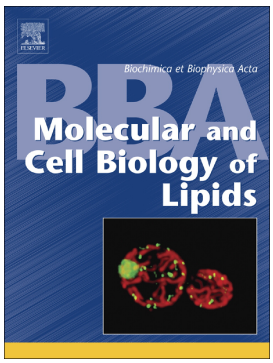

Oxysterol-binding protein: New insights into lipid transport functions and human diseases

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Oxysterol-Binding Protein: new insights into lipid transport functions and human diseases

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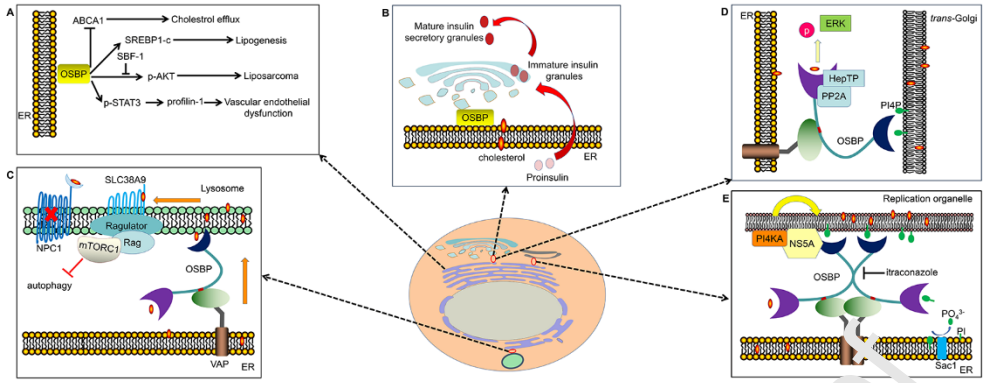
Abstract: Oxysterol-binding protein (OSBP) mediates lipid exchange between organelles at membrane contact sites, thereby regulating lipid dynamics and homeostasis. How OSBP's lipid transfer function impacts health and disease remain to be elucidated. In this review, we first summarize the structural characteristics and lipid transport functions of OSBP, and then focus on recent progresses linking OSBP with fatty liver disease, diabetes, lysosome-related diseases, cancer and viral infections, with the aim of discovering novel therapeutic strategies for common human diseases.

Graphical abstract



OSBP, a lipid-binding/transfer protein, plays an important role in lipogenesis, liposarcoma, vascular endothelial dysfunction and cholesterol efflux (Fig. A). Additionally, OSBP can be translocated to the MCS between ER and TGN or lysosome, regulating insulin granule secretion (Fig. B), mTORC1 activation (Fig. C) and ERK phosphorylation (Fig. D). All of these functions depend on the cholesterol transport ability of OSBP. Finally, in virus-infected host cells, virus hijacks the cholesterol transport pathway of OSBP for replication (Fig. E). These findings highlight the important physiological functions of OSBP and its potential to be targeted for disease treatment.

Highlights:

- Cholesterol is transported from ER to TGN by OSBP at the expense of TGN PI4P.
- OSBP, CERT, PKD, Sac1 and PI4P form a complex regulatory system to maintain the balance of lipid composition in Golgi apparatus.
- OSBP relies on its cholesterol transport function to be involved in lysosome-related disorders, cancer and other diseases.

Key words: OSBP; cholesterol; PI4P; disease; virus.

1. Introduction

Lipids are key components of biological membranes that are mainly synthesized in endoplasmic reticulum (ER). Each organelle has its own unique lipid composition to ensure that membrane-related biochemical reactions and other organelle-specific functions, such as ion transport and signal transduction, can respond and adapt to various challenges. Translocation and exchange of lipids between organelles are mainly through vesicular and non-vesicular transport [1, 2]. Non-vesicular lipid transfer mediated by lipid-binding/transfer proteins (LTPs) usually occurs at membrane contact sites (MCSs, a region separated by 10 – 30 nm between the membranes of two different organelles) [3, 4]. LTPs play an integral role in lipid metabolism and signaling, and are capable of accurately and efficiently transporting lipids [5, 6].

Human oxysterol binding protein (OSBP) and OSBP-related proteins (ORPs) are one of the highly conserved LTP families in eukaryotes [7] with 12 members identified so far [8]. Most members of this family exhibit structural similarities to OSBP, featuring a pleckstrin homology (PH) domain near the N-terminus, a two phenylalanines in an acidic tract (FFAT) motif, and an OSBP-related ligand binding (ORD) domain at the C-terminus. Further, they are divided into six sub groups based on sequence similarities and gene structure. Group I includes OSBP and ORP4, group II includes ORP1 and ORP2 (without PH domain), group III includes ORP3, ORP6 and ORP7, group IV includes ORP5 and ORP8 (without FFAT motif), group V includes ORP9, and group VI includes ORP10 and ORP11 (without FFAT motif). These 12 members of the ORP family can produce 15 proteins from alternative splicing, including OSBP, ORP4L, ORP4S, OPR1L, ORP1S, ORP2, OPR3, ORP6, ORP7, ORP5, ORP8, ORP9L, OPR9S, ORP10, and

ORP11 [9, 10]. L represents “long” isoforms containing a PH domain while S represents “short” isoforms lacking this domain. OSBP/ORPs have the ability to bind to and transfer oxysterols, cholesterol or phospholipids, which can have an impact on lipid metabolism, cell signaling or vesicle trafficking [11, 12]. OSBP, being the founding member of this family, has been extensively studied to understand its role and function. Consequently, this review primarily focuses on OSBP, while also including the functions performed by other members of the ORP family to provide a comprehensive understanding.

2. Structure and lipid transport function of OSBP

Mammalian OSBP was first purified from the cytosol of hamster liver in the 1980s [10, 13, 14]. The OSBP gene, responsible for encoding a protein consisting of 807 amino acids, is located on chromosome 11 in humans and chromosome19 in mice [8]. The similarity between human and mouse OSBP genes is 94%, while the protein similarity is as high as 98% [15]. The OSBP protein contains an N-terminal intrinsically disordered region (N-ter) which is rich in glycine and alanine (Gly-Ala domain). This is followed by a PH domain, and an FFAT motif and an ORD domain at the C-terminus [9, 16, 17] (Fig. 1 A). The N-ter significantly increases the mobility rate of OSBP in the narrow MCS environment without affecting the affinity of adjacent PH domains for PI4P and the mechanism of lipid transfer. In order to prevent misorientation and crowding of OSBPs at MCS, the N-ter further restricts the orientation of OSBP by favoring heterotypic rather than homotypic membrane retention [3]. The PH domain interacts with phosphatidylinositol-4-phosphate (PI4P) to enable OSBP to target Golgi apparatus and other organelle membranes [18-20], and can also interact with GTPase ARF1 in the *trans*-Golgi

network (TGN) [21-23]. The FFAT motif can specifically interact with the major sperm protein (MSP) domain of vesicle-associated membrane protein–associated protein A/B (VAP-A/B) on the cytoplasmic surface of ER [9, 24, 25] (Fig. 1 A). The ligand binding region of the ORD domain contains a highly conserved fingerprint motif EQVSHHPP, a characteristic sequence of the OSBP/ORP family (Fig.1A) [16]. OSBP binds 25-hydroxycholesterol (25OHC) with high affinity (K_d of ~10 nM) and binds other oxysterols with lower affinity. An important aspect is that OSBP has a binding affinity for cholesterol, with a dissociation constant (Kd) of 170 nM. While OSBP primarily localizes to the cytosol or ER, it undergoes translocation to Golgi apparatus in response to cellular exposure to 25OHC.

OSBP facilitates the transport of cholesterol and PI4P in an exchange manner between ER and TGN in mammalian cells [24, 25] (Fig. 1 B). OSBP can be translocated to the MCS between ER and TGN, where it is attached to the ER membrane via the FFAT motif-bound VAP and to the TGN membrane via the PH domain. OSBP-ORD extracts and binds cholesterol from ER, the main site of cholesterol synthesis, and rapidly transports cholesterol to Golgi apparatus[26]. PI4P is synthesized in the TGN by the phosphatidylinositol (PI)-4 kinase IIα (PI4KIIα) and IIIβ (PI4KIIIβ) [27]. OSBP-ORD exchanges cholesterol with PI4P synthesized in the Golgi and transports PI4P back to the ER, where it is hydrolyzed to PI and inorganic phosphate by the phosphoinositide phosphatases Sac1. Thus, the PI4P gradient between Golgi apparatus and ER drives forward transport of cholesterol against its concentration gradient. Here, OSBP utilizes the metabolic energy released by PI4P hydrolysis to achieve the exchange of PI4P and cholesterol [25].

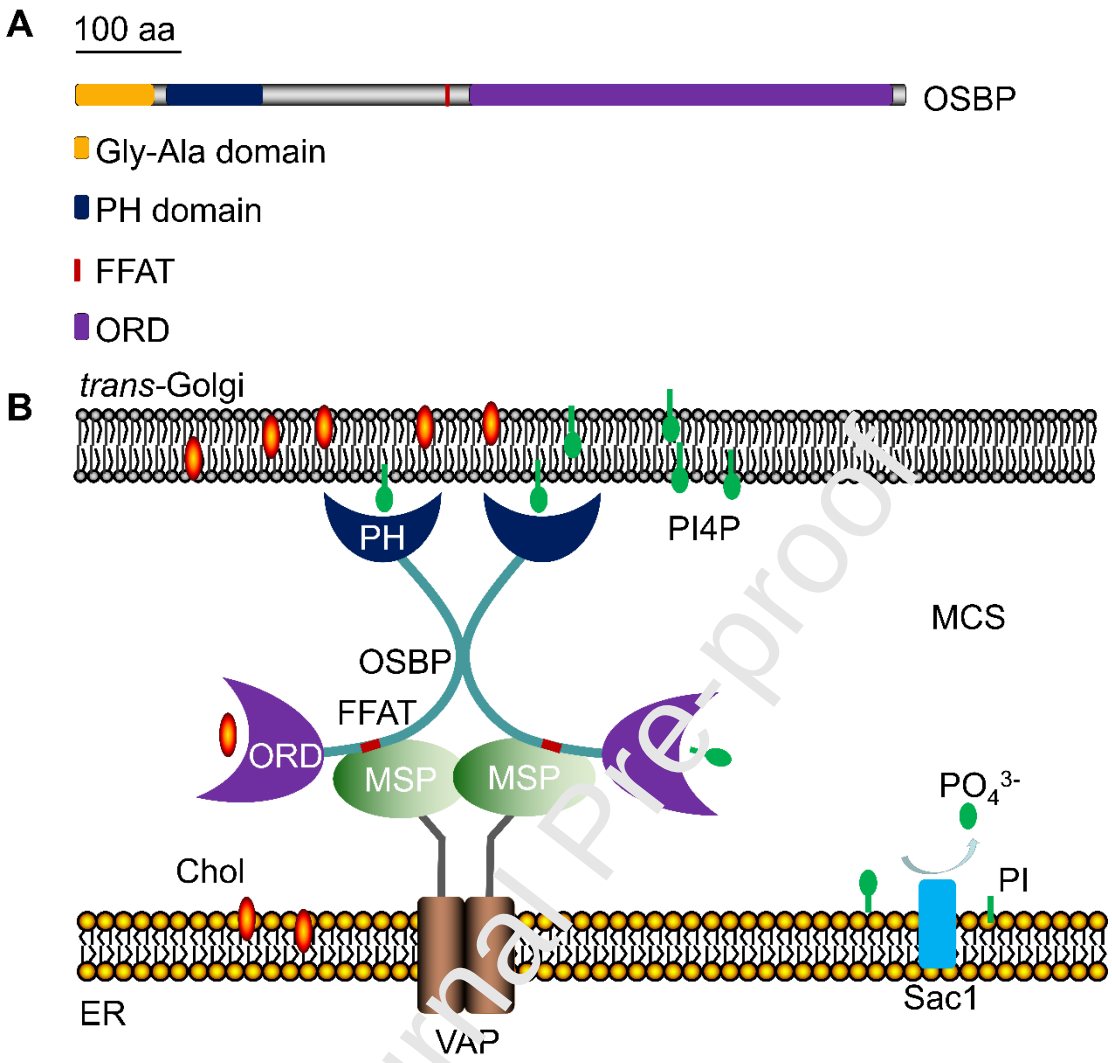


Fig.1 The schematic diagram and lipid transport model of OSBP in mammals. A, the schematic diagram of OSBP protein domains. Gly-Ala domain, glycine and alanine rich domain; PH, pleckstrin homology; FFAT, a two phenylalanines in an acidic tract; ORD, OSBP-related ligand-binding domain. B, a model for the function of OSBP at the ER–TGN MCSs. OSBP connects the ER and TGN by binding VAP of the ER and PI4P of the Golgi apparatus, and uses its ORD to extract cholesterol from the ER and transport it to the Golgi apparatus membrane. OSBP-ORD extracts PI4P from the Golgi apparatus and transports it to the ER. The PI4P retrogradely transported to the ER is hydrolyzed by Sac1 to produce PI and inorganic phosphate. Chol,

cholesterol; MCS, membrane contact sites; MSP, major sperm protein domain.

Additionally, OSBP functions as a lipid sensor in regulating sphingomyelin (SM) synthesis at the ER-TGN junction (Fig. 2). OSBP can facilitate ceramide transport from ER to Golgi by recruiting ceramide transfer protein (CERT) to increase the synthesis of SM [28]. CERT's structure is similar to OSBP: a PH domain at the N-terminus, an FFAT motif in the middle region, and a steroidogenic acute regulatory protein (StAR) related lipid transfer (START) domain which binds or transfers lipid at the C-terminus [29, 30]. The PH domain and VAPA are both indispensable in the process of OSBP regulating SM synthesis, which involves CERT recruitment by OSBP. The heteromeric complex formed by OSBP with CERT and VAP can increase the activity of CERT, but OSBP does not interact directly with CERT [28, 31]. At the same time, ARF recruitment by the PH domain of OSBP can stimulate PI4KIIIβ, which raises the local concentration of PI4P and recruits CERT to the Golgi apparatus [32]. Interestingly, OSBP Ser240 can be phosphorylated by protein kinase D (PKD) upon 25OHC or cholesterol depletion, and phosphorylation of OSBP attenuates its localization in the Golgi apparatus, resulting in a reduction of CERT located in the Golgi apparatus. This further leads to the fragmentation of Golgi apparatus [33]. Furthermore, CERT Ser132 can also be phosphorylated by PKD, impairing its ability to transport ceramides [34]. It has since been found that SM synthesis at TGNS, which is regulated by CERT and OSBP, is negatively regulated by sphingolipid (SL) metabolic flow. Capasso et al. found that CERT can transmit ceramide from ER to TGN to produce SM and DAG at low SL flow or SM synthesis [35]. With higher SL flow or increased SM synthesis, PKD

is activated by DAG, resulting in a transient increase of PI4P. OSBP phosphorylation by PKD then leads to re-translocation of PI4P to the ER and Sac1-dependent dephosphorylation of PI4P. When the levels of PI4P decrease, it reduces the ability of CERT to transport ceramide to TGN, leading to a decrease in SM production. This indicates that OSBP, CERT, PKD, Sac1 and PI4P form a negative feedback loop to maintain the constant lipid composition of the Golgi apparatus. The finding of Capasso et al. further confirm the report that phosphorylation of OSBP by PKD inhibits its localization to the TGN from the side. Hence, OSBP and CERT synergistically regulate the metabolism of cholesterol and SM in the ER-Golgi apparatus. In conclusion, these results suggest that OSBP exerts significant influence over the lipid composition of Golgi apparatus.

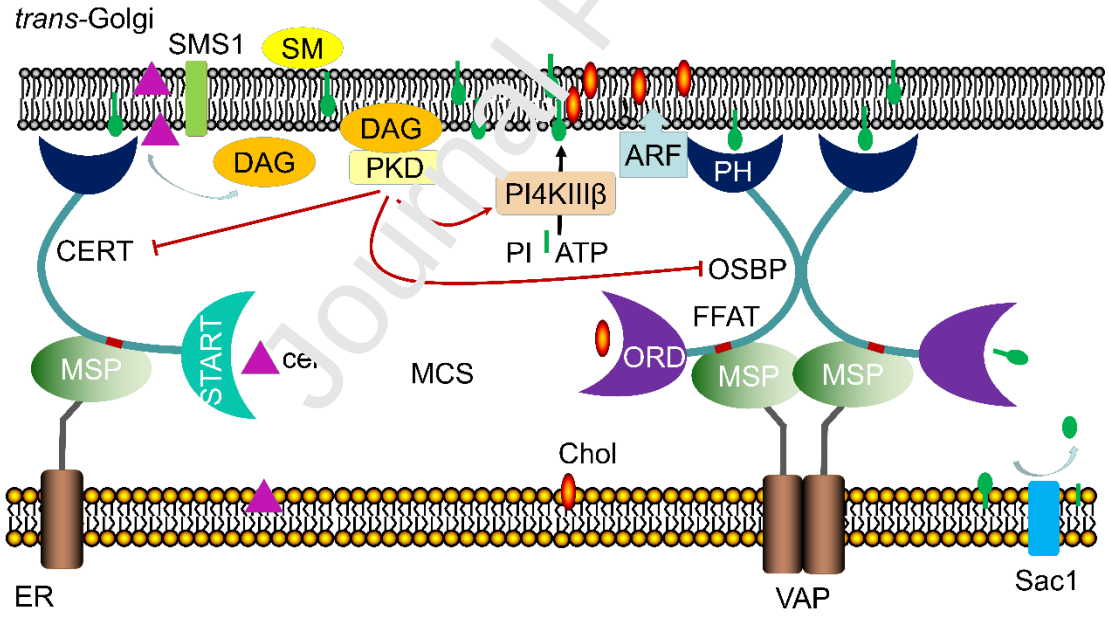


Fig. 2 OSBP regulates the synthesis of SM at the ER-TGN junction. OSBP and CERT associate with the TGN via binding of their PH domains to PI4P. The START domain of CERT promotes the transfer of ceramide from the ER to sphingomyelin synthase SMS1 located in Golgi

apparatus, resulting in SM synthesis in Golgi apparatus. The OSBP-mediated cholesterol transport from ER to TGN reduces the PI4P pool through the coupled reverse-transport of PI4 from TGN to ER. In addition, phosphorylation of OSBP and CERT by PKD decreased the production of SM and DAG. Note that, for simplicity, CERT is represented as a monomer in the schematic model, which is most likely to form homogeneous oligomers in the cells[36, 37] . Chol, cholesterol; cer, ceramide; DAG: diacylglycerol.

3. OSBP and diseases

Lipids contribute to the structural integrity of cell membranes and regulate energy metabolism, which is essential for whole-body function in mammals. Disorders of lipid balance can lead to various diseases [38-40]. OSBP, as a lipid sensing/transfer protein, is essential for lipid transport. It has been discovered that OSBP dysfunction may be involved in many diseases, such as fatty liver disease, diabetes, lysosome-related diseases, cancer and viral infections (Table 1).

Table 1. Roles of OSBP in diseases

Type of disease	**Roles**	**Target molecules or signaling pathways**	**References**
Fatty liver disease	Overexpression of OSBP up-regulates cholesterol synthesis and inhibits	NA	[41]

	cholesterol esterification.		
	OSBP promotes the synthesis of TG, increases the accumulation of lipid droplets in the liver, and upregulates the expression of fat synthesis-related genes.	The insulin signaling cascade	[42]
	Silencing of OSBP results in increased cholesterol efflux activity.	ATP-binding cassette transporter A1 (ABCA1)	[43, 44]
Diabetes	Silencing of OSBP increases cholesterol efflux activity, which in turn could affect insulin secretion and pancreatic β cells function.	ABCA1 and the insulin signaling cascade	[43-46]
	In a diabetic rat model, OSBP interacts with JAK2 and phosphorylation at Tyr-394 results in vascular endothelial dysfunction.	JAK2-STAT3	[47]
	Knockdown of OSBP in pancreatic β cells affects cholesterol transport from ER to TGN, and increases lysosomal degradation of young insulin granules and newly synthesized proinsulin.	The insulin secretory pathway	[48]
Niemann-Pick	OSBP in NPC1-deficient cells promotes	mechanistic	[49]

disease type C (NPC)	cholesterol transport to lysosomes, leading to accumulation of cholesterol on the limiting membranes of lysosomes and triggering autophagy defects.	Target of Rapamycin Complex 1 (mTORC1)	
Breast cancer	OSBP has no effect on proliferation.	NA	[50-53]
Human liposarcoma	OSBP promotes cell growth and inhibits apoptosis.	AKT	[54, 55]
Renal cancer	Favorable prognostic markers	NA	the Human Protein Atlas
Colorectal cancer	Favorable prognostic markers	NA	the Human Protein Atlas
Hepatitis C virus (HCV) infection	The OSBP-mediated cholesterol transport system facilitates viral RNA replication.	PI4KA	[56]
Poliovirus infection	The OSBP-mediated cholesterol transport system facilitates viral RNA replication.	PI4KIII β	[56]
Enterovirus infection	The OSBP-mediated cholesterol transport system facilitates viral RNA replication.	PI4KIII β	[57]
Rhinovirus infection	The OSBP-mediated cholesterol transport system facilitates viral RNA replication.	PI4KIII β	[58]
Aichi Virus infection	The OSBP-mediated cholesterol transport system facilitates viral RNA replication.	Independent of PI4P	[59]
Dengue virus	It is uncertain whether the replication of	Independent of	[52, 56, 60]

infection	viral RNA is dependent on OSBP.	PI4P	

3.1 OSBP and fatty liver

It was initially reported that overexpression of OSBP in Chinese hamster ovary (CHO)-K1 cells up-regulates cholesterol synthesis and inhibits cholesterol esterification, suggesting a potential role of OSBP in cholesterol homeostasis [41]. According to a study by Yan et al. (2007), overexpression of rabbit OSBP in mouse liver using adenovirus-mediated techniques resulted in increased synthesis of triglycerides (TG) and the accumulation of lipid droplets in the liver. Interestingly, this overexpression had no effect on cholesterol synthesis [42]. In addition, transcriptional regulator of fatty acid synthase sterol regulatory element-binding protein (SREBP)-1c and its target genes: acetyl-CoA (CoA) synthase (AceCS), fatty acid synthase (FAS), and stearoyl-CoA desaturase 1 (SCD-1) are upregulated upon OSBP overexpression [42]. In line with these findings, silencing of OSBP in cultured mouse hepatoma cells Hepa1-6 reduces the insulin-induced expression of SREBP-1c and FAS, as well as a reduction in TG synthesis [42]. These results indicate that OSBP plays a novel role in the regulation of TG metabolism and the insulin signaling cascade.

Inactivation of the mouse ATP-binding cassette transporter A1 (ABCA1) increases lipid storage in hepatocytes and leads to the accumulation of sterols in certain tissues [61]. However, ABCA1 does not appear to be associated with non-alcoholic fatty liver disease (NAFLD) [46]. Nonetheless, increased lipid and hepatic cholesterol deposition play a role in the progression of steatosis to nonalcoholic steatohepatitis (NASH) [62]. Vega - Badillo et al. (2016) provided evidence that hepatic microRNA (miR)-33a/144 and its target gene ABCA1 regulate hepatic free

cholesterol content and are associated with NASH [63]. OSBP negatively regulates ABCA1 activity in the cytoplasm by destabilizing ABCA1 through its ORD domain in CHO cells [44]. Silencing of OSBP results in elevated level of ABCA1 protein and increased cholesterol efflux activity [44]. Cadmium (Cd) also increases the stability of cholesterol efflux protein ABCA1 by inhibiting the lysosomal pathway. Conversely, it promotes the degradation of OSBP by enhancing ubiquitination, which promotes cholesterol redistribution and ultimately affects hepatic lipid metabolism [43]. This evidence establishes a clear association between OSBP and lipid metabolism, suggesting that OSBP may cooperate with ABCA1 in the context of fatty liver disease. However, further studies are required to elucidate the role of OSBP in liver disease, specifically its connection to NAFLD/NASH. Further investigation into the precise mechanisms by which OSBP regulates fatty acid and triglyceride synthesis is crucial for comprehending the transcriptional activation of lipid synthesis genes. Such research holds significant importance in identifying new drug targets for the prevention and treatment of liver diseases, including hepatitis and fatty liver.

3.2 OSBP and diabetes

Overloading or deficiency of cholesterol in cell membranes can lead to cellular dysfunction and diseases [64]. Elevated cholesterol levels in pancreatic β cells lead to disruption of pancreas islet function, reduce pancreas islet mass and decrease insulin secretion by interfering with the normal insulin secretory pathway. Pancreatic β-cell-specific cholesterol homeostasis thus plays a key role in insulin secretion and β-cell dysfunction in diabetes [65, 66]. ORP8, as a novel regulator of the insulin signaling system, can promote the activation of the insulin-AKT

signaling pathway, and affect reverse cholesterol transport by regulating ABCA1 expression and cholesterol efflux from macrophages [45, 46]. Interestingly, silencing of OSBP in CHO cells results in high expression of ABCA1 protein and increased cholesterol efflux activity [44]. However, the overall loss of ABCA1 function is not sufficient to cause type 2 diabetes (T2D), and it needs to cooperate with other factors to cause T2D [46]. Therefore, OSBP may regulate ABCA1 via unidentified mechanisms or cooperate with other factors to affect the insulin signaling pathway to regulate pancreatic β-cell cholesterol homeostasis, thereby participating in the occurrence of diabetes.

Romeo et al. discovered that the expression of the actin-binding protein proflin-1 is increased in diabetic endothelium and inhibition of profilin-1 prevents atherosclerosis [67]. Subsequently, they found that the JAK2/STAT3 pathway in aortic endothelial cells is activated in the diabetic macrovasculature, which is closely related to OSBP [47]. In this model, diabetes causes a conformational change in OSBP that allows OSBP to bind to JAK2 [47]. OSBP is subsequently phosphorylated by JAK2 at Tyr394. Then, STAT3 recognizes and binds to the YXXQ motif of OSBP through the Src homology 2 (SH2) domain, thus leading to STAT3 translocation to the nucleus through the non-canonical pathway. After STAT3 enters the nucleus, it binds to the promoter of profilin-1, up-regulates profilin-1 expression, which ultimately leads to diabetic endothelial dysfunction [47]. Phosphorylation of OSBP at Tyr-394 is required for STAT3 activation. This study confirms the role of OSBP in diabetic complications and lays the foundation for evaluating the effect of the OSBP/STAT3 pathway in models of diabetic vascular injury. However, the role of OSBP in pancreatic cholesterol homeostasis and pancreatic β cell

function was not addressed in this study.

In recent years, studies have gradually increased on cholesterol dynamics regulating the insulin secretory pathway, and it has been found that OSBP plays an important role in the formation of insulin granules. Knockdown of OSBP in pancreatic β cells stably expressing human proinsulin containing a superfolder green fluorescent protein insert in the C-peptide domain (hPro-CpepSfGFP) results in reduced cholesterol biosynthesis, cholesterol accumulation in ER, and decreasing cholesterol levels in TGN [48]. Furthermore, OSBP deficiency increases lysosomal degradation of young insulin granules and newly synthesized proinsulin, which cannot be rescued by the addition of exogenous cholesterol [48]. The findings indicate that OSBP plays a role in regulating the insulin secretory pathway, which relies on cholesterol transport from ER to TGN. As a cholesterol transporter influencing intracellular cholesterol levels and distribution, OSBP is expected to have a significant impact on pancreatic cholesterol homeostasis, β cell function, insulin resistance, and diabetes.

3.3 OSBP and lysosome-related diseases

Lysosomes are crucial for many cellular activities, and lysosomal dysfunction is usually closely related to diseases [68, 69]. Niemann-Pick disease type C (NPC) is mainly caused by the inactivation of the NPC1 and NPC2 genes [70, 71], which is characterized by lysosomal accumulation of cholesterol and increased lysosomal dysfunction. Lysosomal cholesterol levels are regulated by NPC1, which enables LDL-derived cholesterol to reach the limiting membrane of lysosomes. High cholesterol levels in this membrane activate the guanine nucleotide exchange factor of Rag GTases, which recruits mTORC to the lysosome surface and then initiates various

activities. In principle, luminal cholesterol derived from LDL cannot be transported to the limiting membrane of lysosomes to stimulate mechanistic Target of Rapamycin Complex 1 (mTORC1) activation when NPC1 is absent. In patients with NPC, there is a significant accumulation of cholesterol in the lumen and limiting membranes of lysosomes [70, 71]. This cholesterol accumulation results in the hyperactivation of mTORC1 at the lysosomes [72-74]. A recent study has shown that OSBP can transfer cholesterol from ER to the limiting membrane of lysosomes. In NPC disease, cholesterol provided by OSBP has the effect of activating the mTORC1 pathway. OSBP inactivation can rescue aberrant cholesterol-dependent mTORC1 signal transduction in NPC1-deficient cells and restore autophagy flux, leading to the clearance of p62, an autophagy adaptor protein [49]. Thus, OSBP may be an effective therapeutic target for correcting lysosome function and restoring NPC as well treatment for diseases driven by excessive mTORC1 signaling. Moreover, the involvement of OSBP in other diseases may also be related to its role in autophagy. Corroborating with the above observations, these functions of OSBP are dependent on its ability to bind cholesterol and PI4P as well as its ER-lysosomal localization, which is consistent with the model of OSBP transferring two lipids in opposite directions in ER-lysosomal MCS [18].

In addition, lysosomal membrane permeabilization (LMP) is a hallmark of lysosome-associated diseases. During the LMP, Ca^{2+} release from damaged lysosomes promotes the accumulation of PI4K2A on the lysosomes to produce large amounts of PI4P. Large amounts of PI4P accumulate on the lysosomes and promotes phosphatidylserine transport to the lysosomes for membrane repair by recruiting and stimulating ORPs family members ORP9/10/11 to

establish extensive membrane contact sites between ER and lysosomes [75]. Cholesterol can improve membrane rigidity and stability, and OSBP, acting as a membrane tether, can transport cholesterol to damaged lysosomes to assist membrane repair [75]. In their research, Radulovic et al. provided evidence supporting the significance of PI4K2A-mediated phosphatidylinositol 4-phosphate (PI4P) production in lysosome repair [76]. Additionally, they highlighted the essential role of cholesterol transfer mediated by ORP1L in lysosome damage repair [76]. While Radulovic et al. did not directly investigate the involvement of OSBP-mediated cholesterol transport in lysosome damage repair, they did observe that OSBP can remove excessive accumulation of PI4P on damaged lysosomes, thereby counteracting the damage response mediated by PI4K2A and preserving cellular viability [76]. These complementary studies collectively underscore the important function of OSBP in repairing lysosome damage. Consequently, targeting the regulation of OSBP holds promise as a potential strategy for restoring NPC protein homeostasis, enhancing lysosomal function, and treating lysosomal-related diseases, including NPC.

3.4 OSBP and Cancer

As the primary structural components of biological membranes and second messengers, lipids are crucial for cell signaling and development. The OSBP/ORPs family plays a key role in controlling cellular lipid trafficking, and abnormal expression of ORPs has been reported in many cancers, such as ORP3 in lymphoma and colorectal cancer [77, 78], ORP5 in renal cell carcinoma [79], ORP8 in hepatocellular carcinoma [80] and OSBP2 (also known as ORP4) in pancreatic ductal adenocarcinoma [81]. In addition, ORP4L is expressed in T-cell acute

lymphoblastic leukemia cells including leukemia stem cells and is essential for leukemia proliferation [82, 83].These suggest that ORPs may be important contributors to cancer development. In the presence of cholesterol, OSBP has been shown to form a phosphatase complex with PP2A and HePTP, two extracellular signal-regulated kinase (ERK) phosphatases, to regulate ERK1/2 activity [84]. Following cholesterol depletion or 25OHC treatment, the phosphatase complex dissociates, resulting in increased levels of ERK phosphorylation. Since the ERK signaling pathway is closely related to cancer development [85, 86], it is speculated that OSBP may play an important role in regulating cell signaling and cancer development.

More than a decade ago, the discovery of ORPphilins, a family of natural molecules, revealed their ability to inhibit the growth of human cancer cells through mechanisms distinct from other known anti-cancer compounds [50, 87, 88]. These findings have opened up new possibilities for potential cancer therapies. OSBP and ORP4L were identified as the targets of ORPphilins through affinity purification and related techniques [50]. However, it remains uncertain whether the impact of ORPphilins on cancer is solely based on their effect on OSBP and ORP4L. Nevertheless, the discovery of ORPphilins has provided valuable insights into the study and understanding of OSBP's role in cancer. One member of the ORPphilins family, the natural compound OSW-1, derived from the *Ornithogalum saudersiae* plant, exhibits a strong affinity for OSBP. OSW-1 inhibits the growth of cancer cells by binding to OSBP and preventing the transport of cholesterol to Golgi apparatus [50-52]. Intriguingly, transient treatment with OSW-1 leads to a significant reduction in OSBP protein levels, although the mechanism underlying this reduction remains unclear. Additionally, the OSW-1 analogue SBF-1 can reduce

the proliferation and adhesion of human liposarcoma cells by promoting the proteasomal degradation of OSBP, thereby inhibiting AKT phosphorylation [54]. Experiments involving ORPphilins and their analogues in cancer cells have demonstrated the association between OSBP and cancer.

Moreover, miR-195 inhibits cell proliferation and migration and promotes apoptosis by reducing OSBP expression in liposarcoma cells and tissues [55]. More importantly, OSBP overexpression reverses the effects of miR-195 on the cell growth, migration and apoptosis [55]. According to a recent study, OSBP translocates from Golgi to the PM where it co-localized with ORP4L via dimerizing with ORP4L. PI4P is transported from Golgi apparatus to PM as a result of this alteration in OSBP distribution, which promotes the synthesis of PI(4,5)P2 and PI(3,4,5)P3. The resulting increase in PI(3,4,5)P3 levels contributes to the overactivation of the PI3K/AKT oncogenic signaling and drives T cell deterioration [89]. These results seem to imply that OSBP, which functions as a lipid pump, might promote the occurrence and development of tumor.

However, the role of OSBP in cancer remains controversial. The Human Protein Atlas (https://www.proteinatlas.org/ENSG00000110048-OSBP/pathology) indicate that low OSBP expression is associated with poor prognosis in both renal and colorectal cancers. OSBP is a prognostic marker for both cancers. According to these data, OSBP function is tissue-specific, and the conflicting findings most likely reflect the complexity of OSBP function in different cell types and physiological contexts. Therefore, understanding how OSBP is involved in cancer is important because it could constitute an emerging class of drug targets for cancer treatment.

3.5 OSBP and virus infection

Infections caused by positive-sense single-stranded RNA virus are a widespread public health issue of great concern. The entry of these viral genomes into cells can cause encephalitis, hemorrhagic fever, gastroenteritis and respiratory diseases, etc. [90]. Positive-sense single-stranded RNA viruses replicate in the cytosol of host cells, using the membrane contact sites (MCSs) of the ER, Golgi and endosomal membranes as a replication platform, while utilizing lipid droplets and lipid metabolism of host cells as replication energy [91, 92]. Closely involved in the formation of the viral replication organelle (RO) in infected cells, OSBP is considered to be involved in the viral replication process.

Hepatitis C virus (HCV), a positive-sense single-stranded RNA virus, can cause an imbalance of lipid homeostasis in host cells during replication [24, 59]. The HCV nonstructural protein NS5A directly interacts with PI4KA in infected cells to generate PI4P, which subsequently triggers the recruitment of OSBP to the RO by binding to the N-terminal domain I of NS5A. OSBP removes PI4P from the RO by exchanging it with cholesterol, which elevates the cholesterol levels near the RO [56]. In order to prevent HCV replication, ORP4 negatively regulates the activity of NS5A in addition to OSBP [93], suggesting that members of ORP family other than OSBP also play a role in this process. Other RNA viruses, including poliovirus [56, 94], enterovirus [95] and rhinovirus [58], also can hijack the cholesterol transport system of OSBP, but they utilize different PI4K to generate PI4P on their RO. Unlike HCV, Aichi Virus hijacks this pathway by recruiting OSBP prior to PI4P through protein-protein interactions [59]. While dengue virus replication is also independent of PI4K, it is uncertain whether it depends on

OSBP [52, 56, 60]. These studies suggest that OSBP-mediated cholesterol transport system is essential for viral replication.

Small-molecule antagonists targeting OSBP have been proposed as a new strategy against viral infection. The natural compound OSW-1 binds to the ORD of OSBP and blocks its exchange activity, thereby inhibiting the replication of enteroviruses [52]. TTP-8307 and T-00127-HEV2 inhibit viral replication by altering OSBP localization [53, 96]. Additionally, the antifungal drug itraconazole (ITZ) binds OSBP to inhibit its ability to transport the cholesterol and PI4P, thereby inhibiting the RNA genome replication of enterovirus and HCV [57]. Posaconazole also affects cholesterol homeostasis by targeting OSBP to inhibit dengue and Zika virus replication [60]. Notably, among these small molecules, OSW-1 is the only OSBP-targeted compound with cell-preventive antiviral activity that also inhibits the growth of human cancer cell lines [53]. Due to its intracellular localization and lipid transport properties, OSBP has recently been hypothesized as a potential target for the treatment of Coronavirus Disease 2019 (COVID-19) [97, 98]. So far, the role of OSBP in coronavirus infection and the efficacy of therapeutic drugs targeting OSBP in COVID-19 have not been investigated. However, genetic screening based on genome-wide CRISPR (clustered regularly interspaced short palindromic repeats) reveals that ORP9L, but not OSBP, is the host factor for coronavirus infection in human Huh7 cell lines [99]. However, since the basis for this screening was cancer cells rather than normal cells, more research is required to verify whether OSBP plays a role in the host infection process of COVID-19.

4. Conclusion and Outlook

In conclusion the OSBP/ORPs family proteins have been established to be primarily involved in disease through their impact on phospholipids and cholesterol metabolism, facilitated by OSBP/ORPs interactions with other proteins. OSBP, as a member of the ORPs superfamily, plays a crucial role in cellular lipid transport and homeostasis. While dysfunctional OSBPs have been implicated in various diseases, the underlying mechanisms still remain largely unclear.

Unraveling the involvement of OSBP in tumorigenesis and its significance as a target for specific anti-tumor drugs requires further investigation. Furthermore, the direct relationship between OSBP's lipid transport activity and disease involvement warrants further research. Understanding the regulation of the OSBP cycle and associated mechanisms under physiological and pathological conditions presents a major challenge for future research. Detailed functional studies of OSBP hold promise for the development of novel therapeutic approaches for various diseases.

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6. Statement of Competing Interest

All authors have declared that there was no conflict of interest.

7. Author contributions

YW and HY provided outlines and guidance; YL, YW and HY wrote and revised the

manuscript; LR and XD reviewed and edited the manuscript. All authors have read and gave consent for the published version of the manuscript.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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