Sorting Nexin 27 as a potential target in G protein-coupled receptor recycling for cancer therapy (Review)

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Abstract. G protein-coupled receptors (GPCRs) are the largest family of membrane receptors and activate several downstream signaling pathways involved in numerous physiological cellular processes. GPCRs are usually internalized and desensitized by intracellular signals. Numerous studies have shown that several GPCRs interact with sorting nexin 27 (SNX27), a cargo selector of the retromer complex, and are recycled from endosomes to the plasma membrane. Recycled GPCRs usually contain specific C-terminal postsynaptic density protein 95/Discs large protein/Zonula occludens 1 (PDZ) binding motifs, which are specifically recognized by SNX27, and return to the cell surface as functionally naïve receptors. Aberrant endosome-to-membrane recycling of GPCRs mediated by SNX27 may serve a critical role in cancer growth and development. Therefore, SNX27 may be a novel target for cancer therapies.

Contents

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1. Introduction to the sorting nexin family

The sorting nexin (SNX) family comprises cytoplasmic and membrane-associated proteins that are involved in endocytosis and protein trafficking through these membranous compartments (1). The hallmark of the SNX family is the presence of a phospholipid-binding motif (PX domain) that contains a conserved sequence of 100-130 amino acids. The PX motif binds to various phosphatidylinositol phosphates and then mediates the transportation of these proteins to specific cellular membranes. At present, 25 human SNXs involved in membrane trafficking regulation have been identified (2). Based on their common domain structures, the SNX family is divided into three subgroups: The first group, including SNX1, 2, 4, 5, 6, 7, 8, 9, 13, 14, 15, 16 and 18, contains 1-3 coiled-coil domains that may be involved in protein-protein interactions as well as homo- and/or hetero-oligomerization with other SNXs. The second group, which includes SNX3, 10, 11, 12, 22, 23 and 24, only contains a PX domain and acts as a cargo protein adaptor in retromer-dependent recycling. The remaining sorting nexins, including SNX17, 19, 21, 25 and 27, contain various membrane targeting sequences, G-protein regulatory sequences or protein-protein interaction sequences, and may be involved in endosomal sorting and signal transduction (1).

The hallmark of SNX27 is the presence of both a PX domain, a postsynaptic density protein 95/discs large 1/zona occludens 1 (PDZ) domain and four-point-one-protein-erzin-moesin-radixin (FERM) domain, which is linked to the endosomal trafficking of a range of transmembrane cargoes (Fig. 1A) (1,2). The PDZ domain, which distinguishes SNX27 from other SNX proteins, allows SNX27 to recognize and bind with internalized proteins with unique PDZ binding motif 'S/T-x-ϕ' (x, any amino acids; ϕ, any hydrophobic amino acids) located in their C-terminal tails (Fig. 1B). Furthermore, SNX27 acts as a 'cargo selector' to form the actin-sorting nexin 27-retromer tubule (ASRT) complex and participates in G protein-coupled receptor (GPCR) recycling from endosomes to the plasma membrane after internalization (3). Components internalized from the plasma membrane can be transported to the plasma membrane for rapid recycling, to lysosomes for degradation, or to endosomes for short-term storage via

endosomal signal transduction before being trafficked to the cell surface (4). Interestingly, several latest research reports have indicated that SNX27 may contribute to cancer development via recycling cancer-associated proteins (5,6).

2. GPCRs and their activation modes

GPCRs, which are also known as seven transmembrane spanning receptors, constitute the largest family of membrane proteins in mammalian cells. On the basis of their sequence homology and functional similarity (7), the GPCR superfamily is divided into six principal classes (A to F) via an alternative classification system called glutamate, rhodopsin, adhesion, frizzled/taste2, secretin. A diverse array of components involved in extracellular stimulation, including light-sensitive compounds, odors, peptides, hormones, neurotransmitters and large proteins are converted into intracellular signals via GPCR activation and regulate various physiological or pathological processes, including proliferation, differentiation, chemotaxis and communication (8). Recently, certain modes of GPCR activation have been used to explain the complexity of their behaviors. The canonical GPCR activation signaling pathway, which is termed the 'GPCR-G protein' activation mode, is dependent on the interaction of GPCRs with intracellular G proteins, which is mediated by GPCR ligands. Compared to the canonical 'GPCR-G protein' activation, GPCRs may be biased towards either G protein-dependent pathways or β-arrestin-dependent pathways, which is referred to as the biased activation mode (8).

In canonical GPCR signaling, each Gα subfamily binds GTP and dissociates from Gβ and Gγ to initiate downstream signaling cascades via second messengers. Gβ and Gγ contribute to the termination of canonical signaling by binding to β-arrestin, which is recruited near the G protein and mediates the internalization of the agonist-GPCR complex. Subsequently, desensitization is mediated by phosphorylation of the GPCR by a specific kinase (4).

In addition to the canonical signaling pathway, accumulating evidence has provided support suggesting that activation of the G protein initiated by a GPCR occurs both in the plasma membrane, but also in endosomes or the Golgi membrane, and this is called intracellular activation. In this mode, β-arrestin serves a central role in mediating GPCR trafficking. In 1999, β2-adrenergic receptor (β2-AR) was shown to initiate ERK1/2 activation following internalization and was found to be mediated by β-arrestin on clathrin-coated pits (9). Similar phenomena have also been found in other GPCRs, such as proteinase-activated receptor 2 (10) and angiotensin II type 1A receptor (11). Prolonging the lifetime of arrestin-bound clathrin-coated pits by inhibiting subsequent endocytosis enhances the intensity of the signal (12). Based on the strength of their interaction with β-arrestin, GPCRs are divided into two classes: Class A (β2-AR and biogenic amine receptors), which exhibit unstable interactions and display transient endocytosis, and class B (including peptide receptors), which form stable complexes with β-arrestin and undergo sustained internalization (13). After internalization, complexes are sorted to endosomes or the Golgi apparatus through the trans-Golgi-network (TGN) by the SNXs family. The receptors then recruit G proteins to active adenylyl cyclase, which

in turn produces cAMP and induces transient or prolonged activation (14).

Other novel modes of GPCR activation, including biased activation, dimerization activation, transactivation and biphasic activation, have been addressed in a recent review (15). These activation modes may serve as potent drug targets to treat types of cancer with this mode of activation (16), as previous studies have shown that GPCRs regulate a broad range of signal transduction pathways that contribute to several characteristics of cancer development, including initiation, growth, invasion, migration and angiogenesis (17,18).

3. SNX27-dependent GPCR endosome-to-plasma membrane recycling is involved in cancer progression and metastasis

Aberrant expression and activation of GPCRs has been linked to oncogenicity since 1986, when the Mas oncogene was identified, which was predicted to encode an internal protein exhibiting the characteristics of a GPCR (19). At present, multiple lines of evidence have shown that several GPCRs may be involved in tumor progression and metastasis via activating excessive signaling cascade (Table I). These cancer-associated GPCRs are recycled from the cytoplasm to the plasma membrane in several types of tumor cells in a SNX27-dependent manner owing to the PDZ binding motifs. Some proteins regulated by SNX27 belong to the classical GPCR superfamily, such as parathyroid hormone receptor (PTHR) (20), β-adrenergic receptor 1 (β1-AR) (21,22) and β2-AR (2). Second, metabotropic glutamate receptors (mGluRs), including mGluR1-8, are class B, synaptic GPCRs that contain large extracellular ligand binding domains and form constitutive dimers. The trafficking and expression of mGluRs in the dorsal horns is primarily dependent on SNX27 modifications (23). Third, the recycling of ion channels, such as protein-coupled inwardly rectifying potassium channels (GIRKs), is regulated by SNX27 through a combination of GIRK subunits (24,25). Additionally, Frizzled receptors (FZDs), which are a family of GPCRs, interact with SNX27 and consequently mediate the canonical Wnt signaling pathway (26).

Classic GPCRs and cancer. PTHR, a receptor of parathyroid hormone or parathyroid hormone-related protein (PTHrP) is occasionally secreted by cancer cells and is involved in cancer progression. Immunohistochemistry analysis has demonstrated that PTHR1 is highly expressed in the plasma membrane of certain cancer cells (27). PTHR typically activates a range of mitogenic pathways, including the ERK1/2, MAPK and PI3K/AKT signaling pathways, and modulates cell cycle progression by inducing the expression of cyclin D1 (28). Additionally, hypercalcemia, which is caused by an increase in PTHrP and PTHR, contributes to epithelial-mesenchymal-transition (EMT) progression and skeletal metastasis of breast and prostate cancer cells (29,30).

As with other classical GPCRs, β-ARs are associated with the transduction of multiple intracellular signals, such as adrenalin/HuR/TGF-β/Smad2 (3), cAMP/PKA/CREB (or VEGF) and cAMP/Ras/ERK, and are involved in the progression of several types of cancer (31-33). β-ARs have also been demonstrated to stimulate tumor progression via several cellular and molecular processes, such as recruitment
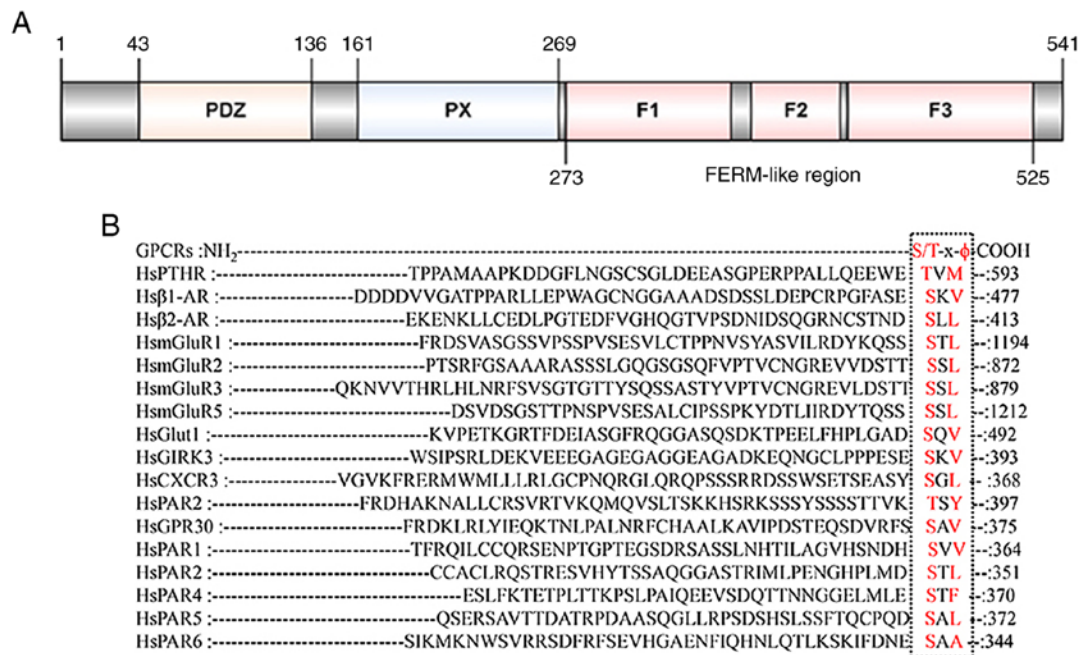
Figure 1. SNX27 structure and PDZ binding motif in GPCRs. (A) Schematic diagram of SNX27. SNX27, sorting nexin 27; PDZ, protein 95/discs large 1/zona occludens; GPCR, G-protein coupled receptor. (B) Several GPCRs contain the PDZ binding motif 'S/T-x-ϕ' in their C-terminal tails, where x indicates any amino acid and ϕ represents any hydrophobic amino acid.

of macrophages into tumor tissues or increasing the expression of inflammatory cytokines (34,35).

Transporters and cancer. Glutamate receptors on neuronal cell membranes are responsible for regulating glutamate-mediated postsynaptic excitation. Interestingly, in the last few decades, it has been reported that glutamate receptors are involved in tumor development in both neural and non-neural cancer tissues (36). Glutamate receptors are composed of two groups, mGluRs and ionotropic glutamate receptors iGluRs. Excluding the activation iGluRs in a G protein-independent manner, mGluRs (mGluR1-8), which belong to the GPCR superfamily, are ubiquitously expressed in both neuronal tissues and various non-neuronal human tissues, such as the skin, liver, heart and adrenal gland (37). Abundant expression of mGluRs has been confirmed to regulate glutamate-mediated signals and enhance malignant tumor phenotypes. For example, both mGluR1 and mGluR5 can be coupled to Gαq, which stimulates phospholipase C β and activated PKC, resulting in the phosphorylation of downstream targets (38). Additionally, mGluR2-4 and 6-8 couples with Gαi/o leading to the inhibition of adenylyl cyclase, which attenuates several different pathways, including the MAPK and PI3K/Akt signaling pathways (39,40). Inhibition of mGluR5 by the selective antagonist MPEP promotes glioma cell death by facilitating the generation of a hypoxic microenvironment (41). Notably, through a PDZ-dependent mechanism, SNX27 promotes the recycling and membrane insertion of the glutamatergic receptor. For example, mGluR5 recycling in a vacuolar protein sorting-associated protein 26 (VPS26)-SNX27-dependent manner serves a role in the development of neuropathic pain (23).

Ion channels and cancer. G protein-coupled inwardly rectifying K⁺ channels (GIRKs), as classical G protein effectors, are special potassium ion channels, the activation of which results in hyperpolarization of the cell membrane, thereby regulating cellular activity. GIRK channels are known to be activated by GPCRs coupled to the Gi/o subclass, which also inhibit voltage-dependent Ca^{2+} channels and adenylate cyclase (42). Over the last decade, numerous studies have suggested that two of the gene loci that encode GIRK subunits in humans are related to tumorigenesis and tumor growth. For example, 69% of patients with non-small cell lung cancers exhibit high levels of GIRK1 gene expression and an increased likelihood of cancer progression compared with patients with low GIRK1 levels (43). Overexpression of KCNJ3 (a GIRK1 subunit) contributes to invasion, metastasis and angiogenesis in breast cancer cell lines (44). Stimulation of GIRK1 or GIRK2 channels may activate the β-adrenergic signaling pathway in both small cell lung cancer and breast cancer (44,45). Interestingly, SNX27 also appears to promote the movement of GIRKs through early endosomes to the cell surface and leads to alterations in their expression (46).

FZDs and cancer. Frizzled receptors are structurally similar to GPCRs, with seven transmembrane-spanning domains, and they serve a vital role in development and tissue homeostasis (47). The canonical Wnt/β-catenin signal cascade, which is a pathway involved in vital aspects of cell proliferation and differentiation, occurs via a combination of a single Wnt ligand and multiple FZDs (26,48). There is some evidence indicating that FZDs are frequently overexpressed in tumor tissues, and this upregulation is associated with a poor prognosis (26). Amino acid sequence analysis has shown that most FZDs contain a PDZ-binding motif at the C-terminal tail (49). Therefore, FZDs may bind to SNX27 through their PDZ domain and are then internalized and undergo vesicular trafficking to regulate canonical Wnt signaling in cancer cells.

Table I. Recycling GPCRs in a SNX27-dependent manner are involved in cancer.

Subfamily	GPCRs	Cancers	Expression	Activating signals	Effects	Refs.
Classic GPCRs	PTHR	Breast cancer Prostate cancer Colorectal carcinomas	High	ERK1/2 MAPK PI3K/AKT	Proliferation Metastasis Invasion	(27-30)
	β1-AR	Breast cancer Melanoma	High	TGF-β/Smad2(3) cAMP/PKA/CREB Ras/ERK	Proliferation Migration	(31-33)
	β2-AR	Squamous cell carcinoma Pancreatic cancer Gastric cancer Breast cancer	High		Proliferation Migration Angiogenesis	(34,35)
Transporter	mGluRSs	Glioma Hepatocellular carcinoma Prostate cancer Melanoma	High	MAPK PI3K/AKT PKC	Proliferation Metastasis	(36-41)
Ion channels	GIRKs	Breast cancer Lung cancer Hepatocellular carcinoma	High	β-adrenergic signaling	Proliferation Migration	(42-46)
FZDs		Prostate cancer Glioma Lung cancer Hepatocellular carcinoma Gastric carcinoma	High	Wnt/β-catenin	Proliferation Metastasis	(26,47-49)
Others	Chemokine receptors	Breast cancer Colorectal cancer	High	ERK1/2/MMP-7	Proliferation Metastasis	(50-52)
	PARs	Prostate cancer Breast cancer Pancreatic cancer Glioma	High	p38 MAPK TGF-β	Metastasis	(53,54)
	LPARs	Breast cancer Liver cancer Gastric cancer Ovarian cancer	High	NOTCH0 PI3K/PAK1/ERK LPAR signaling	Proliferation Migration	(55-58)
	GPR30	Fibroblasts Thyroid cancer Ovarian cancer Endometrial cancer Renal cell cancer	High	ERK1/2 NOTCH	Proliferation Anti-apoptosis	(59)

GPCR, G-protein coupled receptor; SNX27, sorting nexin 27; PTHR, a receptor of parathyroid hormone or parathyroid hormone-related protein (PTHrP); β1-AR, β-adrenergic receptor 1; β2-AR, β-adrenergic receptor 2; mGluRs, metabotropic glutamate receptors; GIRKs, G protein-coupled inwardly rectifying K⁺ channels; FZDs, frizzled receptors; PARs, protease-activated receptors; LPARs, lysophosphatidic acid receptors.

Other cancer-associated GPCRs dependent on SNX27-mediated recycling. As described above, aberrant expression of several SNX27-related GPCRs is closely associated with cancer development and progression. Additionally, there are the other cancer-associated GPCRs which are dependent on SNX27-mediated recycling and trafficking to the membrane as naïve receptors. For example, chemokine receptors are well-documented receptors that facilitate cell growth, survival, migratory capability and cancer metastasis (50-52). Protease-activated receptors (PARs) are a unique class of GPCRs involved in cancer that can transmit signals to extracellular proteases. Thrombin acts on PAR1, 2 and 4, and has been shown to affect cancer progression via activation of the PAR pathway (53,54). Most lysophosphatidic acid receptors (LPARs) are GPCRs and several studies have shown that the activation of the LPAR signaling axis is involved in cell proliferation and invasion in several types of cancer (55-58). Recent studies have shown that activation of GPCR30 (GPR30) results in cancer cell growth, including in breast cancer-associated fibroblasts, thyroid cancer cells, ovarian cancer cells,
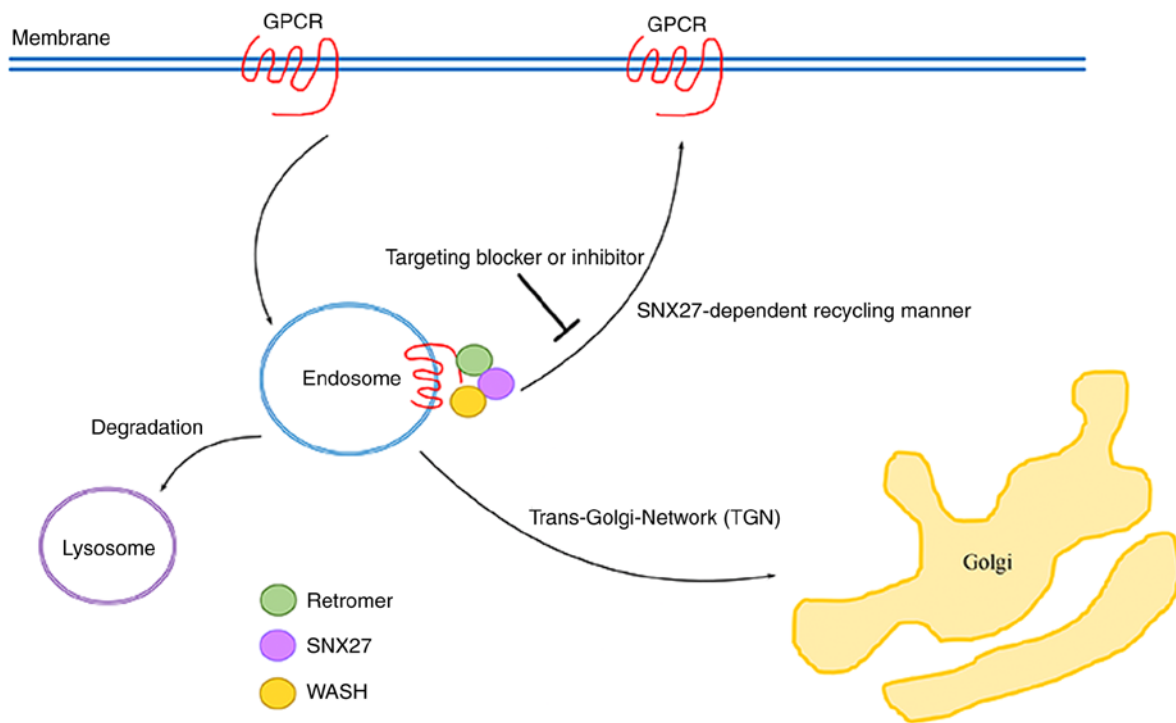
Figure 2. Mode of GPCR trafficking in a SNX27-dependent manner. SNX27 acts as a cargo selector to mediate GPCR recycling from endosomes to the plasma membrane by binding to a PDZ-binding-motif and forming an ASRT complex. Some recycling may be blocked by specifically targeting SNX27 and disrupting its binding with retromer, which in turn reduces the membrane levels of cancer-related GPCRs. SNX27, sorting nexin; GPCR, G-protein coupled receptor; PDZ, protein 95/discs large 1/zona occludens; ASRT, actin-sorting nexin 27-retromer tubule.

endometrial cancer cells and renal cell cancer cells (59). It is currently unknown whether these cancer-associated GPCRs are dependent on endosome-to-plasma membrane recycling via the SXN27-dependent pathway, but it is hypothesized that these cancer-associated GPCRs may undergo SNX27-mediated recycling and trafficking to the membrane as naïve receptors, which is hypothesized to enhance cancer signaling pathways owing to the presence of similar PDZ binding motifs (Fig. 2).

4. SNX27 and cancer

SNX27, as a scaffold protein which mediates protein-protein interaction in membrane remodeling, signaling, intracellular trafficking, tight junctions, organelle motility and cell movement, potentially exhibits its roles sequentially during tumorigenesis, cancer progression and metastasis. Sharma *et al* (6) investigated the expression pattern of SNX27 in datasets obtained from The Cancer Genome Atlas and found significantly higher levels of SNX27 expression in invasive breast tumor tissue compared with normal breast tissue. Furthermore, the higher expression of SNX27 was inversely correlated with patient survival. SNX27 knockdown dramatically decreased cell motility owing to increased expression of E-cadherin and β-catenin, which contributes to adhesion formation and mesenchymal-epithelial transition. Studies have further shown that SNX27 regulates matrix invasion by cancer cells by recycling matrix metalloprotease depending on its direct interaction (6,60). Additionally, SNX27 is involved in regulating energy substance uptake in cancer cells via recycling energy transport receptors. For example, due to the roles for SNX27 in glutamine uptake and amino acid-stimulated mTORC1 activation via modulation of

alanine-, serine-, cysteine-preferring transporter 2 intracellular trafficking, knockdown of SNX27 in breast cancer cells significantly decreased cell proliferation *in vitro,* inhibited tumor growth and prolonged animal survival in xenograft nude mouse models (5,61). Additionally, via the PDZ domain, SNX27 is able to bind to and regulate the localization and expression of GLUT1, which facilitates the transport of glucose across the plasma membrane to support cell growth (62). Taken together, the abundance of SNX27 may serve important roles in tumorigenesis, cancer progression and metastasis.

5. Mechanism of SNX27-dependent GPCR recycling

At present, the mechanisms responsible for the trafficking of internalized GPCRs are not well understood. PTHR, a retromer, is a component of the endosomal sorting complex actin/SNX 27/retromer tubule (ASRT), regulates the sustained generation of cAMP triggered by the internalization of PTHR and results in the movement of internalized receptors from endosomes to the Golgi apparatus (63). In general, internalized receptors do not exert any functions following termination of endosomal signaling. To maintain quantitative receptor homeostasis, inactive receptors undergo two definite modes of postendocytic sorting: First, transfer into the lysosome for degradation and downregulation of receptors, and second, recycling from the endosome to the membrane in an ASRT-dependent manner or via the TGN to the Golgi and then back to the cell surface in an ASRT-independent manner (4,64,65). In the second mode, recycled GPCRs on the cell surface, which act as naïve receptors, may be directly or indirectly ready to receive another stimulus (66).

Accordingly, it is critical to comprehensively understand the molecular basis of GPCR recycling as it may contribute to several receptor-associated diseases. As the SNX27/retromer recycling pathway occurs in multiple tissues, particularly in neurons, the loss of SNX27 contributes to several neurological diseases, such as Alzheimer's disease, Parkinson's disease, Down syndrome, epilepsy and cancer (67-70). Low expression of SNX27 also reduces the membrane levels of β2-AR, NMDARs and AMPARs, resulting in relevant disorders (71,72). Although transfection of SNX27 small interfering (si)RNA does not inhibit the recycling of FLAG-tagged β1-AR in HEK293 cells (22,73), selective depletion of SNX27 reduces recycling of the most relevant GPCRs and results in the subsequent downregulation of membrane receptors. Emerging evidence suggests that SNX27 interacts with a multitude of proteins and forms the ASRT complex to perform GPCR recycling from the endosome to the cell membrane (4). Receptors such as β-2 AR contain a PDZ ligand at the C-terminus called the PDZ-binding-motif (PBM), which can interact with SNX27 and subsequently control the recycling process (74,75). The PDZ domain of SNX27 binds to PBM as a cargo selector, while two Bin-Amphiphysin-Rvs domains interact with retromer, which consists of the vacuolar protein sorting (Vps) proteins Vps26-Vps29-Vps35, constituting the ASRT complex (2). Interaction analysis between GFP-tagged Vps26 and SNX27 indicates that SNX27 directly interacts with retromer via Vps26. The PX and FERM domains of SNX27 are hypothesized to be involved in recruiting the ASRT complex to the Wiskott-Aldrich syndrome protein and SCAR homologue complex, which activates Arp2/3-mediated actin polymerization on endosomes (62,76). These findings strongly support the relevant mechanism that SNX27, as a cargo selector, serves important roles in recycling GPCRs from endosomes to the plasma membrane.

6. Concluding remarks and future perspectives

Several GPCRs have been demonstrated to undergo recycling in a SNX27-dependent manner and are suggested to critically regulate cancer progression and development. GPCRs have been used as potential targets for cancer treatment. Several humanized monoclonal antibody drugs, such as mogamulizumab, which targets CCR4 have been approved by FDA to treat T-cell lymphoma. Several small molecules, such as plerixafor, which targets CXCR4, brigatinib which targets EGFR have been used to treat myeloma and lung cancer patients. In addition, several known GPCR-targeted drugs such as β-blockers, are reported to contribute to improvement in the prognosis of numerous cancers, which is currently in phase II clinical trials (17). Additionally, since the PDZ domain is involved in protein-protein interactions and abnormal intracellular signaling, small molecule drugs, including intrabodies, peptides and siRNA, have been used to block the interaction between proteins and PDZ domains for cancer treatment (77). The specific small-molecule inhibitor compound 3289-8625 strongly binds the disheveled PDZ domain and effectively blocks Wnt/β-catenin signaling, which impacts the growth rate of prostate cancer cells (78). The cell-permeable lipopeptide CR1166 blocks the PDZ domain of GIPC, and prevents pancreatic and breast cancer development (79). The peptide

PSD95, which binds to syntenin tandem PDZ domains (PDZ1 and PDZ2) with high affinity, significantly inhibits cancer cell proliferation, migration and invasion (80).

Recent research has indicated that a specific small-molecule inhibitor that targets the PDZ1 domain of MDA-9/Syntenin (SDCBP) reduces prostate cancer cell invasion, migration and metastasis, thus exhibiting significant therapeutic potential (81). Additionally, the association of SNX27 with retromer (VPS26) can be mechanistically blocked by PTEN via PTEN-PDZ binding motif, which controls the Glut1 recycling pathway and contributes to the tumor-suppresser function of PTEN (82). Hence, the interaction between cancer-associated proteins and SNX27 via recognition of the PDZ binding motif can be a therapeutic drug target for cancer treatment. Selective inhibition of the PDZ domain can prevent cancer progression by blocking SNX27-dependent recycling manner and reducing aberrant expression of cancer-associated proteins at the membrane.

Thus, further research is required to identify the universality of SNX27-dependent recycling, and whether it applies across the GPCR superfamily and also other membrane proteins, particularly those associated with tumorigenesis. Additionally, understanding whether different types of cancer express elevated levels of SNX27 and its relationship with prognosis of cancer patients will provide sufficient evidence that SNX27 and the PDZ binding motif are potential anticancer drug targets.

In summary, several highly expressed GPCRs on the plasma membrane of cancer cells are primarily dependent on SNX27-mediated endosome-to-membrane recycling, and are involved in cancer progression. As GPCR recycling participates in cancer progression and GPCRs are currently the most extensively investigated drug targets in pharmaceutical studies, the targeting of SNX27 may be of great pharmaceutical interest, as endosome-to-plasma membrane recycling occurs in a SNX27-dependent manner. Therefore, the discovery of compounds, antibodies or small molecules that bind to functional SNX27 may provide novel avenues for targeted therapy of cancer.

Availability of data and materials

All information included in this Review is documented by recent and valid references.

Authors' contributions

ZB and HZ conceptualized and co-wrote the manuscript. ZB and SZ searched the literature, organized and wrote various

sections of the manuscript. HZ is the PI and grant holder. All authors read and approved the final manuscript

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

On behalf of all authors, the corresponding author states that there are no competing interests.

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