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– VPS10P domain receptors in cardiovascular and metabolic diseases

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ABSTRACT

VPS10P domain receptors are a unique class of sorting receptors that direct intracellular transport of target proteins in neurons and that play central roles in neurodegenerative processes. Surprisingly, genome-wide association studies now implicate the very same receptors in cardiovascular and metabolic disturbances. In this review, we discuss current findings that uncovered some of the molecular mechanisms whereby sorting receptors, such as SORLA, sortilin, and SORCS1 control homeostasis in cardiovascular and metabolic tissues, and how they promote hypercholesterolemia, atherosclerosis, obesity, and diabetes, when being altered.

Introduction

Genome-wide association studies (GWAS) have been widely used to identify loci associated with cardiovascular and metabolic diseases in humans and animal models. These studies have confirmed well-known culprits such as the low-density lipoprotein receptor (LDLR), proprotein convertase subtilisin/kexin type 9 (PCSK9), or the peroxisome proliferator-activated receptor-γ, just to name a few [1]. However, GWAS also uncovered unexpected perpetrators in cardiovascular and metabolic dysfunctions. One prominent example are VPS10P domain receptors, a group of intracellular sorting factors that direct target proteins between secretory and endocytic compartments in many cell types. The role of VPS10P domain receptors as causative agents in neurodegenerative diseases has long been appreciated (reviewed in [2, 3]), yet their genetic implication in cardiovascular and metabolic disturbances came as a surprise. Here, we will discuss recent studies that have substantiated the involvement of VPS10P domain receptors in disturbances of the cardiovascular system and the metabolism, including hypercholesterolemia, atherosclerosis, obesity, and diabetes.

The complex cell biology of sorting receptors

VPS10P domain receptors were initially identified in a quest for new lipoprotein receptors that may share structural resemblance with the LDLR. These studies led to the cloning of two type-1 transmembrane proteins termed sortilin[4] and sortilin-related receptor with A-type repeats (SORLA, also known as LR11) [5, 6]. Although SORLA and sortilin bound apolipoproteins, they did not share much structural similarity to prototypical lipoprotein receptors of the LDLR gene family (Fig. 1A). Rather, both receptors exhibited a structural motif in their extracellular domain that had been identified in a sorting receptor in Yeast, the vacuolar protein sorting 10 protein

(VPS10P). This VPS10P domain represents a 700 amino acid module that folds into a ten-bladed β-propeller and that serves as a binding site for ligands [7, 8]. Cloning of SORCS1, SORCS2, and SORCS3 (sortilin-related receptor CNS expressed) added three more mammalian members to the VPS10P domain receptor gene family (Fig. 1A) [9, 10].

VPS10P serves as a sorting factor that moves newly synthesized hydrolases from the Golgi compartment to their place of action in the vacuole (the yeast lysosome)[11]. An even more complex trafficking path has been identified for mammalian VPS10P domain receptors that are able to shuttle between the cell surface and endocytic and secretory compartments of cells (Fig. 1B). Sorting of VPS10P domain receptors is guided by cytosolic adaptors that bind to distinct motifs in the intracellular domains of these receptors and determine their trafficking path. Sorting also determines proteolytic processing of the receptors, pivotal to activate ligand binding and to shed soluble receptor domains (see Fig. 1B for details). For more detailed discussions, the reader is referred to recent reviews on the molecular concepts of VPS10P domain receptor trafficking[2, 12].

All mammalian VPS10P domain receptors are expressed in neurons of the central and peripheral nervous system. Thus, earlier work mainly focused on the neurobiology of these receptors uncovering their ability to sort a number of target proteins in control of neuronal cell death and survival. Neuronal ligands for VPS10P domain receptors include neurotrophins and their receptors, or the amyloid precursor protein and progranulin, etiologic agents in Alzheimer's disease and in frontotemporal lobar degeneration, respectively (reviewed in [2, 3]). However, VPS10P domain receptors are also expressed in peripheral tissues with relevance to cardiovascular and metabolic processes. For example, SORLA is produced in adipose tissue and in

smooth muscle cells [6, 13]. Sortilin is found in hepatocytes [14], while SORCS1 is expressed in pancreatic islets [15]. In contrast to the situation in the nervous system, the expression patterns for VPS10P domain receptors in peripheral tissues are largely non-overlapping suggesting unique functions for each receptor in cardiovascular and metabolic processes. This hypothesis received recent support from genetic studies documenting association of loci close to *SORL1* (encoding SORLA) with hypertriglyceridemia, obesity, and vessel disease, *SORT1* (encoding sortilin) with hypercholesterolemia and risk of myocardial infarction, and *SORCS1* and *SORCS3* with type 1 and type 2 diabetes (Tab. 1). Although all SNPs were non-coding variants and the disease gene in question remained unclear at times, functional studies in cell and animal models have now confirmed the importance of VPS10P domain receptors for systemic metabolism. In the following, we will focus on three main aspects of such receptor functions, on SORLA in triacylglyceride metabolism and progression of atherosclerosis, on sortilin in control of systemic cholesterol levels, and on SORCS1 in glucose homeostasis and insulin secretion.

SORLA impacts vascular integrity and promotes atherosclerosis

SORLA is a 250 kDa receptor that harbors a VPS10P domain but also displays structural elements found in the LDLR and other members of the LDLR gene family (such as a β-propeller and complement-type repeats). The encoding gene had been mapped as a pro-atherogenic locus in inbred strains of mice [16]. The relevance of SORLA for atherosclerotic processes was further substantiated by correlating circulating levels of the shedded ectodomain (considered as a diagnostic marker of receptor levels in tissues) with intima-media thickness in dyslipidemic subjects [17], with coronary artery disease [18], and with acute coronary syndrome [19]. Genetic

association of *SORL1* with cerebral lesions in hypertensive patients lend further support to a role of this receptor in vascular pathology [20].

Currently, there are two main hypotheses how SORLA may impact vascular integrity and atherosclerotic lesion formation. One model suggests a role for SORLA in control of plasma triacylglyceride levels through regulation of lipolysis. Triacylglyceride-rich lipoproteins are pro-atherogenic particles. Their turnover is determined by hydrolysis of triacylgylcerides to free fatty acids through lipoprotein lipase (LPL) in the circulation. Defects in lipolytic activity result in hypertriglyceridemia and in premature atherosclerosis as exemplified in familial deficiencies of LPL [21], or of apolipoprotein (apo) C-II [21] and apoA-V [22, 23], two activators of this lipase. SORLA has been shown to mediate endocytosis of apoA-V in cells [24, 25], suggesting its ability to modulate levels of this factor in the circulation. SORLA-dependent control of lipolytic activity through clearance of apoA-V was substantiated by documenting loss of SORLA binding in apoA-V variants encoded by *APOA5* mutations in individuals with severe hypertriglyceridemia [26]. SORLA has also been shown to direct anterograde trafficking of newly synthesized LPL molecules to lysosomes, thereby reducing the levels of the enzyme secreted by cells [27]. Thus, either through control of LPL or apoA-V levels, SORLA may inhibit lipolysis and elevate the levels of pro-atherogenic lipoprotein particles in the circulation.

An alternative model suggests a more direct role for SORLA in atherosclerotic processes in the vessel wall. This model is based on the documented expression of SORLA in intimal smooth muscle cells (SMC), and on up-regulation of its expression in the lesioned vessel wall [13, 28]. In the vessel wall, SORLA stimulates proliferation and migration of SMC and monocytes, processes that accelerated intimal thickening and atherosclerotic plaque formation [29, 30]. The molecular basis for SORLA's action in

cell migration is the ability of this receptor to regulate cell surface expression of the urokinase receptor (uPAR) (Fig. 2A) [31, 32]. The uPAR is a glycosylphosphatidyl inositol-anchored receptor for urokinase, a protease that activates plasminogen to plasmin, which, in turn, breaks down the extracellular matrix. Binding of urokinase to uPAR on the surface of target cells increases the local proteolytic potential and facilitates cell migration. Binding of SORLA to uPAR delays endocytosis of uPAR/urokinase complexes from the cell surface, possibly by blocking interaction of uPAR/urokinase complexes with the endocytic receptor LRP1 (low-density lipoprotein receptor related protein 1) [32]. The ability to regulate surface exposure uPAR is seen for full-length SORLA but also for its secreted ectodomain, suggesting both cell autonomous and non-autonomous modes of action [32, 33]. The therapeutic potential of modulating SORLA levels in treatment of atherosclerosis was highlighted in a study using conjugated linoleic acids (CLA), athero-protective ligands for PPARs. CLA potently reduces SORLA expression in monocytes *in vitro* and in the aortas of treated mice, inhibiting monocyte migration [30].

Of note, recent studies have also associated *SORL1* with several metabolic traits (e.g., obesity, waist circumference) in humans and mouse models (Tab. 1). The mechanism of SORLA action in control of adiposity still remains to be elucidated.

Sortilin, a risk factor for hypercholesterolemia and myocardial infarction

Sortilin is a 95 kDa receptor solely comprising a VPS10P domain as its ectodomain [7]. Several GWAS have associated the encoding locus *SORT1* at 1p13.3 with plasma levels of cholesterol and risk of myocardial infarction in humans, implicating sortilin in systemic cholesterol homeostasis [34-37]. Subsequently, a SNP at 1p13.3 was identified that represents a binding site for the transcription factor C/EBP. The minor

allele variant (correlated with reduced plasma cholesterol) resulted in higher transcriptional activity in a luciferase reporter assay and translated into approximately 80% increased hepatic sortilin levels as compared with the homozygous major allele genotype [38].

Human gene expression data argued for sortilin as a protective factor attenuating circulating levels of cholesterol. In support of this hypothesis, knockdown of receptor expression in the liver increased plasma cholesterol in mice [38]. Unexpectedly, this working model was challenged by studies in two independent mouse models with ubiquitous genetic inactivation of *Sort1* in which loss of sortilin reduced (rather than increased) circulating cholesterol levels [14, 39]. Based on the experimental model, several mechanisms are currently discussed how sortilin may impact cholesterol homeostasis, all of which suggest a function for this receptor in control of hepatic lipoprotein handling (Fig. 2B). The main type of lipoprotein particle secreted by hepatocytes is the very low-density lipoprotein (VLDL) that is produced by lipidation of apoB100. VLDL particles are released into the circulation where they are converted to low-density lipoproteins (LDL), the main carriers of cholesterol in the human circulation. Circulating LDL are cleared by the LDL receptor in the liver and other tissues [40]. Proposed functions for sortilin in reducing plasma cholesterol comprise its action as a hepatic clearance receptor for LDL [39, 41] or a role in anterograde sorting of nascent lipoprotein particles from the TGN to lysosomes, effectively reducing the output of VLDL by the liver [38, 42]. In contrast, a possible activity of sortilin in increasing plasma cholesterol involves its ability to facilitate secretion of VLDL from hepatocytes [14]. In addition, a recent study documented a function for sortilin in promoting the release of PCSK9 from the liver [43]. PCSK9 binds to the LDL receptor and causes its lysosomal degradation, either in

the biosynthetic pathway of the cell or following secretion of the protease into the extracellular space (reviewed in [44]). Hepatocytes are the main source of circulating PCSK9 and high plasma levels of the protease correlate with low LDL receptor activity (and hence higher LDL concentrations). Sortilin interacts with PCSK9 in the TGN of hepatocytes and promotes secretion of this protease. Loss of sortilin in *Sort1*-deficient mice reduces whereas hepatic overexpression of the receptor increases circulating PCSK9 levels causing diminished LDL receptor expression and increased plasma LDL. The amount of the sortilin ectodomain in the blood stream correlates with circulating PCSK9 levels in healthy subjects suggesting a similar function for sortilin in PCSK9 handling in humans as in mouse models [43].

At present, the contradiction between proposed modes of sortilin action in murine versus human lipoprotein metabolism remains unresolved. Potentially, sortilin performs both promoting and inhibiting actions in cholesterol homeostasis, and the net balance of these competing actions varies by pathophysiological context and experimental models. In support of the complex actions of this receptor in cardiovascular processes, new studies highlight the potential of sortilin to promote atherosclerotic processes in the vessel wall independent of its action in hepatic lipoprotein metabolism. Thus, deficiency of sortilin in the hematopoetic system attenuates atherosclerosis in mice, a mechanism attributed to the ability of this receptor to control release of pro-inflammatory cytokines from lymphocytes [45, 46] and/or facilitate macrophage uptake of LDL and foam cell formation [47].

Although not substantiated by human genetic data as yet, functional studies in cell and mouse models have suggested additional functions for sortilin in control of glucose homeostasis and onset of obesity that warrant further elucidation. A function

for sortilin in glucose metabolism had been noted early on when the receptor was identified as a major constituent of vesicle carrying the glucose transporter (Glut) 4 [48, 49]. Following insulin stimulation, Glut4 moves from intracellular storage vesicles to the cell surface to facilitate uptake of glucose in muscle and adipose tissues. In line with its role as an intracellular sorting factor, sortilin was subsequently shown to interact with the Glut4 protein and to trigger its inclusion in storage vesicles [50, 51]. In adipocytes, this sortilin activity proved essential to confer insulin responsiveness of Glut4 storage vesicles [52]. A possible role for sortilin in body weight control is suggested by studies documenting protection from diet-induced obesity and from fatty liver disease (hepatic steatosis) in mice lacking this receptor [53]. Possible receptor functions implicated in these processes include the intracellular trafficking of acid sphingomyelinase, a modulator of insulin signaling in adipose tissue, or of delta-like 1 homologue, an inhibitor of adipogenesis [54].

SORCS1, a diabetes risk factor and regulator of insulin secretion

SORCS1, the gene encoding the 130 kDa receptor SORCS1 is closely linked with *SORCS3* on the distal chromosome 19 in the murine genome. This locus has been mapped as a quantitative trait locus affecting fasting insulin levels in obese mice [15]. The syntenic regions in rats [55] and humans [56] are also associated with fasting insulin levels and insulin secretion. Similarly to the situation in mice, association in humans is strongest in overweight women. Remarkably, *SORCS1* is not only associated with phenotypes consistent with type 2 diabetes (T2D), but was also mapped as a locus for glycemic control in patients with type 1 diabetes [57].

Because SORCS1 is expressed in pancreatic islet cells and expression is increased 10-fold in mice susceptible to T2D [15], a function for this receptor in insulin

secretion from β-cells seems plausible. This hypothesis is supported by studies in *Sorcs1 null* mice in which loss of SORCS1 coincides with an insulin secretory dysfunction. This secretion defect is likely caused by a failure to replenish secretory granules (SG) following repeated stimulation of islets with various secretagouges[58]. The molecular mechanism of SORCS1 action in SG biogenesis in islets remains enigmatic. However, recent progress in the understanding of SORCS1 functions in neurons may provide some interesting food for thought. Thus, in neurons, SORCS1 interacts with the synaptic adhesion molecule neurexin and controls trafficking of glutamate receptors, a process critical for plasticity of the synapse [59]. Remarkably, neurexin-1α and its ligand neuroligin are also expressed in pancreatic β-cells where they interact with components of the SG docking machinery to modulate insulin secretion rates [60-62]. Consequently, loss of expression of neurexin-1α [62] or neuroligin-2 [63] alters islet morphology, pancreatic insulin content, and insulin secretion in mice. Although speculative at present, proper sorting and docking of SG in islet cells may dependent on the interaction of neurexins with the sorting receptor SORCS1.

Conclusion

Ample evidence from association studies in humans and rodents implicate several members of the VPS10P domain receptor gene family in (patho)physiological processes of the cardiovascular system and the metabolism. Similar to the situation in neurons, functions for these receptors in cardiovascular and metabolic cell types involves uptake and intracellular trafficking of target proteins. Interestingly, ectodomain shedding, producing soluble receptor fragments capable of ligand binding, emerges as an important aspect of receptor function. This fact provides exciting

opportunities for diagnosis of receptor levels in patients but also for the development of therapeutics based on the structure of the ectodomains.

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TABLE: Genetic association of VPS10P domain receptors with cardiovascular and metabolic traits in humans and animal models.

Receptor	**Association**	**Cohort**	**Reference**
SORLA	Cerebral small-vessel disease	Human	[20]
	Obesity	Human Mouse	[64] [65]
	Atherosclerosis	Mouse	[16]
sortilin	Hypercholesterolemia	Human	[34-37]
SORCS1	Type 1 diabetes	Human	[57]
	Type 2 diabetes	Mouse Rat Human	[15] [55] [56]
	Obesity	Human	[66]
SORCS2	Biomarkers of cardiovascular disease	Human	[67]
SORCS3	Type 2 diabetes	Rat	[55]

FIGURE LEGENDS

Figure 1: Structure and cell biology of VPS10P domain receptors

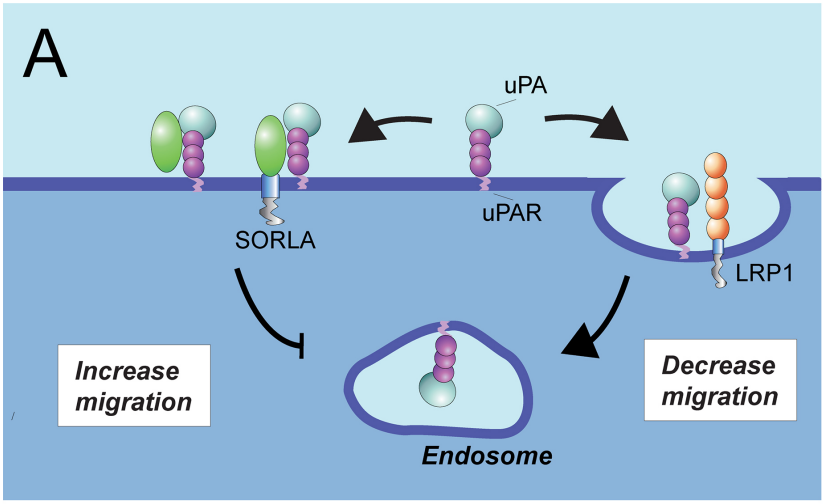
(**A**) Structural organization of VPS10P domain receptors from yeast (VPS10P) and mammals (sortilin, SORLA, SORCS1, SORCS2 and SORCS3). The extracellular domains of the receptors are composed of one or two VPS10P domains, and may carry additional modules for protein-protein interaction (leucine-rich domains, complement-type repeats, EGF-type repeats and fibronectin-type III domains) or regulation of ligand binding (β-propeller). The structure of the low-density lipoprotein receptor (LDLR) is shown for comparison. (**B**) VPS10P domain receptors are synthesized as precursor proteins harboring a 40 - 55 amino acid pro-peptide that act as intrinsic chaperones for proper folding and prevent premature ligand binding. Removal of the pro-peptide by proprotein convertases in the trans-Golgi network (TGN) activates nascent receptor molecules (step 1). From the TGN, mature VPS10P domain receptors follow at least three alternative trafficking routes. Firstly, they may be directed to the cell surface via constitutive secretory vesicles (step 2). Some receptor molecules at cell surface are subject to shedding, releasing the soluble ectodomain to act as diffusible regulator by sequestering ligands [33, 68]. Intact receptor molecules at the cell surface may perform clathrin-dependent endocytosis of ligands, a process facilitated by binding of the adaptor protein (AP-2) (step 4) [69, 70]. From endosomes, internalized receptors (and some of their cargo) return to the TGN (step 5) [71, 72]. This retrograde sorting path requires the interaction with the adaptor complex retromer [71-75] and with PACS1 [76]. A second route for exiting the TGN involves anterograde movement of VPS10P domain receptors to endosomes (step 6), employing the monomeric clathrin adaptors GGA1, GGA2, and GGA3 (Golgi-

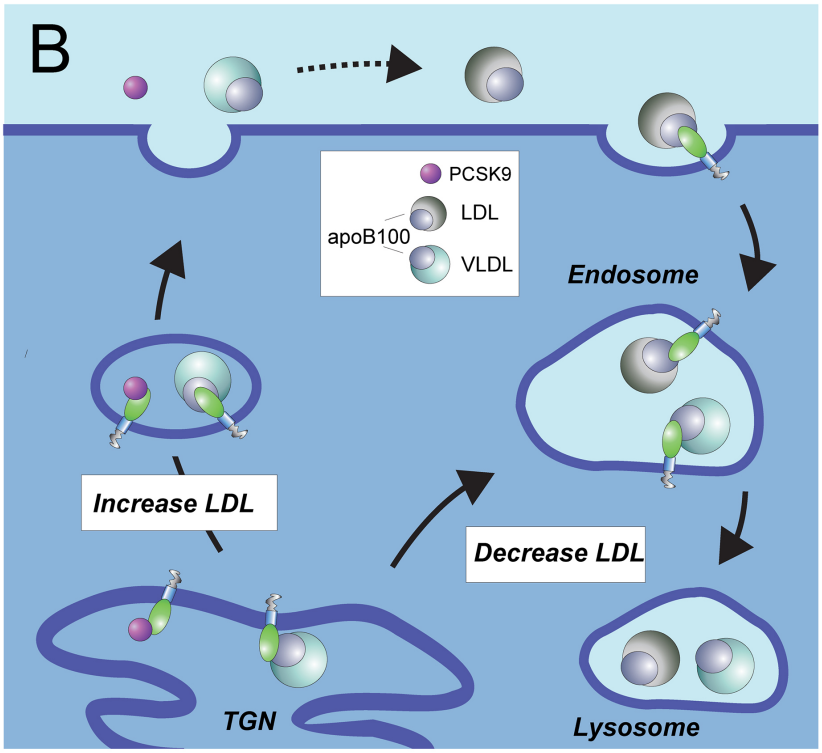
localizing, g-adaptin ear homology domain, ARF-interacting proteins) [70, 77, 78]. From endosomes, ligands, and in some instances even the receptor, may be targeted for lysosomal degradation (step 7) [78]. A third pathway for TGN export exists in cells capable of regulated secretion whereby receptors move endogenous ligands from the TGN to secretory granules (step 8). The scheme summarizes trafficking paths identified for various VPS10P domain receptors, but not every route has been confirmed for each receptor. Figure 1B adopted from [2].

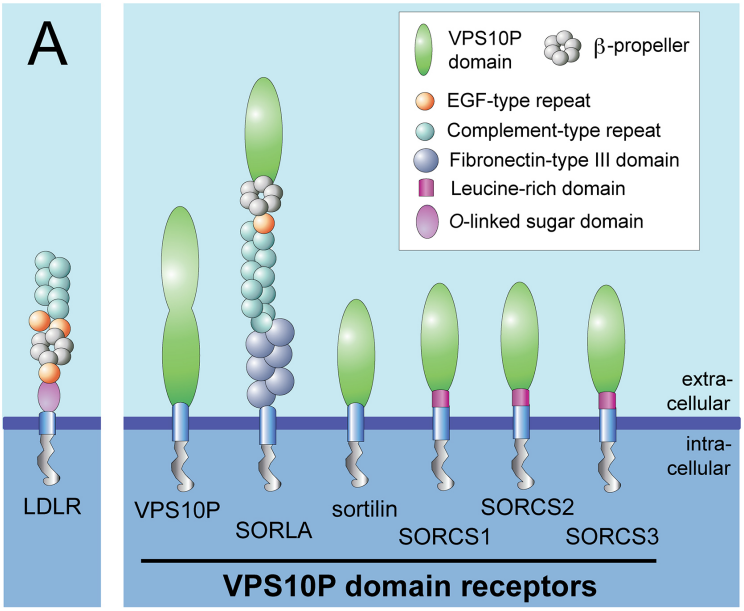
Figure 2: Functions for SORLA (A) and sortilin (B) in cholesterol homeostasis and atherosclerosis

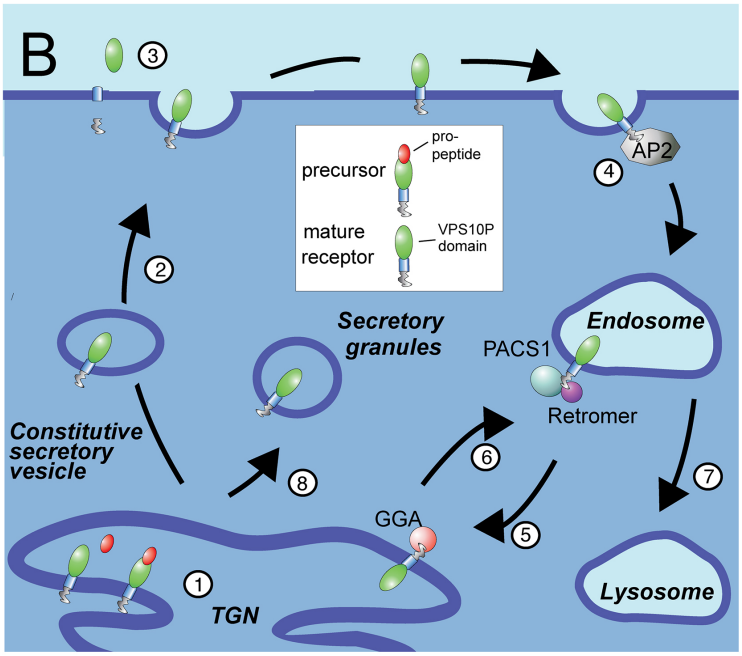
(A) Urokinase (uPA) bound to the urokinase receptor (uPAR) on the cell surface provides cells in the vessel wall with the ability to locally activate plasminogen to plasmin and to breakdown extracellular matrix components to enable cell migration. The surface exposure of uPA/uPAR complexes is reduced through endocytosis by the endocytic receptor LRP1, decreasing cell migration. By contrast, binding of full-length SORLA or the soluble ectodomain to uPAR prevents LRP1 interaction and delays removal of uPA/uPAR complexes from the plasma membrane. As a consequence, cell migration is increased. **(B)**. In the TGN, sortilin interacts with PCSK9 and with nascent VLDL particles to facilitate their secretion from hepatocytes. Both mechanisms increase plasma LDL, either through enhanced output of VLDL, the precursor of LDL, or through raising circulating levels of PCSK9 that causes proteolytic degradation of LDL receptors. Simultaneously, decrease of plasma LDL may be achieved by sortilin through endocytic clearance of LDL or by anterograde

sorting of newly synthesized VLDL particles to the endosomal/lysosomal system. Figure 2B adopted from [79].


A
uPA
uPAR
SORLA
LRP1
Increase migration
Decrease migration
Endosome


B
PCSK9
LDL
apoB100
VLDL
Endosome
Increase LDL
Decrease LDL
TGN
Lysosome


A
VPS10P domain
β-propeller
EGF-type repeat
Complement-type repeat
Fibronectin-type III domain
Leucine-rich domain
O-linked sugar domain
extra-cellular
intra-cellular
LDLR
VPS10P
SORLA
sortilin
SORCS1
SORCS2
SORCS3
VPS10P domain receptors


B
precursor
pro-peptide
mature receptor
VPS10P domain
AP2
Secretory granules
Endosome
PACS1
Retromer
Constitutive secretory vesicle
GGA
TGN
Lysosome
1
2
3
4
5
6
7
8