

Lysosomal Solute Carrier Transporters Gain Momentum in Research

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Emerging evidence indicates that lysosome function extends beyond macromolecular degradation. Genetic and functional defects in components of the lysosomal transport machinery cause lysosomal storage disorders implicating the lysosomal solute carrier (SLC) transporters as essential to vital cell processes. The pathophysiology and therapeutic potential of lysosomal SLC transporters are highlighted here, focusing on recent discoveries in autophagic amino acid sensing (SLC38A9), phagocytic regulation in macrophages (SLC29A3, SLC15A3/A4), adenosine triphosphate (ATP) exocytosis in neurotransmission (SLC17A9), and lysosomal transport of mianserin catabolites into the cytoplasm (SLC46A3).

LYSOSOMAL SLC TRANSPORTERS IN HEALTH AND DISEASE

Solute carrier (SLC) transporters are increasingly recognized as essential for ion, molecule, and other solute transport to support lysosomal function. To date, more than 30 lysosomal SLC transporters have been genetically identified, molecularly characterized, and investigated for a role in health and disease. Despite these advances, there remain lysosomal transport systems for which the molecular identity is obscure, and, conversely, orphan lysosomal SLC transporters with functional relevance unresolved. As the scope of lysosomal SLC transporters is broad, expression, functional characteristics, and disease associations for known family members are summarized in **Table 1**. Instead, we focus on recent discoveries in the lysosomal SLC transporter field that provide novel mechanistic insight into autophagy, phagocytosis, and exocytosis processes (**Figure 1**).

Distinct amino acid transport systems exist in the lysosomal membrane to transit cystine, cysteine, cationic amino acids, dicarboxylic amino acids, and small and large neutral amino acids, as elucidated over the last 3 decades. Similarly, the process of autophagy – lysosomal degradation of defective intracellular material during cellular stress – was recognized well over 3

decades ago. Yet, the mechanism for precisely relaying cellular nutrient status to lysosomes to regulate the autophagy process was unclear. Previous work predicted that the species and quantity of amino acids inside the lysosomal lumen were somehow sensed by the lysosome to allow docking of a master regulator of autophagy (mammalian target of rapamycin complex 1 [mTORC1]) to the lysosomal surface to initiate an autophagy signal. However, this putative lysosomal nutrient sensor for mTORC1 remained unresolved. Three groups have now independently discovered SLC38A9 as an arginine sensor at the lysosomal membrane, identifying a central role for this transporter in regulating the steps preceding autophagy.

SLC38A9 is an 11-pass lysosomal transmembrane protein and a low capacity neutral amino acid transporter that directly interacts with Ragulator–Ragulator GTPases conveying the nutrient status of the cell to the mTORC1 complex.¹ The initiation and formation of double-membraned autophagosomes tightly links mTORC1 regulation to this nutrient-sensing mechanism. Thus, identification of SLC38A9 as an amino acid sensor was a critical step to understanding lysosomal signaling processes in autophagy. It is not yet known whether additional amino acid sensors or analogous sensors for sugars, lipids, or nucleotides exist within or outside lysosomes, but these discoveries drive the quest to find new sensors for autophagy initiation and termination. Moreover, modulation of lysosomal SLC38A9 transporters may offer a new therapeutic intervention in neurodegenerative and aging disorders in which autophagy is perturbed.

Lysosomes also serve as the terminal compartment for cellular endocytic machinery and degradation of endocytosed material. Besides other functions, phagocytosis is essential for internalizing pathogens for degradation and for orchestrating immune responses. The handling of pathogens by dendritic cells for the engagement of adaptive immune response is intricately tied to endolysosomal function. Among lysosomal SLC transporters known to facilitate this process, the endolysosomal peptide transporters, SLC15A3 and SLC15A4, in dendritic cells are reported

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Table 1 Major lysosomal solute carrier transporters

Gene name	Protein name	Substrate	Km	Localization	Tissue with high expression		Cellular function	Associated diseases	References (Pubmed ID)
SLC1A2	EAAT2	L-glutamate, L and D-aspartate	52.7 ± 7.5 μM for L-glutamate	Plasma membrane, Endo-lysosomes	Brain (Astrocytes)		Maintains glutamate clearance for synaptic function	Schizophrenia	9539131 (1998); 10602327 (1999); 25064045 (2015)
SLC2A8	GLUT8	D-Glucose, D-Fructose	2 mM for Glucose	Endo-Lysosomes, Endoplasmic Reticulum	Testes, Brain, Liver, Lung		Regulates facilitative glucose transport	-	10821868 (2000); 19523115 (2009); 22822162 (2012)
SLC7A14	CAT	Cationic L-amino acids	1.9 ± 0.2 μM for Arginine	Lysosomes, Plasma membrane	Brain (mRNA expression)		Mediates lysosomal uptake of cationic amino acids	Autosomal Recessive Retinitis Pigmentosa	22787143 (2012); 24670872 (2014)
SLC9A6	NHE6	Na ⁺ /H ⁺ (Exchanger)	5 to 50 mM for Na ⁺	Endosomes, Mitochondria	Brain, Skeletal muscle, heart		Maintains pH homeostasis in organelles	Angelman Syndrome and Christianson Subtype	9507001 (1998); 11940519 (2002); 26515654 (2016)
SLC11A1	NRAMP1	Mn ²⁺ , Fe ²⁺ other divalent ions	4 μM for Fe ²⁺	Phagolysosomes	Macrophages, Neutrophils		Regulates iron homeostasis and host resistance to certain pathogens	Mycobacterium Tuberculosis	7964458 (1994); 19500110 (2009); 26353180 (2015)
SLC11A2	DMT1	Fe ²⁺ , Cd ²⁺ , Co ²⁺ , Cu ²⁺ , Mn ²⁺ , Ni ²⁺ , Pb ²⁺ , Zn ²⁺	3 μM for Fe ²⁺ ; 1 μM for Mn ²⁺ ; 1 μM for Cd ²⁺	Endo-Lysosomes, Plasma membrane	Ubiquitous, Duodenal enterocytes, Erythroid cells, Kidney		Mediates iron transport from endosomes to cytosol of duodenal enterocytes	Microcytic Anemia	16737442 (2006); 22313374 (2012)
SLC12A9	CIP1	Cation Cl ⁻ (cotransporter)	-	Lysosomes, Plasma membrane	Ubiquitous		Regulates intracellular chloride concentration	Bartter disease	10871601 (2000); 23325410 (2013); 24802699 (2014)
SLC15A3	PHT2	Di and Tripeptides, Histidine	1.3 ± 0.5 μM for dipeptides, 43 μM for Histidine	Endo-Lysosomes, Vesicles	Intestine, Spleen, Lung, Thymus		Transports histidine and oligopeptides from inside the lysosome to the cytosol	-	11741232 (2000); 24695226 (2014); 24754256 (2014)
SLC15A4	PHT1	Di and Tripeptides, Histidine	17 μM for Histidine	Endo-Lysosomes, Vesicles	Intestine, Eye, Brain		Transports histidine and oligopeptides from inside the lysosome to the cytosol	SLE	9092568 (1997); 25238095 (2014); 25310967 (2015)
SLC16A12	MCT12	Monocarboxylic Acid, Creatine	567.4 μM for Creatine	Plasma membrane, Vesicles	Retina, Lung, Kidney, Testes		Transports monocarboxylic acids and creatine	Cataract and Glucosuria	18304496 (2008); 23578822 (2013)
SLC17A5	Sialin	Sialic Acid, L-Aspartate, L-Glutamate	4 ± 2 mM for N-acetyl Neuraminic acid; 5 ± 2mM for Glucuronic acid	Lysosomes, Plasma membrane	Ubiquitous		Transports sialic acid and glucuronic acid out of lysosomes	Salla Disease	10581036 (1999); 20424173 (2010); 23889254 (2013); 23900835 (2014)

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Gene name	Protein name	Substrate	Km	Localization	Tissue with high expression	Cellular function	Associated diseases	References (Pubmed ID)
SLC17A9	VNUT	Purine Nucleotides	0.61mM for ATP	Lysosomes, Secretory Vesicles	Brain (Neurons), Adrenal, Thyroid	Regulates vesicular ATP storage and exocytosis	Prokeratosis8	22052906 (2008); 18375752 (2008); 25596766 (2015)
SLC26A11	KBAT	HCO ₃ ⁻ , Cl ⁻ , SO ₄ ²⁻	-	Lysosomes, Plasma membrane	Endothelial cells, Renal cells, Brain	Regulates intracellular electrolyte balance	Chronic Myeloid Leukemia	12626430 (2003); 17002596 (2007); 25910210 (2015)
SLC29A3	ENT3	Nucleosides	1.9 ± 0.3 mM for Adenosine; 2.0 ± 0.4mM for Uridine	Endo-Lysosomes	Ubiquitous	Mediates nucleoside efflux across lysosome membrane	H Syndrome; PHID; Rosai Dorfman disease	15701636 (2005); 20595384 (2010); 22174130 (2012); 24535606 (2014)
SLC30A2	ZnT2	Zn ²⁺	14-16 μM for Zn ²⁺	Vesicles, Lysosomes, Plasma membrane	Breast, Pancreas, Kidney, Testis, Small intestine	Functions to secrete zinc into breast milk	Acrodermatitis Enteropathica	8617223 (1996); 9655614 (1998); 24456035 (2014)
SLC31A1	CTR1	Cu ²⁺	3.6 ± 0.8 μM for Copper	Endo-Lysosomes	Ubiquitous (mRNA expression)	Facilitates copper transport in late endosomes and lysosomes	Wilson disease	26945057 (2016); 12034741 (2002); 9207117 (1997)
SLC31A2	CTR2	Cu ²⁺	11.0 ± 2.5 μM for Copper	Plasma membrane, Endosomes	Gastro-intestinal Tract, Ubiquitous	Mediates low affinity copper uptake	-	17617060 (2007); 24522273 (2014)
SLC32A1	VIAAT	GABA, L-Glycine	0.8 mM for GABA; 2.8 mM for Glycine	Vesicles	CNS tissues	Mediates GABA and glycine uptake in synaptic vesicles	Hyperexcitability Disorders	12115694 (2002); 19843525 (2009); 25749864 (2015)
SLC36A1	PAT1/LYAAT1	Glycine, Alanine, Proline, Serine, Alanine, GABA	7.0 ± 0.7 mM for Glycine; 7.5 ± 0.6 mM for L-Alanine; 2.8 ± 0.1 mM for L-Proline; 69 ± 5 mM for L-Serine; 6.3 ± 0.7 mM for D-Alanine; 3.1 ± 0.2 mM for GABA	Lysosomes, Plasma membrane, Endoplasmic Reticulum	Brain, Intestine, Kidney, Spleen	Transports small amino acids L-Glycine, L-Alanine and L-Proline across lysosomes	Iminoglycinuria Digenic	12893527 (2003); 16373326 (2005); 25880931 (2015)
SLC37A2	SPX2	Glucose-6-Phosphate (G6P)	-	Plasma membrane, Endoplasmic Reticulum	Ubiquitous	Functions as phosphate-linked G6p antiporter	-	17356011 (2007); 21949678 (2011)
SLC38A7	SNAT7	Neutral L-Amino acids	-	Endo-lysosomes	Brain, Liver, Muscle Uterus	Regulates L-glutamine transport in neurons	-	18418736 (2008); 21511949 (2011)
SLC38A9		Low Capacity neutral L-Amino acid transporter	39mM for Arginine	Lysosomes, Late endosomes	Ubiquitous	Acts as a sensor for L-arginine in lysosomes	Upregulated in Lung Cancer	25567906 (2015); 25963655 (2015); 25561175 (2015)

Table 1 Continued on next page

Table 1 Continued

Gene name	Protein name	Substrate	Km	Localization	Tissue with high expression	Cellular function	Associated diseases	References (Pubmed ID)
SLC39A8	ZIP8	Zn ²⁺	0.3 μM for Zn ²⁺ ; 0.5 μM for Cd ²⁺	Vesicles, Plasma membrane	Ubiquitous	Regulates zinc influx	-	18270315 (2008); 26637978 (2015)
SLC40A1	FPN1	Fe ²⁺	-	Lysosomes, Plasma membrane	Macrophages, Liver, Duodenum,	Mediates iron export from duodenal epithelial cells	Hemochromatosis	10693807 (2000); 15114483 (2004); 24304836 (2014); 26059880 (2015)
SLC45A2	MATP	Uncharacterized, transports substances for melanin synthesis	-	Melanosomes, Plasma membrane	Melanocytes, Skin, Eye	Regulates melanosome Ph	Oculocutaneous Albinism Type IV	26016411 (2015); 26057890 (2015)
SLC46A3	FKSG16	Uncharacterized	-	Lysosomes, Plasma membrane	Kidney, Liver, Placenta	Mediates antibody drug conjugate-maytansine efflux from lysosomes	-	26631267 (2015)
SLC48A1	HRG-1	Heme	125 μM	Endo-Lysosomes	Liver, Heart, Muscle, Small Intestine	Regulates intracellular heme availability through endo-lysosomal compartment	-	16143108 (2005); 18418376 (2008); 23395172 (2013)

CAT1, cationic amino acid transporter 1; CIP1, cation-chloride cotransporter-interacting protein 1; CTR2, copper transporter 2; DMT1, divalent metal transporter 1; EAAT2, excitatory amino-acid transporter 2; ENT3, equilibrative nucleoside transporter; FPN1, ferroportin1; Glut8, glucose transporter 8; HRG-1, heme-responsive gene 1; KBAT, kidney brain anion transporter; K_{an}, apparent substrate affinity LYAAT1, lysosomal amino acid transporter 1; MATP, membrane-associated transporter protein; MCT12, monocarboxylate transporter 12; NHE6, sodium hydrogen exchanger; NRAMP1, natural resistance-associated macrophage protein 1; PAT1, proton/amino acid transporter 1; PHT1, peptide histidine transporter 1; PHT2, peptide histidine transporter 2; SNAT7, sodium-coupled neutral amino acid transporter 7; SPX2, sugar phosphate exchanger 2; URLC11, upregulated in lung cancer 11; VIAAT, vesicular inhibitory amino acid transporter; VNUT, vesicular nucleotide transporter; ZIP8, zinc transporter 8; ZnT2, zinc transporter 2.

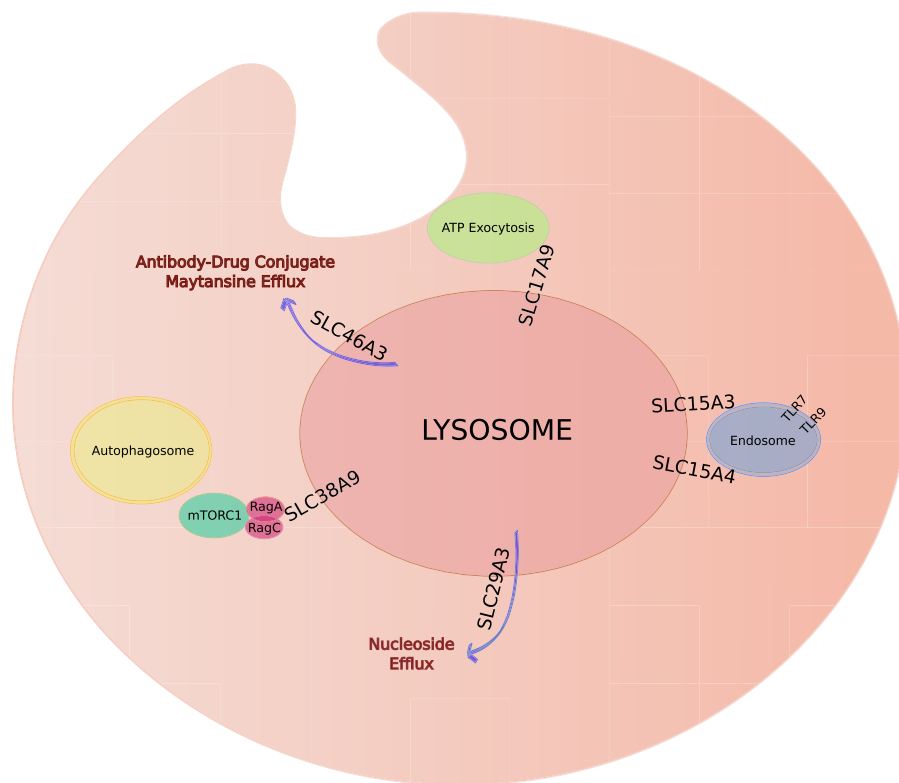


Figure 1 A schematic model of the lysosomal solute carrier (SLC)ome: emerging roles in cellular pathophysiology and pharmacology. A multitude of lysosomal SLCs across lysosomal membrane is involved in the lysosomal transport of solutes. Significant advances in understanding the lysosome proteome has revealed novel roles for lysosomal SLCs, including SLC38A9 as an amino acid sensor (by direct interaction with mTOR complex), SLC17A9 as a mediator of adenosine triphosphate (ATP) exocytosis, SLC15A3/A4 as a participant in toll-like receptor (TLR) signaling, SLC29A3 as a regulator of nucleoside salvage, and SLC46A3 as a facilitator of antibody-drug conjugate maytansine efflux. mTOR, mammalian target of Rapamycin; RagA, Ragulator A; RagC, Ragulator C.

to mediate the intracellular sensing of pathogens after toll-like receptor stimulation.² Both transporters assist in the egress of bacterially derived components, particularly muramyl dipeptide, to facilitate the NOD2-mediated immune response. Consistently, single nucleotide polymorphism of SLC15A4 is found to associate with autoimmune disease systemic lupus erythematosus. Similarly, SLC11A1 (formerly *Nramp1*), a divalent metal transporter, is reported in dynamic host-pathogen interactions essential for conferring resistance to certain pathogens. Mutations in SLC11A1 associate with infectious (e.g., tuberculosis, leprosy) and inflammatory (e.g., rheumatoid arthritis) diseases.

Recently, the role of an acidic pH-dependent lysosomal nucleoside transporter, SLC29A3, has been linked to macrophage phagocytic function.³ SLC29A3 is predicted to salvage lysosomal nucleobases, nucleosides, and nucleotides, presumably derived from encapsulated pathogens and host macrophage-derived nucleic acids. Accumulating evidence reveals that SLC29A3 mutations can cause a spectrum of human genetic disorders due to abnormal lysosomal nucleoside buildup and increased intralysosomal pH. These diseases include: H syndrome (progressive scleroderma, hyperpigmentation, hypertrichosis, facial telangiectases and dermal and subcutaneous fibrosis); pigmented hypertrichotic dermatosis and insulin-dependent diabetes syndrome; familial Rosai-Dorfman disease; familial histiocytosis; and sinus

histiocytosis with massive lymphadenopathy. SLC29A3 spectrum disorders are allelic, share common mutation(s), and share overlapping manifestations that display an intriguing resemblance to lysosomal storage disorders. Although the basis of these monogenic disorders is only beginning to be appreciated, further studies on the involvement of SLC29A3 in lysosomal and cellular homeostasis should clarify the role of macrophages and other cell types in the pathogenesis of SLC29A3 spectrum disorders.

The lysosome participates in membrane trafficking and exocytosis, wherein the lysosome expels its cargo outside the cell. Lysosomal exocytosis is also important in plasma membrane repair and cholesterol homeostasis. Deficiency of lysosomal proteins in Niemann–Pick type C1 disease or Niemann–Pick type C2 diminish cholesterol efflux from the lysosomal compartment, leading to abnormal lysosomal cholesterol accumulation. Correspondingly, a recent finding identified SLC17A9 in lysosome-mediated adenosine triphosphate (ATP) release.⁴ Because ATP is an important neurotransmitter, lysosomal exocytosis maintains regulated ATP release from neurons and/or astrocytes. This lysosomal ATP release is essential as a perturbation of SLC17A9 significantly affected neurotransmission. ATP facilitates synaptic efficiency and plasticity in neurons, and its dysregulation associates with central nervous system pathologies, including brain ischemia, inflammation, and stroke.

POTENTIAL THERAPEUTIC APPROACHES TARGETING LYSOSOMAL SLC TRANSPORTERS

The localization of SLC transporters on the lysosomal membrane represents a potential class of “druggable” targets for treating lysosomal disorders. However, success, to date, is limited due to the initial requirement of endocytosis for drugs to reach the lysosome. The absence of an SLC transporter crystal structure also slows the drug discovery process. Nevertheless, alternative strategies to effectively utilize or target lysosomal SLCs for development of improved therapeutics are underway. For instance, antibody-drug conjugates are emerging as a cancer-specific treatment to avoid off-target toxicities observed with conventional chemotherapeutic agents.

Upon binding of antibody-drug conjugates to surface antigens on cancer cells, they are endocytosed and accumulate in lysosomes. The antibody component is catabolized in the lysosome-generating active drug. However, the active drug now faces the impending challenge of finding its way out of the lysosome to reach its target site (nucleus, cytoplasm, cytoskeleton, etc.) for action. Because oligonucleotide (siRNA, miRNA) therapeutics and nanoparticle-based therapeutics face the same drug-exit impediment, lysosomal accumulation and degradation have become a widespread concern.

Cleavable linker technology, proton-sponge effect, ion pair formation, and hydrophobic modification of vectors or cargos are some of the techniques being currently used for effective drug delivery. In some cases, such strategies are harmful, disrupting (endo) lysosomal membranes. In this regard, lysosomal SLCs offer great promise to aid safe efflux of therapeutic cargos from lysosomes. A recent discovery of SLC46A3 facilitating transport of a noncleavable antibody-drug conjugate catabolite to export cargo from the lysosome validates the feasibility of this strategy.⁵ Identification and characterization of orphan SLC46A3 identified maytansine as a substrate for SLC46A3, further clarifying the mechanism of noncleavable maytansine-based antibody drug conjugates, including ado-trastuzumab emtansine. The discovery of SLC46A3 as a means for antibody-drug conjugates to target cancer cells suggests future investigation will identify other lysosomal SLC transporters with substrates amenable to therapeutic conjugation for lysosomal exit. In another example, SLC31A2, the lysosomal copper transporter, regulates sensitivity to cisplatin treatment. Measurement of copper flux with new intracellular copper sensors enables testing for copper-dependent sensitivity to chemotherapeutic agents.

Enhancement of transporter activity is a treatment option for diseases with loss of transporter function. The lysosomal glutamate transporter, SLC1A2 (EAAT2), is involved in glutamate clearance in astroglial cells, and its protein expression decreases in neurological disorders, like Alzheimer disease, amyotrophic lateral sclerosis, and schizophrenia. The antibiotic ceftriaxone increases transcription of SLC1A2 through modulating nuclear factor-kappa B signaling and riluzole, an amyotrophic lateral sclerosis

therapeutic, reportedly increases transport activity of SLC1A1 and SLC1A2, supporting this strategy. Furthermore, SLC12A9 is a member of the sodium chloride cotransporter family (SLC12) that regulates ion gradients across renal tubules and maintains cell volume. Mutations in SLC12A9 associate with Bartter disease, characterized by a defect in the thick ascending limb of the loop of Henle, hypokalemia, and alkalosis. Diuretic drugs, like bumetanide and furosemide, inhibit multiple SLC12 transporters; thus, directed development of novel diuretics that target specific SLC12 transporters may offer more effective disease management.

Finally, various lysosomal storage disorders are caused by an aberrant accumulation of undigested material within lysosomes. Classic examples involving lysosomal transporters include SLC17A5 and MFSD8 in Salla disease and neuronal ceroid lipofuscinosis, respectively. Although there are no curative treatments yet, enzyme replacement therapy and gene therapy are promising options. Some treatment options for lysosomal storage disorders, like Niemen-Pick disease, Gaucher disease, and alpha-mannosidosis using enzyme replacement therapy are already approved or are under active (pre)clinical evaluations.

Overall, targeting and utilization of SLC transporters for better treatment and management of lysosomal disorders or drug delivery is becoming a reality and research on lysosomal SLCs is gaining momentum to realize new therapeutic possibilities.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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