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COMMENTARY AND VIEWS

SQSTM1, lipid droplets and current state of their lipophagy affairs

Ankit Shroff and Taras Y. Nazarko

Department of Biology, Georgia State University, Atlanta, GA, USA

ABSTRACT

SQSTM1/p62 (sequestosome 1) is a well-established indicator of macroautophagic/autophagic flux. It was initially characterized as the ubiquitin-binding autophagic receptor in aggrephagy, the selective autophagy of ubiquitinated protein aggregates. Recently, several studies correlated its levels with the abundance of intracellular lipid droplets (LDs). In the absence of a bona fide receptor for the selective autophagy of LDs (lipophagy), a few studies demonstrated the role of SQSTM1 in lipophagy. Our analysis of these studies shows that SQSTM1 colocalizes with LDs, bridges them with phagophores, is co-degraded with them in the lysosomes, and affects LD abundance in a variety of cells and under diverse experimental conditions. Although only one study reported all these functions together, the overwhelming and complementary evidence from other studies suggests that the role of SQSTM1 in lipophagy via tagging, movement, aggregation/clustering and sequestration of LDs is rather a common phenomenon in mammalian cells. As ubiquitination of the LD-associated proteins under stress conditions is increasingly recognized as another common phenomenon, some other ubiquitin-binding autophagic receptors, such as NBR1 and OPTN, might soon join SQSTM1 on a list of the non-exclusive lipophagy receptors.

Abbreviations: LD: lipid droplet; LIR: LC3-interacting region; PAT: Perilipin, ADRP and TIP47 domain; SAR: selective autophagy receptor.

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SQSTM1/p62 (sequestosome 1) is a macroautophagic/autophagic receptor that bridges ubiquitinated proteins and the growing autophagic membrane, the phagophore, via its ubiquitin-associated (UBA) domain and LC3-interacting region (LIR). SQSTM1 was originally found to mediate aggrephagy, the selective autophagic degradation of ubiquitinated protein aggregates [1,2]. It was the first example of receptor-mediated selective autophagy in mammals making SQSTM1 the first mammalian selective autophagy receptor (SAR). Later, SQSTM1 was found to oligomerize via its Phox and Bem1 (PB1) domain and result in aggregation of its substrates. The roles of the SQSTM1 receptor in autophagy and the ubiquitin-proteasome system have been reviewed [3,4]. Importantly, SQSTM1 itself is an autophagic substrate and accumulates when autophagy is blocked. Therefore, its levels, in combination with the levels of lipidated MAP1LC3B (microtubule associated protein 1 light chain 3 beta), which reports about the abundance of autophagosomes, can be used to measure autophagic flux [5]. Since 2009, SQSTM1 was used as an autophagic marker in numerous studies with some of them also showing the accumulation of both SQSTM1 and intracellular lipid droplets (LDs). Like SQSTM1, these lipid storage organelles can be degraded by selective autophagy (lipophagy [6]; reviewed in [7–12]) and not only by cytosolic lipolysis. Interestingly, some cytosolic lipases that act on LDs, such as PNPLA2/ATGL (patatin like phospholipase domain containing 2) and LIPE/HSL (lipase E, hormone sensitive type), have multiple putative LIRs [13] and are proposed to act as lipophagy receptors [14]. However, they bind mostly to the cytosolic (not lumenal) face of autophagosomes [13]. Moreover, PNPLA2 localizes to LDs in a LIR-dependent manner [13] suggesting that autophagic membranes mediate the recruitment of these cytosolic lipases to LDs and not *vice versa*. Therefore, a question of the LD-specific SAR remains open and of great interest to the field. Because a few recent studies suggested the involvement of SQSTM1 in lipophagy, we reviewed this one aspect of SQSTM1 function and evaluated a possibility of SQSTM1 acting as a non-exclusive lipophagy receptor.

To be considered a SAR, a protein should meet several requirements: 1) it must colocalize and physically interact with its substrate, 2) it has to bridge its substrate with the phagophore, 3) it must be degraded together with its substrate in the lysosome, and 4) lack or overexpression of this protein should result in accumulation or disappearance of its substrate in the cytosol, respectively. We applied these criteria to SQSTM1 in relation to LDs and summarized our findings in Table 1. Several studies observed colocalization of SQSTM1 with LDs in different models and under a variety of LD induction conditions [15–19]. The recruitment of SQSTM1 to LDs is mediated by the LD-associated protein, PLIN1 (perilipin 1) [17]. Because LDs are ubiquitinated under those conditions, it is not clear if PLIN1 recruits SQSTM1 directly or via ubiquitin. Interestingly, SQSTM1 co-immunoprecipitates with PLIN1 [20] and two other LD proteins, PLIN2/ADRP (perilipin 2) [15] and PLA2G4A/cPLA2-alpha (phospholipase A2 group IVA) [18]. However, the nature of these interactions was not clarified. Because ubiquitination of LDs is rather a common phenomenon in mammalian cells [17,19], these interactions might have been mediated by

CONTACT Taras Y. Nazarko tnazarko@gsu.edu Department of Biology, Georgia State University, P.O. Box 4010, Atlanta, GA 30303-4010, USA

Table 1. The summary of SQSTM1 and LD relations regarding the role of SQSTM1 as a lipophagy SAR.

		SQSTM1				
Model	LD inducer	Does it colocalize with LDs?	Does it bridge LDs and phagophores?	Is it co-degraded with LDs in lysosomes?	Does it affect LD abundance?	Ref
Rat L6 myocytes	Oleate and palmitate	Yes, it colocalizes with LDs; also, it co-IPs* with PLIN2	n/a	Yes (modulated by Rapa and BafA1)	n/a	[15]
Male Wistar rat livers	Ethanol	Yes, it cofractionates with LDs, especially on ethanol diet	Maybe; it cofractionates with MAP1LC3B on LDs	n/a	n/a	[16]
Mouse AML12 hepatocytes	Ethanol	Yes, it colocalizes with LDs via PLIN1; also, it colocalizes with PLIN1 and Ub on LDs	Yes, it is required (together with PLIN1) for LDs-MAP1LC3B colocalization	Yes (modulated by Rapa, CBZ, 3-MA, Wort, CQ and ATG5)	Yes, its KD leads to lipid accumulation	[17]
Human C13, HeyA8MDR and OVCAR5 cells	Increased p-PFKFB3	Yes, it colocalizes with LDs and PLA2G4A; also, it co-IPs with PLA2G4A	n/a	Yes (modulated by PFK158 and BafA1)	n/a	[18]
Human THP-1 and mouse peritoneal or bone marrow-derived macrophages	agLDL or oleate	Yes, it colocalizes with LDs; Ub also colocalizes with LDs	Maybe; it colocalizes with MAP1LC3B on LDs	n/a	Maybe; its KD reduces cholesterol efflux	[19]
Human HepG2 cells	Oleate	n/a	Yes, its LIR is required for LD degradation	n/a	Yes, its OE promotes LD degradation	[25]
Human H4 cells	None	No, but it forms contact sites with LDs via DYNLRB1 and moves together with LDs	n/a	n/a	Yes, its OE promotes LD degradation	[27]

*3-MA: 3-methyladenine; agLDL: aggregated low-density lipoprotein; BafA1: bafilomycin A_1; CBZ: carbamazepine; co-IP: co-immunoprecipitate; CQ: chloroquine; KD: knockdown; OE: overexpression; Rapa: rapamycin; Ub: ubiquitin; Wort: wortmannin.

ubiquitin. Indeed, both PLIN1 and PLIN2 are polyubiquitinated and degraded by the proteasome in the delipidated culture medium [21,22]. Also, PLIN1 is polyubiquitinated [20] and degraded by the autophagy-lysosomal pathway induced with TNF (tumor necrosis factor) [20] or nelfinavir [23]. In addition, lipophagy in mammalian cells depends on HTT (huntingtin), the selective autophagy scaffold that binds SQSTM1 and promotes its attachment to (1) polyubiquitinated proteins with the K63-linked ubiquitin chains and (2) MAP1LC3B [24]. Therefore, it seems very likely that the SQSTM1-LD interactions are at least partially mediated by ubiquitin (Figure 1).

SQSTM1 also satisfies the second requirement of a lipophagy SAR by bridging LDs with the phagophore. It has been shown that both PLIN1 and SQSTM1 are required for colocalization of LDs with the phagophore/autophagosome marker, MAP1LC3B [17]. In addition, SQSTM1 and MAP1LC3B were found to cofractionate [16] and colocalize [19] with each other on LDs. As mentioned above, the SQSTM1-MAP1LC3B interaction is facilitated by the HTT scaffold [24]. Also, it has been established that the LIR motif of SQSTM1, which is responsible for the SQSTM1-MAP1LC3B binding, is required for LD degradation upon SQSTM1 overexpression [25]. However, it might not be just a simple bridging of individual LDs and phagophores. Using its UBA and PB1 domains, SQSTM1 can form the LD-containing aggresomes rich in HDAC6 (histone deacetylase 6). HDAC6 is recruited to the aggresomes via its binder of ubiquitin zinc finger (BUZ) domain and this recruitment is required for LD degradation upon SQSTM1 overexpression [25]. In conclusion, SQSTM1 does bridge the LDs or LD-containing aggresomes with the phagophores.

But is SQSTM1 co-degraded with LDs in the autophagy-lysosomal pathway, as is the case with other SARs? To study this, autophagy was modulated either pharmacologically or genetically and the effects on SQSTM1 and LDs were monitored [15,17,18]. Activation of autophagy with rapamycin [15,17], carbamazepine [17] or PFK158 [18] causes the decline

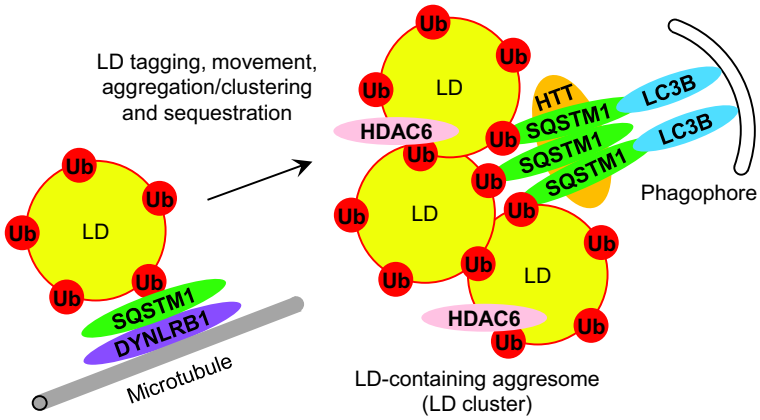

Figure 1. The role of SQSTM1 in lipophagy via tagging, movement, aggregation/clustering and sequestration of LDs. See text for details. LC3B: MAP1LC3B; Ub: ubiquitin.

in both SQSTM1 and LDs. The opposite is true when autophagy is inhibited with bafilomycin A_1 [15,18], 3-methyladenine, wortmannin, chloroquine or knockdown of *ATG5* (autophagy related 5) [17]: SQSTM1 and LDs accumulate. The co-accumulation of SQSTM1 and LDs in the presence of autophagosome-lysosome fusion inhibitors, bafilomycin A_1 and chloroquine, suggests that they are indeed co-degraded in the lysosomes. Thus, SQSTM1 meets the third requirement of a lipophagy SAR.

The forth criterium (role in lipophagy) is also important, because as we have recently shown, the ubiquitin-binding SARs, such as Cue5 in yeast, can (1) accumulate on LDs in a ubiquitin-dependent manner, and (2) follow LDs to the lytic compartment for degradation, but be dispensable for it [26]. Therefore, it is essential to test if the LD-associated SAR is indeed "driving" lipophagy, and is not just one of many LD "passengers". Importantly, the knockdown of *SQSTM1* does increase lipid abundance [17] and decreases the lipophagy-dependent cholesterol efflux [19]. In addition, overexpression of *SQSTM1* promotes LD degradation [25,27]. Interestingly, the knockdown or overexpression of *DYNLRB1* (dynein light chain roadblock-type 1) has the same effect on LD abundance as the knockdown or overexpression of *SQSTM1* [27] suggesting that SQSTM1 might cooperate with its binding partner, the dynein motor complex, for LD movement and degradation.

In conclusion, various studies showed that SQSTM1 has all the characteristics of a lipophagy SAR (Table 1). Although the four requirements of a SAR together were addressed for SQSTM1 only in a single study [17], the cumulative evidence from other studies suggests that SQSTM1 might act as a lipophagy receptor in different mammalian cells and under a variety of experimental conditions. Moreover, when fused to the LD-binding PAT (Perilipin, ADRP and TIP47) domain of PLIN3/TIP47 (perilipin 3) the $SQSTM1^{T352A}$ variant with disabled KEAP1 (kelch like ECH associated protein 1) binding induces lipophagy in both mammalian cells and fertilized mouse embryos [28]. The PAT-$SQSTM1^{T352A}$ fusion also causes LD clustering and their movement to the cell periphery during early embryonic development. Surprisingly, the non-mutated SQSTM1 without PAT domain does not localize to LDs or cause these effects, but the PAT domain itself also fails to localize to LDs during embryonic development [28]. Therefore, it is not clear how the PAT-$SQSTM1^{T352A}$ fusion localizes to LDs and drives lipophagy. Nevertheless, the studies discussed in this article implicate SQSTM1 in LD tagging, movement, aggregation/clustering, and sequestration (Figure 1). However, more *in vivo* work is needed to strengthen this conclusion.

This paradigm is consistent with other findings in the field, e.g. (1) accumulation of PLIN2 and PLIN3 in the mouse embryonic fibroblasts lacking NFKB1/p50 (nuclear factor kappa B subunit 1) due to reduced expression of SQSTM1 [29] and (2) overaccumulation of LDs in the mouse primary adipocytes lacking EIF2A (eukaryotic translation initiation factor 2A) due to their inability to express SQSTM1 upon differentiation [30]. Excitingly, the knockdown of some other ubiquitin-binding SARs, such as NBR1 (NBR1 autophagy cargo receptor) and OPTN (optineurin), also reduces the lipophagy-driven cholesterol efflux [19]. Moreover, both NBR1 and OPTN colocalize with ubiquitin on LDs suggesting that SQSTM1 might not be the only autophagic receptor that has lipophagy affairs with LDs. It will be interesting to see if these other ubiquitin-binding receptors also satisfy all the criteria of a lipophagy SAR.

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ORCID

Ankit Shroff http://orcid.org/0000-0001-7612-1122
Taras Y. Nazarko http://orcid.org/0000-0001-5322-8496

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