the ability to bind lipids within a hydrophobic fold for lipid transfer, substrate presentation or signaling [2]. Oxysterol-binding protein (OSBP) and OSBP-related proteins (ORPs) constitute a large family of eukaryotic LTPs that bind cholesterol, oxysterols and anionic phospholipids within a conserved hydrophobic binding domain. Additional membrane-targeting motifs allow OSBP/ORPs to simultaneously associate with a diverse set of protein and lipid partners in organelle membranes, thus positioning the lipid-binding domain in close apposition to donor and acceptor membranes to facilitate transfer or signaling functions. The potential for redundancy in this large gene family adds complexity to any analysis of individual OSBP/ORP function in normal and disease states. This review will focus on our current understanding of mammalian OSBP/ORP function at the cellular and systemic level and how these functions are exploited by pathogens and contribute to disease pathology.

Structural organization of OSBP/ORPs

OSBP was identified in 1985 by Taylor and Kandutsch as a soluble receptor for oxysterols [3], which are oxidized cholesterol derivatives that promote cholesterol esterification and efflux and inhibit de novo cholesterol synthesis and uptake [4]. Cloned in 1989, OSBP was initially thought to be an oxysterol-regulated transcription factor due to a central leucine repeat reminiscent of some DNA binding proteins [5]. However, OSBP was later shown to undergo sterol-dependent localization to the Golgi apparatus [6]. In 1999, Ikonen and colleagues cloned the cDNAs for 6 human OSBP-related proteins (termed ORPs) [7], and the remaining members of the 12-gene family were cloned in 2001 [8]. Mammalian OSBP/ORPs contain a conserved C-terminal lipid-binding domain designated the OSBP homology domain (OHD) that harbors a highly conserved motif (EQVSHHPP) that is found in homologs across species (Fig. 1a). All but one of the *OSBP* genes encode full-length or long (L) forms that contain additional membrane-targeting domains (Fig. 1a). For example, all full-length OSBP/ORPs have an N-terminal pleckstrin homology (PH) domain that recognizes phosphatidylinositol phosphates (PIPs) with varying degrees of specificity and affinity. OSBP and ORPs 1, 2, 3, 4, 6, 7 and 9 also contain a two phenylalanines-in-an-acidic tract (FFAT) motif that interacts with the resident endoplasmic reticulum (ER) protein vesicle-associated membrane protein-associated protein (VAP). As a result, OSBP/ORPs that contain PH and FFAT motifs are peripherally anchored

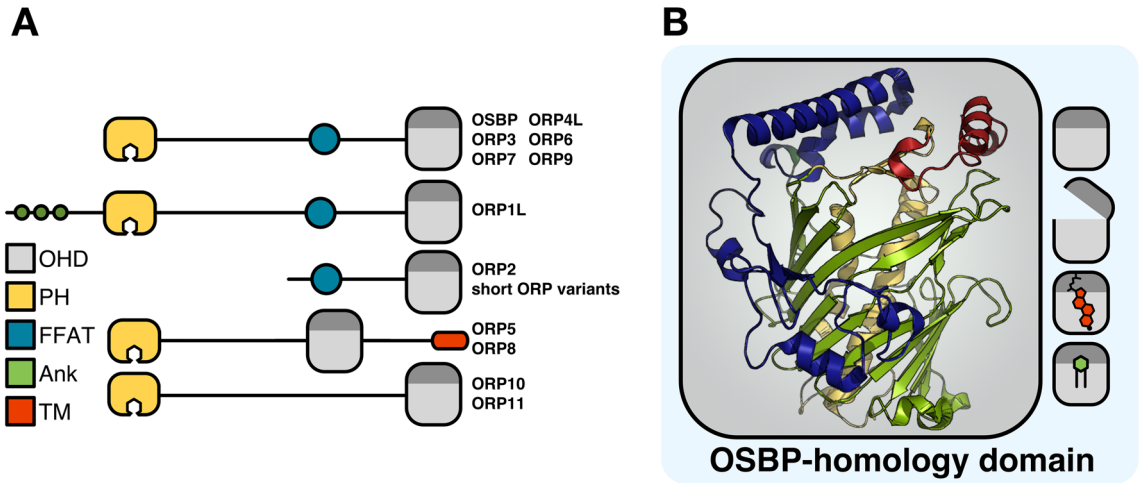

Fig. 1 Structural organization of the OSBP/ORP family. **a** Domain structure of the OSBP/ORP family. *OHD OSBP homology domsin, PH* pleckstrin homology, *FFAT* two phenylalanines in an acidic tract, *Ank* ankyrin, *TM* transmembrane. **b** Shown is the structure of the *S. cerevisiae* Osh4p OHD composed of an incomplete β-barrel (green) flanked by two central helices (yellow), N-terminal region (blue) and α-helical lid (red). The OHD binds sterols and phospholipids competitively and in opposite orientations; the acyl chains of phospholipids are buried in the pocket, while the iso-octyl side chain of sterols interacts with the lid

to the ER and to organelles enriched in PIPs, such as the Golgi apparatus, plasma membrane (PM) and endosomes. ORP5 and ORP8 provide a variation on this theme: both have C-terminal membrane-spanning domains that anchor them to the ER as well as PH domains that facilitate interaction with other organelles (Fig. 1a). Collectively, these dual membrane-binding properties could facilitate transient association–dissociation with individual membranes or simultaneous association at a membrane contact site (MCS) between organelles [2]. Based on experimental evidence, the localization of OSBP/ORPs at organelle MCS and with cytoskeletal networks is depicted in Fig. 2. How this unique subcellular localization is involved in lipid transfer and regulation is discussed below.

Much of the structural and functional information we have for the OSBP/ORP family is derived from studies of the *S. cerevisiae* oxysterol-binding protein homolog (Osh) proteins (see "Structure and specificity of the OSBP homology domain"). Osh1p and Osh2p are long forms that contain N-terminal PH, FFAT and ankyrin motifs, Osh3p has PH, FFAT and Golgi dynamics (GOLD) motifs, while and Osh4p-Osh7p contain only the OHD. While the seven Osh proteins associate with different membranes and cellular processes [9], they are functionally redundant such that any one Osh protein can rescue the growth-arrest phenotype caused by deletion of all seven Osh proteins [10].

Studies in the cholesterol auxotrophs *C. elegans* and *D. melanogaster*, which each express four ORP homologs, revealed similar functional redundancy. Deletion of individual *C. elegans obr1-to obr-4* homologs did not affect viability but deletion of all four caused embryonic lethality [11]. Deletion of individual *Osbp* homologs in *D. melanogaster* also yielded viable mutants [12]. However, *Osbp* deletion or overexpression in *D. melanogaster* caused aberrant cholesterol accumulation in the Golgi apparatus with resultant deleterious effects on spermatogenesis and wing expansion. These results also indicate that OSBP/ORPs are essential for lipid homeostasis in cells that lack de novo sterol biosynthesis [12, 13].

Structure and specificity of the OSBP homology domain

Osh4p was the first to have its structure solved by X-ray crystallography to reveal an OHD comprising an incomplete 19-strand beta-barrel (Fig. 1b, green) with an alpha-helical lid (Fig. 1b, red), which together form a hydrophobic binding cavity that accommodates cholesterol and oxysterols

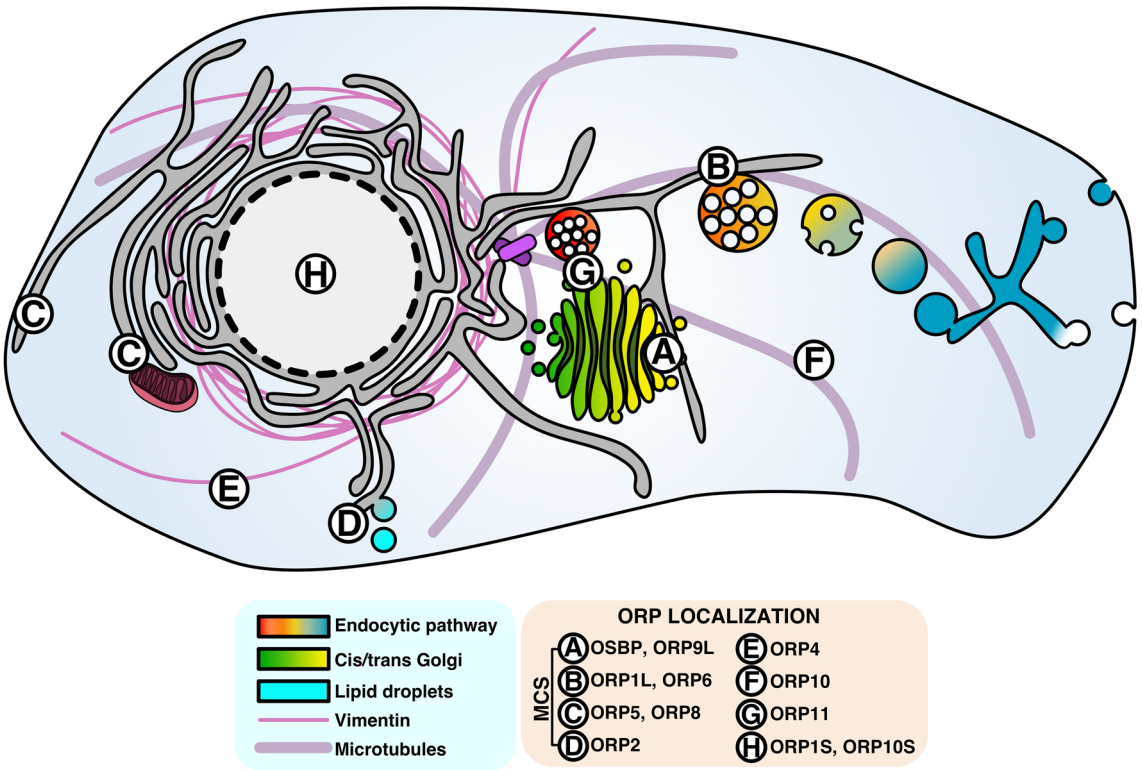

Fig. 2 Subcellular localization of the OSBP/ORP family. Aside from ORP5 and ORP8, all ORPs display partial cytosolic localization, but most also have discrete localization patterns, including membrane contact sites between organelles, on cytoskeletal elements and in the nucleus (see figure for details). For simplicity, only well-characterized localization patterns relevant to known or predicted protein functions have been included. ORP3 and ORP7 do not have well-defined localization patterns and have been omitted

[14]. Cholesterol co-crystallized in the Osh4p OHD pocket is oriented 'head-first' (Fig. 1b); the 3-hydroxyl is coordinated at the bottom of the pocket while the iso-octyl side chain interacts with the lid in a closed position, making the pocket inaccessible to other ligands. Interestingly, protein–sterol interactions are mediated by water molecules that line the binding pocket, bridging basic residues at the base of the pocket with the 3-hydroxyl of the bound sterol. Indirect, water-mediated binding may explain the broad range of sterols that are bound by OSBP/ORP and Osh proteins.

Despite similarities in the sequence and structure of OHDs amongst the Osh and OSBP/ORP family, it is apparent that sterol binding is not a core activity [15]. Crystallization of the OHDs of Osh4p [16] and Osh3p [17] revealed that phosphatidylinositol 4-phosphate (PI(4)P) occupies the OHD 'tail-first' (Fig. 1b); the acyl chains extend into the binding pocket while the inositol 4-phosphate headgroup interacts with two histidine residues close to the entrance of the binding fold. Notably, the two histidine residues are part of the OSBP/ORP signature motif and not required for sterol binding [17], suggesting a primary function that involves the binding and transfer of anionic phospholipids. The "tail first" positioning of PI(4)P in the OHD suggests that the alpha-helical lid of the OHD is capable of inserting into membranes to excise embedded lipids for binding in the observed orientation [18]. This configuration for PI(4)P was confirmed for Osh1p, which also binds ergosterol [18]. While the structure of a mammalian OHD has not been solved, in vitro assays of lipid binding and extraction from liposomes have shown that mammalian OSBP/ORPs also competitively bind both sterols and phospholipids, including phosphatidylserine (PS) and PI(4)P (summarized in Table 1 and reviewed in [19]).

Dual-binding specificity for phospholipid and sterol ligands allows an Osh/OSBP/ORP to potentially exist in binary states; each ligand induces conformational changes in the OHD that direct protein and membrane association to potentially drive lipid exchange between organelles. This concept was first demonstrated for Osh4p, a prototypical OHD-only protein that does not physically bridge donor and acceptor membranes during a lipid transfer cycle. Liposome-based Osh4p lipid transfer assays showed that the rate of delivery of ergosterol from a donor membrane to an acceptor membrane increased when the acceptor membrane was enriched with anionic lipids [16]. However, Osh4p only dissociated from the acceptor membrane when bound to a molecule of ergosterol, therefore resulting in no net change in the ergosterol content of the acceptor membrane. However, when the acceptor membrane was enriched with PI(4)P, which is an anionic lipid and Osh4p ligand, ergosterol delivery to acceptor membranes was increased while the back-transfer of ergosterol was inhibited by competitive binding of PI(4)P, thereby driving directional transport of ergosterol to the acceptor membrane. Complementation studies in yeast suggest that Osh4p functions similarly in vivo (Fig. 3), transferring ER-derived cholesterol to the Golgi apparatus in exchange for Golgi-derived PI(4)P, which is then hydrolyzed in the ER by the PI(4)P phosphatase Sac1p [20]. In yeast, the phosphatidylinositol (PI) transfer protein Sec14p drives PI(4)P synthesis by providing substrate for the PI 4-kinase Pik1p. In the absence of Sec14p, unchecked Osh4p-mediated PI(4)P depletion causes cytotoxic failure of the secretory system [21].

Lipid transport and metabolic regulation by OSBP at ER/Golgi contact sites

Oxysterol-binding protein is a prototypical dual membrane-interacting protein that transfers lipids and regulates lipid metabolism at MCS. Over a decade of research has described OSBP as a sterol-sensitive regulator of sphingomyelin (SM) and PI(4)P synthesis at the ER/Golgi interface (Fig. 3). OSBP is a cytosolic protein that associates transiently with the *trans*-Golgi and *trans*-Golgi network (TGN) via the PH domain [22], and the ER via interaction between its FFAT motif and the ER-resident protein VAP-A [23]. When cells are treated with exogenous oxysterols (a signal of excess cholesterol) or depleted of cholesterol to stimulate de novo synthesis, OSBP is associated with ER/Golgi MCS [6, 24]. There, OSBP transports ER-derived cholesterol to the Golgi and Golgi-derived PI(4)P to the ER, where it is degraded by the PI4P phosphatase Sac1 [25]. Cholesterol enrichment in the Golgi apparatus leads to recruitment of phosphatidylinositol-4-kinase (PI4K)IIα [26] and, consequently, a local increase in PI(4)P, which in turn leads to recruitment of ceramide transport protein (CERT) via PH and FFAT domain-dependent targeting to the MCS [27]. The transport of ceramide to the Golgi apparatus provides substrate for the synthesis of SM, thereby matching the increased transport of cholesterol to maintain the relative proportions of SM and cholesterol-rich microdomains, which are important for protein and lipid sorting in the secretory pathway [28]. For example, the biogenesis of carriers of the *trans*-Golgi network to the cell surface (CARTS) requires VAP localization to ER/Golgi MCS and lipid transfer mediated by OSBP [29]. Reduction in the levels of VAP, OSBP or CERT prevented the secretion of CARTS cargo, as did expression of OSBP or CERT with mutations in the FFAT motifs. This suggests a requirement for OSBP in the maintenance of cholesterol and sphingolipid-enriched membranes at TGN/ER contacts and in the resulting directional transport of CARTS cargoes.

Less than 1% of cellular cholesterol is present in the ER, where the sterol regulatory element binding protein (SREBP) pathway is subject to negative feedback inhibition by cholesterol and oxysterols [30]. Thus, subtle changes in sterol

Table 1 OSBP/ORP localization and function

Name	Isoforms	Domains	Tissue expression	Localization	Ligands (k_d)	Intracellular functions
OSBP (*OSBP*)		OHD, FFAT, PH	Ubiquitous [133]	Golgi, cytosol [6], ER [23]	25OH (8 nM) [3, 5, 51], cholesterol (173 nM) [51], PI(4)P [25]	Catalyzes sterol/PI(4)P counter-current transport at ER/Golgi. Recruits CERT [24, 25, 27] and regulates SM synthesis [134]
ORP1 (*OSBPL1*)	ORP1L	OHD, ANK, PH	Brain, lung [50]	LEL, ER [50, 52]	25OH (83–97 nM) [40, 56], cholesterol (1.4 μM), PI(4)P [56]	Endosome positioning [52] and cholesterol efflux [56] via Rab7/RILP/p150^{GLUED} assembly [53]
	ORP1S	OHD	Heart, skeletal muscle, macrophages [50]	Cytosol [50], nucleus [51]	25OH (8.4 nM) [50], PI(4)P [56]	LXR regulation [40] and sterol transport from PM to LD [125]
ORP2 (*OSBPL2*)		OHD, VAP	Ubiquitous, enriched in CNS [62]	LD, ER, Golgi [62]	22(R)OH (14 nM), 7-ketocholesterol (160 nM), cholesterol [58], 25OH (3.9 μM) [40]	Cholesterol efflux [62], endocytosis [63] and PM-to-ER sterol transport [125]. Regulates triglyceride hydrolysis [59] and actin dynamics [73]. Binds LXR and regulates cortisol biosynthesis [60]
ORP3 (*OSBPL3*)		OHD, VAP, PH	Kidney, lymph nodes, thymus [135], macrophages [50]	Cytosol, ER, PM [135], nuclear envelope [136]	Photo-25OH (weak), photo-cholesterol [40]	Regulates actin dynamics via interaction with R-Ras [137], and PI levels [138]
ORP4 (*OSBP2*)	ORP4L	OHD, FFAT, PH	Brain, heart, testis, skeletal muscle [111]	Cytosol, ER, vimentin [111]	25OH (17–27 nM) [112, 113], cholesterol (68 nM) [113]	Regulates Ca^{2+} homeostasis [90, 115, 116]. Murine KO has oligo-astheno-teratozoospermia and sterility [117]. Silencing in cell culture causes apoptosis [113]
	ORP4M	OHD, FFAT	N/A	Vimentin aggregates [113]	N/A	
	ORP4S	OHD	Brain, heart [111]	Vimentin aggregates [111]	25OH (23 nM), cholesterol (60 nM) PI(4)P [113]	
ORP5 (*OSBPL5*)		OHD, PH, TM	Heart, brain, lung, liver, kidney [139]	ER [39], PM [140], mitochondria [37]	Photo-25OH [40], All PIPs (0.8–26.8 μM) [36], PS [141]	Cholesterol efflux from endosomes. PS/PI(4)P counter-current transport [39] at ER/PM [140]. PS transport to mitochondria [37]
ORP6 (*OSBPL6*)		OHD, FFAT, PH	Brain, skeletal muscle [135]	Nuclear envelope, cytosol, ER, PM [135], LEL [49]	Photo-25OH and photo-cholesterol (weak) [40]	Regulated by LXR. Involved in cholesterol efflux from early endosomal compartment [49]
ORP7 (*OSBPL7*)		OHD, FFAT, PH	Gastrointestinal tract [135]	Cytosol, ER, PM [135]	Photo-25OH and photo-cholesterol (weak) [40]	Promotes proteosomal degradation of Golgi snare GS28 via 25OH-dependent interaction with GS28 partner protein GATE-16 [108]
ORP8 (*OSBPL8*)		OHD, PH, TM	Macrophages, spleen, kidney, liver, brain [91]	ER, PM [140], mitochondria [37]	25OH [91], All PIPs (1.7–6.0 μM) [36], PS [141]	Counter-current transport of PS/PI(4)P at ER/PM [140]. PS transport to mitochondria [37]. Involved in macrophage motility and hepatic lipid efflux [91, 93]

Table 1 (continued)

Name	Isoforms	Domains	Tissue expression	Localization	Ligands (k_d)	Intracellular functions
ORP9 (*OSBPL9*)	ORP9L	OHD, FFAT, PH	Brain, heart, kidney, liver [142]	ER, Golgi [142]	Cholesterol [143], PI(4)P, cholestatrienol, dehydroergosterol [144]	Recruits ORP11 to the Golgi [137]. Regulates Golgi organization via cholesterol [143] or PI(4)P trafficking [144]
	ORP9S	OHD, FFAT	Liver [142]	ER [142]	PI(4)P, cholestatrienol, dehydroergosterol [144]	Involved in Golgi organization, growth inhibition [143] and PI(4)P regulation [144]
ORP10 (*OSBPL10*)	ORP10L	OHD, PH	N/A	Microtubules [85, 87], Golgi [87]	Photo-25OH [58], cholesterol [87], PS [141]	Dimerizes with ORP9L [87]. Negative regulator of hepatic lipid biosynthesis and apoB-100 secretion [85, 87]
	ORP10S	OHD	N/A	Cytosol, nucleus [87]	N/A	N/A
ORP11 (*OSBPL11*)		OHD, PH	Ovary, testis, brain, liver, kidney, stomach, adipose tissue [145]	Golgi, LEL [145]	N/A	Dimerizes with ORP9L [145]. involved in cellular triglyceride storage and induced during adipocyte differentiation [99]

levels in the ER can initiate rapid changes in cholesterol biosynthetic capacity. While the ER is relatively cholesterol poor, there is a cholesterol gradient from the *cis*/medial to *trans*-Golgi to PM, which constitutes approximately 60–80% of cellular cholesterol. A model for the maintenance of heterogenous cholesterol distribution between the ER and TGN posits that OSBP couples the transport of PI(4)P down its concentration gradient from the TGN to ER to power the counter-transport of cholesterol against its concentration gradient from the ER to TGN. The hydrolysis of the 4-phosphate of PI(4)P by Sac1 in the ER maintains the PI(4)P gradient, effectively harnessing the energy of PI(4)P synthesis and hydrolysis to drive cholesterol transport. Inhibition of OSBP with OSW-1, a member of the antineoplastic saponins (termed ORPphilins) that compete for sterol binding to the OHD of ORP4 and OSBP [31], doubled the cellular mass of PIP, of which PI(4)P is the predominant species. This finding suggests that an OSBP-mediated 'lipid pump' mechanism could consume up to 50% of cellular PI(4)P mass to power this cholesterol transport process. Preventing the replenishment of PI(4)P by pharmacological inhibition of PI4KIIIβ caused oscillation of PI(4)P levels at the TGN. In this scenario, OSBP drives consumption of PI(4)P and, consequently, dissociates from the TGN until PI4KIIα activity eventually replenishes the TGN PI(4)P pool, at which point OSBP is recruited to MCS and restarts the cycle [32].

The phenotypes of OSBP silencing in cultured cells have indicated an important role in ER-to-Golgi cholesterol transport. These include reduction in TGN cholesterol content [26] leading to the mislocalization of Golgi tether proteins [33], and accumulation of PI(4)P in Golgi and endosomal membranes leading to disruption of retromer-mediated transport and increased actin nucleation [34]. Silencing of OSBP expression had no effect on the morphology of the *cis*/medial-Golgi or TGN; however, the perinuclear intra-Golgi v-SNARES GS28 and GS15 become dispersed in the cytosol. GS28 and GS15 traverse the Golgi in COP-I transport vesicles, the fusion of which requires cholesterol-dependent conformational changes in SNARE proteins. Treatment of cells with lovastatin, an inhibitor of cholesterol biosynthesis, replicated the effects of OSBP silencing on GS28 and GS15 localization, suggesting that mislocalization is the result of aberrant cholesterol distribution due to the absence of OSBP [34].

Early reports that OSBP overexpression increased cholesterol biosynthesis and inhibited cholesterol esterification suggested a role in cholesterol transport or regulation in the ER [22]. Recently, inhibition of OSBP by OSW-1 was shown to increase lipid droplet formation indicative of ER cholesterol accumulation and enhanced esterification. However, OSBP silencing in Chinese hamster ovary cells had no effect on cholesterol regulation or esterification in the ER [27]. Similarly, OSBP silencing in HeLa cells had no effect

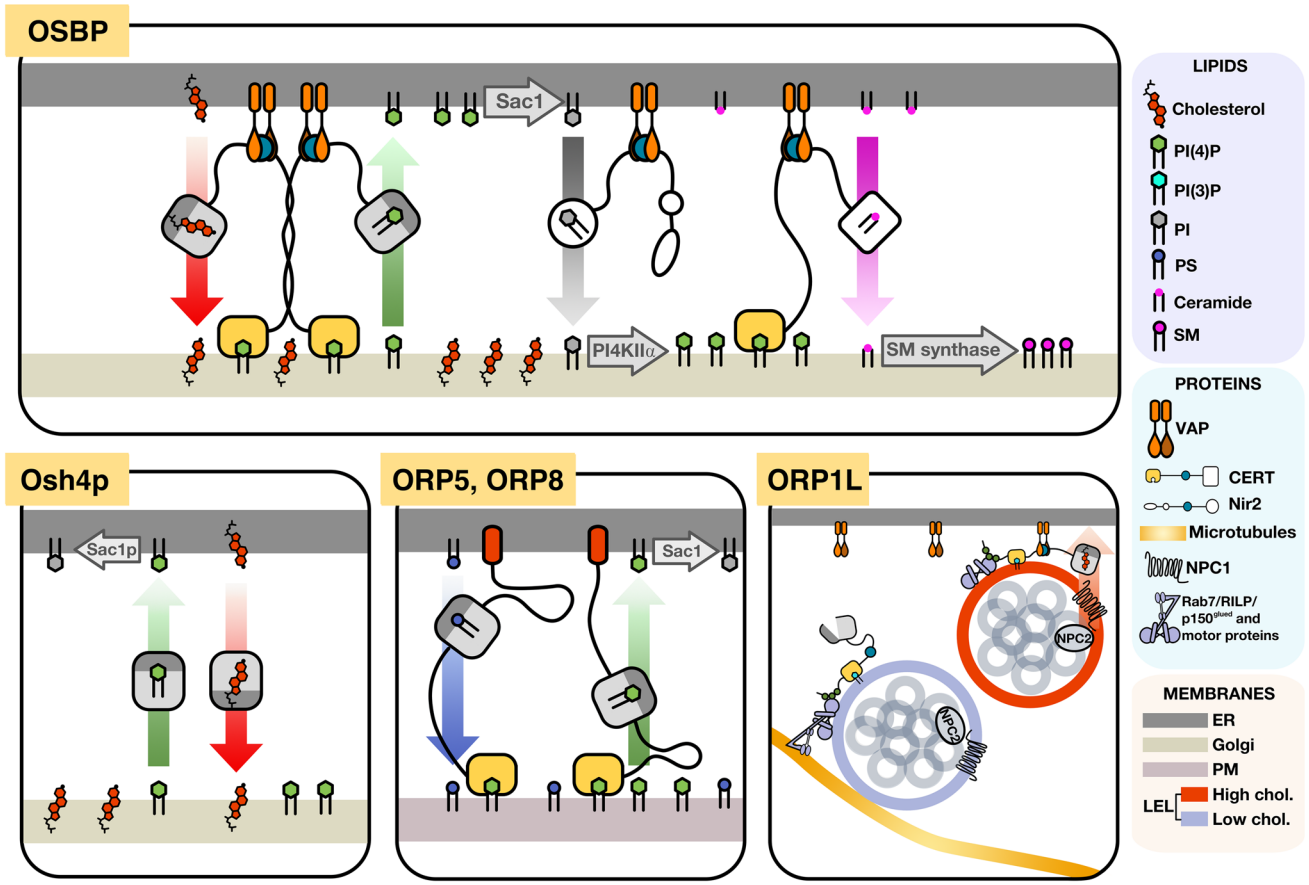

Fig. 3 Models of ORP function at membrane contact sites. OSBP localizes to closely apposed ER/Golgi membranes, where it exchanges PI(4)P and cholesterol. The resulting cholesterol enrichment on the Golgi activates PI4KIIα, which phosphorylates PI delivered by Nir2, into a PI(4)P pool that recruits the ceramide transfer protein CERT, resulting in ceramide delivery to sphingomyelin (SM) synthase and consequent SM synthesis. Consumption of PI(4)P in the endoplasmic reticulum (ER) by the Sac1 phosphatase maintains a concentration gradient that drives the PI(4)P/cholesterol countercurrent transport. Yeast Osh4p functions similarly to OSBP but without tethering to the ER or Golgi apparatus. ORP5 and ORP8 are ER-tethered proteins that extend to the plasma membrane via PH domain interaction with PIPs, using the transport of PI(4)P to the ER to drive phosphatidylserine transport to the PM. ORP1L is localized to the late endosomes/lysosomes (LEL) via interaction between N-terminal ankyrin repeats and Rab7. When cholesterol is low in the limiting membrane of LEL, ORP1L promotes the assembly of a microtubule-tethering complex (Rab7/RILP/p150^{glued}) that facilitates minus-end movement of the LEL. When cholesterol on the LEL limiting membrane is elevated, the complex dissembles and ORP1L tethers the LEL to the ER via VAP and facilitates cholesterol efflux to the ER

on regulation of cholesterol biosynthesis by oxysterols [35]. These data suggest that OSBP-mediated cholesterol export to the Golgi apparatus may involve a relatively small fraction of the ER cholesterol pool that has minimal impact on esterification and the SREBP regulatory machinery.

Lipid transfer by ORP5 and ORP8 at membrane contact sites

ORP5 and ORP8 associate with the ER via tail-anchored C-terminal transmembrane domains while the N-terminal PH domains interact with di- and tri-phosphorylated PIPs in the PM (Fig. 3) [36]. ORP5 and ORP8 are also localized at ER/mitochondria contact sites, but this activity is PH domain independent. Silencing of ORP5 and ORP8 caused defects in mitochondrial function and morphology without affecting the number of ER/PM or ER/mitochondrial MCS [37]. Together, these results suggest that ORP5 and ORP8 may also transfer PS from the ER to the mitochondria where it is decarboxylated to phosphatidylethanolamine required for mitochondrial structure and function [38].

The identification of ORP5 as a PS/PIP transfer protein contrasts an earlier study claiming that ORP5 was responsible for the transfer of low density lipoprotein (LDL)-cholesterol from the late endosomes/lysosomes (LEL) to the ER [39]. siRNA silencing of ORP5, but not ORP8, in HeLa cells caused accumulation of cholesterol in the limiting membrane of LEL and prevented cholesterol delivery to the ER, as measured by acyl-CoA: cholesterol acyltransferase activity [39]. Consistent with this activity, the ORP5 OHD

displayed cholesterol binding and transfer activity in vitro [39, 40]. ORP5 interacted with the LEL cholesterol transports protein Niemann–Pick type C protein 1 (NPC1) [39] and, thus, could receive cholesterol from NPC1 at the LEL/ER membrane interface. Recently, the identification of an interaction between the mammalian target of rapamycin (mTOR) and the ORP5 OHD indicates that ORP5 could have a more complex role in nutrient sensing at LEL [41], perhaps related to the regulation of the mTOR by cholesterol [42]. Therefore, ORP5 appears to have lipid transfer activities at multiple MCS, but whether these are discrete or related activities is presently unclear.

Sterol transfer by ORP1L and ORP6 at endosome/ER contact sites

In addition to de novo cholesterol biosynthesis in the ER, cells also acquire exogenous cholesterol by the uptake and processing of lipoproteins, primarily LDL, in the endo-lysosomal pathway. After receptor-mediated endocytosis, the cholesterol esters in LDL are hydrolyzed in LEL [43]. The bulk of LDL-derived cholesterol is exported from the late endosomes before lysosomal maturation, as evidenced by the discrepancy in cholesterol content between the two compartments [44]. Cholesterol export from the LEL lumen to the external leaflet of the limiting membrane occurs by the concerted action of NPC protein 2 (NPC2), a soluble protein that binds cholesterol in the LEL lumen, and NPC1, a multi-pass transmembrane protein that receives cholesterol from NPC2 and transfers it to the limiting membrane of the LEL [45]. Once on the external leaflet of the limiting membrane, cholesterol is transferred to other organelles by poorly understood mechanisms. The bulk of LDL-derived cholesterol appears to flow to the ER [43], potentially at contact sites with LELs [46]. Indeed, quantitative imaging studies show that almost 100% of Rab7-positive late endosomes have frequent and sustained interactions with the ER [47]. The dual membrane-targeting ORP1L and ORP6 have been implicated in sterol transfer at these contacts.

Initial studies showed that ORP6 mRNA is increased in response to cholesterol loading in cultured cells [48]. Later studies confirmed that expression of ORP6 is regulated by the liver X receptor (LXR) and miR-33 and -27b, and increased under conditions of cholesterol loading in macrophages and animal models [49]. In ORP6-silenced and cholesterol-loaded macrophages, cholesterol accumulated in abnormally clustered early endosomes and cholesterol esterification in the ER was inhibited. Conversely, overexpression of ORP6 stimulated cholesterol esterification and cholesterol efflux from cells. Collectively this indicated that LXR activation of ORP6 is a mechanism to promote the efflux of sterols from early endosomes and suppress cholesterol biosynthesis.

OSBPL1 encodes two variants due to alternate promoter start sites: full-length ORP1L, which has N-terminal ankyrin repeats and a PH domain, and the truncated variant ORP1S that contains only the OHD [50]. ORP1S is a soluble sterol receptor that localizes to the nucleus in response to ligand binding and enhances the activity of LXR [51]. Initial studies of ORP1L described interactions with Rab7 in the LEL via the ankyrin motif and with VAP in the ER via the FFAT motif [52]. The functionality of ORP1L at ER/LEL contacts appears to depend on the level of cholesterol in the limiting membranes of each compartment. In instances where LDL is absent and LEL cholesterol is low, ORP1L, Rab7 and VAP are implicated in the transfer of cholesterol at contacts between the ER and a subpopulation of multivesicular endosomes involved in epidermal growth factor receptor sorting into intraluminal vesicles [46]. These contacts are formed by interaction between annexin A1 and S100A11 and are calcium dependent. In *C. elegans*, deletion of all four *obr* genes also resulted in abnormal multivesicular bodies with reduced cholesterol content, supporting a role for ORP1L in LEL maturation [11].

In a related sterol-sensing function of ORP1L, elevated cholesterol in the LEL (as in the case of NPC disease) initiates the formation of an ORP1L, Rab7 and Rab7-interacting lysosomal protein (RILP) complex that recruits the dynactin subunit $p150^{Glued}$ and βIII-spectrin to initiate minus-end transport on microtubules to the perinuclear region [53, 54]. Reduction of LEL cholesterol promotes a conformational change in ORP1L that increases VAP interactions and displaces $p150^{Glued}$, leading to plus-end transfer of LEL. This sterol-sensing function of ORP1L thus regulates the positioning of LEL based on cholesterol content as well as endosome maturation via interaction with the homotypic fusion and vacuolar sorting (HOPS) complex [55].

ORP1L is also involved in the transfer of LDL-cholesterol from LEL-to-ER. ORP1L knockout cells display sequestration of perinuclear endosomes, defective cholesterol delivery to the ER and increased de novo cholesterol biosynthesis [56]. Contrary to a sensing function, ORP1L interacts with VAP on the ER when cholesterol is present on the limiting membrane of the LEL to establish a peripheral distribution of LELs and mediate cholesterol shuttling to the ER (Fig. 3). When the limiting membrane is depleted of cholesterol, ORP1L dissociates from VAP and promotes assembly of motor complexes that drive minus-end microtubule transport of LEL toward the perinuclear region [53, 55]. Thus, ORP1L has multiple transfer and regulatory activities that could result from its association with numerous, functionally distinct LEL-ER contact sites. However, as a cholesterol transfer protein, ORP1L appears to facilitate the bi-directional transfer of cholesterol between the LEL and ER depending on the sterol gradient.

ORP2 at ER/lipid droplet contact sites

The ER contains acyltransferases that synthesize cholesterol esters and triacylglycerol that coalesce within the bilayer of ER membranes, eventually budding to form specialized storage organelles called lipid droplets (LD) [57]. The lipids stored in LDs are a source of fatty acids for oxidation or can be packaged into lipoproteins for secretion and transport to other cells. ORP2 is composed of an OHD, which binds cholesterol and oxysterols, and an FFAT motif [58]. ORP2 is present at ER/LD contact sites [59] and dissociates from LDs upon ligand binding [60]. The function of ORP2 at ER/LD contacts is not well understood, but silencing of ORP2 leads to increased cellular cholesterol levels [60] and aberrant metabolism of triglycerides [58, 59] and fatty acids [61]. Overexpression of ORP2 increased cellular cholesterol efflux [62, 63] and LD dispersion [60]. Together, these studies suggest that ORP2 regulates LD positioning by establishing ER contacts in a sterol-dependent manner, which facilitates lipid exchange between the organelles.

Involvement of OSBP/ORPs in human disease

OSBP/ORPs display both ubiquitous and tissue-specific expression patterns (summarized in Table 1). Despite the size of the family and the potential for redundant function within specific tissues, high-throughput genetic and targeted functional studies have implicated OSBP/ORPs in a range of rare and common human disorders (summarized in Table 2). Genetic screens of cancerous tissues and cell lines have detected elevated expression of ORP1 [64], ORP2 [64], ORP4 [65, 66], ORP5 [67], ORP7 [64] and ORP10 [68, 69] and revealed that ORP3 is frequently mutated in metastatic versus nonmetastatic breast cancers [70]. In some cases, targeted research on individual ORPs has led to a limited mechanistic understanding of their contribution to the disease phenotype. The following section and Table 2 outline our current understanding of how OSBP/ORPs contribute to a range of human disorders.

ORP2 and hearing loss

ORP2 is ubiquitously expressed but enriched in cells of the central nervous system as well as cochlear inner and outer hair cells [71]. Whole-exome sequencing identified frameshift mutations in *OSBPL2* that have been linked to familial autosomal dominant non-syndromic hearing loss [71, 72]. These mutations were predicted to introduce truncations and deletions in ORP2 that are deleterious to activity, but this was not confirmed experimentally and a functional link to the auditory system was not established [72]. However, ORP2 was recently identified as a regulator of cell morphology, attachment, motility and proliferation via actin remodeling through the RhoA signaling pathway [73]. CRISPR-Cas9-mediated ORP2 knockout in HUH7 hepatocarcinoma cells affected the expression of several RhoA effectors, including the Rho GTPase-activating protein ARHGAP12 and diaphanous-1, with which ORP2 interacts [73, 74]. Interestingly, ARHGAP12 is expressed at inner ear hair cell–cell junctions [75], while mutations in diaphanous-1 are causative in autosomal dominant, non-syndromic hearing loss in an extended Costa Rican family [76]. These studies suggest that interaction between ORP2 and diaphanous-1 could be essential to the function and morphology of cochlear hair cells. The connection between this disease phenotype and the purported role of ORP2 in LD function (see "ORP2 at ER/lipid droplet contact sites") remains to be resolved.

ORP3 and OSBP in heritable amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a poorly understood sporadic neurodegenerative disorder of motor neurons in which 10% of cases are attributed to heritable genetic components [77]. One of these is ALS8, a subtype caused by a loss-of-function point mutation in the major sperm protein domain of VAP-B [78, 79], which interacts with the FFAT motif of partner proteins such as OSBP/ORPs. Mutant VAP-B forms aggregates with itself and wild-type VAP-A and VAP-B in detergent-insoluble vacuoles that sequester ER proteins, leading to mislocalization of Sac1 and defects in retrograde transport due to perinuclear and PM accumulation of PI(4)P [80]. Interestingly, overexpression of ORP3 in HeLa cells prevented mutant VAP-B aggregation and rescued both PI(4)P accumulation and secretory phenotypes [80]. Similar results were obtained by co-expressing mutant VAP-B and the *D. melanogaster* homolog of OSBP [81]. It is unclear whether the lipid-transport functions of ORP3 and OSBP contributed to the rescue of mutant VAP-B phenotypes, or if their overexpression titrates the mutant VAP-B, freeing wild-type VAP-A and B to interact with partner proteins.

ORPs and dyslipidemia

Dyslipidemia is defined as elevated levels of one or more lipoprotein species in the blood, which predisposes individuals to cardiovascular events, hepatic disease and metabolic syndrome. Dyslipidemias have genetic and lifestyle-related contributions, and can result from increased lipid production, decreased lipid clearance or increased lipid absorption from the diet. In cases of hypercholesterolemia, elevated low-density lipoprotein (LDL) imposes cardiovascular risk

Table 2 OSBP/ORPs in human pathologies

Protein	Pathology	Notes	Study type	References
	Cholangiocarcinoma	Increased expression	qRT-PCR and immunohistochemistry	[64]
OSBP	Amyotrophic lateral sclerosis	Overexpression rescues mutant VAP-B phenotype	Drosophila study	[81]
ORP1	Dyslipidemia	Nonfunctional mutant is associated with HDL cholesterol < 1st percentile	Small cohort study ($n = 80$), genome sequencing and blood analysis	[88]
ORP2	Autosomal dominant non-syndromic hearing loss	Truncation/deletion due to frameshift mutation causes hereditary hearing loss	Whole-exon sequencing of affected Chinese and German families	[71, 72]
	Dyslipidemia	Expression inversely correlated with levels of hepatic steatosis marker Hsa-miR-855-5p and HDL cholesterol	microRNA profiling	[84]
	Amyotrophic lateral sclerosis	Overexpression rescues mutant VAP-B ER stress phenotype	Cell culture study	[80, 81]
ORP3	Glioblastoma	Expression associated with positive treatment response	RNA-seq	[146]
	Breast cancer	More frequently mutated in metastatic breast cancer than early breast cancer	Next-gen seq	[70]
	Dyslipidemia	Gain-of-function mutation in LRH-1 induces NAFLD in mice via ORP3 expression	Mouse study	[83]
	Pancreatic ductal carcinoma	High expression associated with poor prognosis	Microarray database analysis, immunohistochemistry	[147]
	Metastasis	Highly expressed in disseminated tumor cells	Differential display PCR	[66, 110]
ORP4	T-cell acute lymphoblastic leukemia	High ORP4 expression provides energetic advantage via calcium signaling	Cell culture study, including patient samples	[116]
	Chronic myeloid leukemia	ORP4 elevated in 80% of CLM patients studied	RT-PCR of patient samples	[65, 109]
	Spermatogenesis	ORP4 KO causes male infertility by oligo-astheno-teratozoospermia	Mouse KO study	[117]
	Cholangiocarcinoma	Increased expression	qRT-PCR and immunohistochemistry	[64]
	Pancreatic cancer	Expression positively correlated with cancer cell invasiveness and poor prognosis	Cell culture studies based on patient gene expression profiles, includes patient samples	[67, 102]
	Failures in meiosis II leading to recurrent triploidy	Rare *OSBPL5* mutation may contribute to recurrent IVF failure by triploidy	Genotyping, whole-exome seq, methylation analysis	[147]
ORP5	Metastatic lung cancer	Increased expression in metastatic lung vs nonmetastatic lung tumor	2D-DIGE/antibody screen, tissue microarrays, cell culture studies	[148]
	Alcohol dependence	SNPs in *OSBPL5* are associated with alcoholism	GWAS	[149]
	Dyslipidemia	Expression level in leukocytes is positively correlated with plasma lipid levels	Microarray expression profiling	[150]
ORP6	Dyslipidemia	Expression level is positively correlated with HDL cholesterol levels	Cell culture studies and patient screen	[49]
	Alzheimer's	SNPs in *OSBPL6* are associated with familial Alzheimer's	GWAS	[151]
ORP7	Cholangiocarcinoma	Increased expression	qRT-PCR and immunohistochemistry	[64]
	Dyslipidemia	SNPs in *OSBPL7* are associated with total cholesterol and LDL levels	GWAS and genotyping	[152, 153]
	Gastric cancer	Reduced expression	Cell culture study based on patient screen	[154]

Table 2 (continued)

Protein	Pathology	Notes	Study type	References
	Cholangiocarcinoma	Increased expression	qRT-PCR and immunohistochemistry	[64]
	Dyslipidemia	SNPs in *OSBPL8* associated with HDL cholesterol levels	GWAS	[155]
		Obesity-related miRNA-143 reduced insulin-induced PKB activation in liver cells via reduction of ORP8 levels	Mouse study	[94]
ORP8		Activin A from epicardial adipose tissue reduced ORP8 levels via production of miRNA-143	Cell culture study with primary rat cells	[95, 96]
		ORP8 KD reduced arterial lesion size by 20% in HFD-fed LDLR-KO mice	Mouse study	[92]
		Lenz-Majewski disease mutations in PS synthesis alter counter-current transport by ORP8	Cell culture study	[156]
		Expression level in leukocytes is negatively correlated with plasma lipid levels	GWAS	
	Gastric cancers and hepatocellular carcinoma	ORP8 expression is low in gastric and hepatic cancers; overexpression decreases tumor growth and increases apoptosis by mitochondrial and ER stress	Cell culture and mouse studies based on patient expression profiles	[107, 154]
ORP9	Colorectal cancer	Elevated expression is positively correlated with survival	Whole genome expression profiling	[157]
	Dyslipidemia	SNPs in *OSBPL9* may be associated with increased risk of cerebral infarction	PCR–RFLP screen of Chinese cohort	[158]
	Dyslipidemia	SNPs in *OSBPL10* associated with extreme high triglyceride levels (>95th percentile), silencing increases lipogenesis and apoB-100 secretion in hepatic cell line	Cell culture experiments based on genotyping in Finnish dyslipidemic families	[85, 87]
		SNPs in *OSBPL10* associated with hypercholesterolemia	Japanese cohort study	[159]
ORP10		RNA expression in leukocytes is positively correlated with plasma lipid levels	GWAS	[150]
		SNP in *OSBPL10* intron strongly associated with peripheral arterial disease	GWAS	[86]
	Prostate cancer	Potential cancer marker	Microarray analysis	[68, 69]
	Hypertension	SNPs in *OSBPL10* may contribute to hypertension	Next-generation linkage association	[160]
	Dyslipidemia	SNPs in *OSBPL11* associated with metabolic syndrome	Genotyping of obese individuals	[97]
ORP11		Granular cholesterol staining in dermal tissue of individuals with homozygous *OSBPL11* missense mutation	Case study of family with rare neurodegenerative disease	[98]

while elevated high density lipoprotein (HDL) is considered beneficial [82]. HDL and LDL levels are dependent on factors that regulate their metabolism in the circulation as well as by cellular cholesterol synthesis, uptake, processing and secretion. Genetic screens of dyslipidemic patients and functional studies on mouse and cell culture models have confirmed a role for the OSBP/ORP family in all steps affecting lipoprotein metabolism.

Studies have implicated OSBP/ORPs in lipid dysregulation without identifying specific mechanisms. ORP3 was recently identified as a liver receptor homolog-1 (LRH-1) target gene responsible for the increased de novo cholesterol synthesis and hepatic steatosis observed in mice expressing a gain-of-function LRH-1 mutant [83]. In a genetic screen of 871 Finnish adults, increased levels of the hepatic steatosis marker hsa-miR-885-5p were found to be inversely correlated with ORP2 expression and HDL cholesterol levels [84]. Studies of hyperlipidemic Finnish [85] and Japanese [86] families identified single nucleotide polymorphisms (SNPs) in the *OSBPL10* gene that were linked to elevated plasma triglyceride and LDL levels. A correlation between SNPs in the *OSBPL10* gene and incidence of peripheral arterial disease was also noted [86]. These findings prompted cell culture studies in which ORP10 was found to associate with microtubules and the Golgi apparatus to promote lipoprotein secretion [85, 87]. However, the connection between the *OSBPL10* SNPs and plasma lipid levels remains obscure.

A heterozygous loss-of-function mutation in ORP1L, which delivers lipoprotein-derived cholesterol from the LEL to the ER for storage and efflux (see "Sterol transfer by ORP1L and ORP6 at endosome/ER contact sites"), was identified in patients with extremely low levels of HDL cholesterol [88]. Individuals with the loss-of-function ORP1L mutation exhibited decreased plasma levels of apoA-1 as well as decreased cholesterol efflux to available apoA-1. Therefore, it remains unclear whether the mutation is associated with impaired cholesterol efflux by ATP binding cassette (ABC)A1 to apoA-1 or impaired secretion of apoA-1 from the liver or other tissues. The former mechanism is supported by ORP1L silencing experiments in cultured macrophages that showed significantly reduced efflux of [^{3}H] cholesterol to apoA-1in the media [89]. Overall, these findings are consistent with a model in which ORP1L-mediated export of cholesterol from the LEL is coupled to efflux from cells by an ABCA1-dependent pathway. ORP6 may have a related function since its expression was positively correlated with HDL cholesterol levels in healthy human subjects and decreased in the atherosclerotic plaques of LDL receptor knockout mice [49].

Homozygous knockout of ORP4 in mice was also shown to reduce atherosclerotic lesion size by a mechanism independent of cholesterol efflux. Rather, ORP4 promotes macrophage survival in atherosclerotic plaques, attenuating oxysterol-induced cytotoxicity by a pro-survival calcium signaling pathway ("ORP4 in leukemia and metastasis") [90]. ORP8 expression is increased in human atherosclerotic plaques [91] and the macrophage-specific knockout of ORP8 in LDLR-knockout mice attenuated arterial lesion size and inflammation [92]. A potential link between ORP8 and lesion formation was strengthened by the finding that ORP8 negatively regulated cholesterol efflux by cultured macrophages via transcriptional suppression of ABCA1 [91]. Consistent with this, chow-fed male *OSBPL8* knockout mice had significantly elevated HDL levels relative to female mice on the same diet and both genders displayed significantly elevated HDL when fed a high-fat diet [89]. The regulatory activity of ORP8 in cultured hepatocytes could be mediated by interaction with the nucleoporin Nup62 [93], or via obesity-associated miR-142 and attenuation of insulin-stimulated protein kinase B/AKT activity in liver and smooth muscles cells [94–96]. Like ORP8, SNPs in *OSBPL11* were associated with altered metabolism of lipids and glucose in obese populations [97], and siblings homozygous for a loss-of-function *OSBPL11* missense mutation display abnormal dermal cholesterol deposits [98]. Interestingly, ORP8 and ORP11 normally follow opposing expression patterns during adipocyte differentiation (reduced and increased, respectively), and in cell culture studies the overexpression of ORP8 or silencing of ORP11 impedes triglyceride storage [99]. In general, these studies show that select ORPs are involved, either directly or indirectly, in pathways that remove or prevent accumulation of cholesterol in cells of the cardiovascular system, leading to reduced risk of atherosclerosis.

OSBP/ORPs in cancers

While genetic screens have associated the expression and/or mutation of individual ORPs with various cancers (summarized in Table 2), only ORP5, ORP8, ORP4 and OSBP were investigated to confirm a functional role in malignancy. Here, we review the current understanding of how these ORPs contribute to cancer cell invasion, evasion of apoptosis and proliferation.

ORP5 and invasiveness in pancreatic ductal carcinoma

Chemical induction of pancreatic ductal carcinoma in hamsters yielded two cell lines, PC1 and the more invasive PC1.0, that are used as models of the human disease [100, 101]. Analysis of mRNA profiles of the two cell lines revealed that *OSBPL5* was differentially expressed in PC1.0 cells. Silencing of ORP5 in PC1.0 cells significantly decreased invasiveness while the converse result was obtained by ORP5 overexpression in PC1 cells [67]. Similar results were obtained in human pancreatic cancer

cell lines [102]. In pancreatic cancer patients, increased *OSBPL5* expression was correlated with a 50% reduction in 1-year survival, and a nearly 70% reduction in median survival time in patients with stage I, II and III cancers [67].

The mechanism by which ORP5 increases cancer cell invasiveness could involve upregulation of oncogene expression through effects on the cholesterol biosynthetic pathway. Under cholesterol-replete conditions, SREBP2 is sequestered in ER membranes, whereas lowering ER cholesterol promotes SREBP translocation to the Golgi apparatus for proteolytic processing to release the soluble transcription factor, which translocates to the nucleus and binds sterol response elements in gene promoters [103]. The promoter region of the histone deacetylase 5 gene *HDAC5* has a sterol response element, placing oncogenic HDAC5 expression under sterol control [104]. Treatment with the HDAC inhibitor trichostatin-A significantly reduced cell growth in ORP5-positive pancreatic cancer cell lines [102]. However, the mechanistic link between ORP5 and SREBP2 is uncertain; ORP5 is implicated in the delivery of LDL-derived cholesterol to the ER (Sect. 2.4) [39, 40], which would potentially restrict SREBP2 activation and subsequent *HDAC5* expression. Also, there is a synergistic relationship between the trichostatin-A and the cholesterol synthesis inhibitor simvastatin [102], which would have opposing functions if HDAC expression was being induced by the SREBP2 [104]. Future studies to evaluate how ORP5 affects SREBP2 processing in the ER, and the link to expression of oncogenic genes, are warranted.

ORP8 promotes apoptosis by the Fas receptor pathway

Fas receptor binding of autocrine or paracrine-secreted Fas ligand leads to the initial activation of caspase 8 and a resulting cascade of caspase autoproteolysis that ultimately leads to cell death [105]. Immunohistochemical analysis of liver tissue from patients with hepatocellular carcinoma (HCC) revealed that malignant cells contained less cell surface and more internal Fas receptor than non-malignant counterparts, implying that HCC cells may avoid apoptosis due to insufficient Fas receptor presentation [106]. ORP8 was identified as a necessary factor for the PM presentation of Fas in HCC cell lines and patient tissue samples in which ORP8 expression are reduced by miR-143 [107]. Expression of ORP8 in HCC cells induced Fas-mediated apoptosis by shifting cytosolic Fas to the PM and reduced the size of mouse tumor xenografts. However, the apoptotic effect of ORP8 may be specific to HCC; ORP8 expression was increased in a hamster model of cholangiocarcinoma [64], and ORP8 expression in HepG2 cells mediated cell-cycle inhibition by 25-hydroxycholesterol but did not induce apoptosis [108].

ORP4 in leukemia and metastasis

Elevated ORP4 transcripts in disseminated tumor cells and the leukocytes of patients with chronic myeloid leukemia first pointed to this ORP as a potential bio-marker or regulator of metastasis [65, 66, 109, 110]. Alternate promoter start sites in *OSBPL2* produce three ORP4 variants; full-length ORP4L, and ORP4M and ORP4S that have truncated and deleted N-terminal PH domains, respectively [113]. Like OSBP, the ORP4 OHD binds cholesterol, oxysterols and PI(4)P [40, 111, 112], and ORP4L has a PH domain that recognizes Golgi-associated PI(4)P [113]. However, ORP4 is the only member of the ORP family required for the proliferation of cultured cells [113]. Silencing of ORP4L induced growth arrest and cell death in immortalized and cancerous cell lines, which was exacerbated by knockdown of all three variants. Moreover, steroidal antineoplastic saponins called the ORPphillins were identified as ORP4 antagonists that compete for sterol binding to the OHD [31].

The correlation between ORP4 expression and tumor dissemination may involve the epithelial-to-mesenchymal transition (EMT), the process by which epithelial cells develop mesenchymal characteristics such as increased motility and decreased cell–cell contact. EMT is accompanied by increased vimentin intermediate filament expression, which leads to increased motility and invasiveness that is oncogenic outside the context of development [114]. All three ORP4 variants interact with the vimentin intermediate filament network but ORP4M and ORP4S collapse the network, suggesting negative regulation of this interaction by the ORP4L PH domain [111–113]. Considering the regulatory role of vimentin in cell adhesion and motility, further study of a functional relationship with ORP4 in the context of metastasis is needed.

Yan and colleagues identified a potential mechanism for ORP4L-mediated proliferation in T cell acute lymphoblastic leukemia, in which ORP4L scaffolds a unique PM-associated phospholipase C (PLC)3β activation complex that produces inositol triphosphate required for ER calcium release and mitochondrial ATP production [115, 116]. In the absence of ORP4L, inositol triphosphate signaling is attenuated, leading to mitochondrial dysfunction and cell death. Importantly, ORP4L is virtually absent from normal T-cells, where inositol triphosphate production occurs via a G-protein-independent pathway involving PLCγ1. Thus, malignant T-cells and other cancerous cells that express abundant ORP4L appear to have acquired a novel signaling pathway that provides increased energy production for survival and proliferation. ORP4 knockout mice develop normally except for male sterility due to defective sperm elongation [117], which is consistent with high levels of ORP4 expression in testis. However, it is unclear why other tissues that express

ORP4, such as the brain and retina, are unaffected in the knockout animals.

To summarize, many studies implicating ORPs in cancers are large-scale genomic analyses that have associated ORP gene SNPs with either cancer progression or survival (summarized in Table 2). However, the relation of these SNPs to gene transcription and protein expression is unknown. The limited number of analyses available suggests that those ORPs implicated in cancer initiation or progress do so downstream of their primary lipid binding/transfer functions. However, the well-defined role for ORP4L in the survival of lymphoblastic T-leukemia cells indicates that other scaffold/signaling mechanisms may apply.

OSBP/ORPs in viral infection, replication and release

Single-stranded RNA viruses replicate in the cytosol of host cells and employ the Golgi apparatus, ER and/or endosomal membranes as replication platforms, altering the morphology of the organelles using a combination of viral and host factors. The morphology of the hijacked host membranes, termed replication organelles, depends on the infecting virus. For example, dengue virus forms double-membrane vesicles of ER origin, while the single-membrane vesicles formed by poliovirus are ER-, Golgi- and lysosome-derived and arranged in rosettes [118]. As outlined below, OSBP, ORP1L, ORP4 and ORP10 contribute to viral egress or replication processes.

Currently, the most well-studied example of viral dependence on ORP function is the requirement for OSBP and ORP4 in the development the hepatitis C virus (HCV) replication organelle. HCV replication involves the translation of polyproteins, cleavage into active peptides and the establishment of a replication organelle, called the membranous web, from host membranes. A screen for host proteins associated with non-structural HCV proteins revealed that the ER-anchored non-structural viral protein NS5A interacts with both OSBP and VAP-A, and established that OSBP is required for the replication and egress of HCV particles [119]. Tai and colleagues determined that OSBP supplies the membranous web with cholesterol in a PI(4)P-dependent manner, similar to the exchange between the Golgi apparatus and ER (Sect. 2.1) [120]. The requirement for OSBP to supply cholesterol extends to the development of replication organelles by poliovirus and dengue [120], with concurrent studies showing similar results in picornavirus [121] and encephalomyocarditis virus infections [122]. These mechanistic findings conceptualized OSBP as a target for known and novel antiviral compounds; OSBP is an identified target of the anti-enteroviral compounds itraconazole and TTP-8307 [123, 124] and the antiviral properties of the ORPphilin OSW-1 are dependent on OSBP and ORP4 [125]. Interestingly, despite the dependence of OSW-1 antiviral activity on ORP4, ectopic expression of ORP4L or ORP4S inhibited the replication of HCV and produced infection-related lipid droplets on the membranous web [126], suggesting that ORP4 may contribute to cellular lipid metabolism by a different mechanism than OSBP. In addition to viral replication and egress, OSBP has also been implicated in viral entry. Treatment of cells with interferon-inducible transmembrane protein 3 inhibited the interaction between OSBP with VAP-A and cholesterol egress from the ER, leading to aberrant accumulation of cholesterol in the endocytic system that prevented cytosolic release of endocytosed vesicular stomatitis virus [127].

A study of dengue susceptibility in African, Asian and European populations revealed that SNPs which affect the expression of *OSBPL10* are protective in African populations [128]. Functional studies using THP-1 monocytic leukemia cells demonstrated that ORP10 silencing significantly reduced viral replication and secretion [128].

Expression of an OHD deletion mutant of ORP1L reduced the infectivity of vesicular stomatitis virus presenting the Ebola glycoprotein by 50%, suggesting a requirement for ORP1L-mediated endosome motility in Ebola infection [55]. ORP1L was also identified as an interacting partner of a 2′–5′ oligonucleotide synthase viral host defence pathway against flavivirus infection, but the mechanism of action is unknown [129]. ORP1L was implicated in another innate immune response to adenovirus infection [130]. In this case, the viral receptor internalization and degradation complex protein RIDα, a GTP-Rab7 mimic, interacts with ORP1L to direct the transfer of LDL-cholesterol from LEL-to-ER independently of NPC1 [131]. The RIDα-ORP1L pathway for cholesterol transfer did not affect cholesterol biosynthesis in the ER, but directed cholesterol to lipid droplets and attenuated lipid raft-dependent pro-inflammatory signaling by Toll-like receptor 4 [130].

Concluding remarks

Changes in ORP expression and function are implicated in common and rare human disorders, and infections by common pathogenic viruses. An emerging recognition that OSBP/ORPs mediate lipid transport and regulate metabolism at MCS suggests that these pathologies may arise due to deleterious changes in the intracellular distribution of cholesterol, phospholipids and/or sphingolipids. Alternatively, considering that many ORPs exhibit nanomolar affinity for oxysterols, ORPs may act as high-affinity receptors that regulate signaling pathways via protein–protein or protein–membrane interactions. While both are attractive possibilities as primary functions of the ORP family, their

multivalent roles suggest that these could be dichotomous functions. For example, sterol-binding by ORP1L [55] and ORP4L [90] alters interactions with their respective protein partners, making them candidate sterol-regulated scaffolding proteins for functional complexes at membranes. However, both have also been shown to bind and transport lipids in vitro [56, 113]. ER-to-Golgi cholesterol transfer by OSBP is required for synergy between sterol and sphingolipid metabolism, but OSBP also acts as a sterol-regulated scaffold for the dephosphorylation of phosphorylated extracellular signal-regulated kinase [132].

Despite being named for the founding member OSBP that binds oxysterols with high affinity, structural and binding analysis clearly indicates the Osh/OSBP/ORPs also bind phospholipids. OSBP/ORP function is contingent on the specificity of the OHD for lipid ligands, which is currently defined by in vitro binding of a limited set of available ligands that may not recapitulate in vivo specificity. Continued elucidation of the structure of OHDs and their associated ligands, particularly those from mammalian family members, will help define the repertoire of lipid ligands. Further studies are required to then reconcile in vitro analysis of lipid transport with in vivo studies of OSBP/ORP function to define their roles in normal and pathological conditions at the cellular and organismal level.

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