The molecular structure and function of Sorting nexin 10 (SNX10) in skeletal disorders, cancers and other pathological conditions

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Abstract

SNX10 is a member of the phox homology (PX) domain containing family of phosphoinositide-binding proteins. Intracellularly, SNX10 localises to endosomes where it mediates intracellular trafficking, endosome organization and protein localization to the centrosome and cilium. It is highly expressed in bone and the gut where it participates in bone mineral and calcium homeostasis through the regulation of osteoclastic bone resorption and gastric acid secretion; respectively. Not surprisingly, patients harbouring mutations in SNX10 mutation manifest a phenotype of autosomal recessive osteopetrosis (ARO) or malignant infantile osteopetrosis (MIOP), which is clinically characterized by dense bones with increased cortical bone into the medullary space with bone marrow occlusion or depletion, bone marrow failure and anaemia. Accordingly, SNX10 mutant osteoclasts exhibit impaired bone resorptive capacity. Beyond the skeleton, there is emerging evidence implicating SNX10 in cancer development, metabolic disorders, inflammation and chaperone-mediated autophagy. Understanding the structural basis through which SNX10 exerts its diverse biological functions in both cell and tissue specific manners may therefore suggest new therapeutic opportunities towards the treatment and management of SNX10-related diseases.
Introduction

Sorting nexins (SNXs) are members of a structurally diverse protein family of up to 49 members unified by the presence the phox homology domain, which mediates binding to phosphoinositide-enriched membranes where each member selectively participates in cargo sorting and protein trafficking along the endocytic pathway (Seet and Hong 2006, Teasdale and Collins 2012). The importance of SNXs in endocytic cargo selectivity and recycling was originally highlighted by sorting nexin 1 (SNX1), which was found to act as a peripheral membrane protein capable of mediating the cell-surface expression of the epidermal growth factor receptor (EGFR) (Kurten, Cadena et al. 1996, Cozier, Carlton et al. 2002). SNX1 was further shown to co-localize with autoantigen 1 protein, an early endosome marker, implying involvement in endosomal membrane function (Teasdale, Loci et al. 2001). Indeed, through physical interaction with membranes enriched with phosphatidylinositol 3-phosphate (PtdIns(3P)) or phosphatidylinositol 3,5-bisphosphate (PtdIns(3,5)P2), SNX1 mediates intracellular protein trafficking (Cozier, Carlton et al. 2002) in conjunction with the multimeric retromer recycling complex (Gullapalli, Wolfe et al. 2006). Searching human databases for sequences homology with SNX1 identified several additional SNXs, including sorting nexin-10 (SNX10), which contained conserved arg-arg-tyr-X-asp/glu and pro-pro-X-pro-X-lys motifs (Teasdale, Loci et al. 2001).

Structurally, SNX10 belongs to the ‘Phox–homology (PX) domain containing only’ SNX family (Teasdale and Collins 2012, Clairfeuille, Norwood et al. 2015). SNX10 binds to phosphoinositide 3-phosphate through amino acid residue 53 (arginine) and has been shown to prompt the formation of endosomal-related vacuoles in cells upon forced expression (Qin, He et al. 2006). Further, SNX10 is required for the formation of primary cilia in cultured cells, and
regulates ciliogenesis and patterning of visceral organs in zebrafish (Chen, Wu et al. 2012). While the regulatory effectors of SNX10 remain unclear, it has been shown to bind ATP6V1D and modulate ciliary trafficking and formation of cilia together with the small Rab8 GTPase (Chen, Wu et al. 2012). Consistent with this position, SNX10 has been localized to centrioles and the base of cilia in human renal carcinoma cells RCC10/VHL (Chen, Wu et al. 2012).

Autosomal recessive osteopetrosis (ARO), also called malignant infantile osteopetrosis (MIOP), or the most severe form of osteopetrosis, is an osteoclast-rich form diagnosed at childhood and often lethal in untreated patients due to bone marrow failure (Sobacchi, Schulz et al. 2013). ARO is characterised by increased bone mass accompanied with poor and lack of bone marrow cavity (Sobacchi, Schulz et al. 2013). ARO patients present with low plasma calcium, high alkaline phosphatase, high parathyroid hormone (PTH), high 1,25(OH)2D, high creatine kinase BB, severe anaemia, deafness and bone marrow failure (de Vernejoul and Kornak 2010, Marcucci and Brandi 2015, Masi, Agnusdei et al. 2015).

In this review, we summarize the molecular structure, expression and regulation of SNX10. We focus our discussion on the role of SNX10 in ARO, including osteoclastic bone resorption, ruffled border assembly in osteoclast, bone mineralization, calcium ion homeostasis and development of osteopetrosis. Further, the emerging roles of SNX10 in cancer and other pathological conditions are also explored.

**The molecular structure and expression of SNX10**

A search of nucleotide sequence using National Center for Biotechnology Information (NCIB) data base shows that human SNX10 gene is denoted with GenBank AF121860.1 (Teasdale,
Loci et al. 2001). It is mapped to chromosome 7p15.2 based on a blast search alignment of the SNX10 sequence (GenBank: AF121860.1). It is designated with an Online Mendelian Inheritance in Man (OMIM) number of #615085, and referred to as autosomal recessive osteopetrosis-8 (OPTB8), which is a typified form of ARO and displays a similar phenotype to the mutation of TCIRG1 subunit of the vacuolar proton pump on chromosome 11q13 that has been designated as OMIM, #604592, or referred as OPTB1.

Sequence analysis reveals that human SNX10 gene encodes a protein of 210 amino acid residues. Multiple sequence analyses shows that the SNX10 shares strong amino acid sequence identity among species of human, mouse, bovine, danre and rat, indicating its conservative nature among species (Fig. 1A-B). Structural analysis reveals that it contains a PX domain between amino acid residues 10-127, which mediates interaction with membranes enriched in phosphatidylinositol 3-phosphate (Qin, He et al. 2006). It also encodes a region of amino acid residues 8-125 that is required for interaction with ATP6V1D (Chen, Wu et al. 2012) (Fig. 2A). A 2.8 Å resolution crystal structure of human SNX10 further revealed that SNX10 contains the extended phospho-homology domain (Xu, Xu et al. 2014). As shown in Figure 2 (B-D), two full-length human SNX10 molecules, from amino acid 8 to 158, were observed in a dimeric structure which might be induced by crystal packing (PDB: 4PZG, molecule A and molecule B in an asymmetric unit). The structure clearly shows a PXe domain very similar to SNX11: A conventional PX domain (pale green in molecule A and light blue in molecule B) with two extended α-helices (α4 and α5) in its C-terminus. The PX domain contains three antiparallel β-sheet (β1, β2 and β3), three α-helices (α1, α2 and α3) and a poly-proline PXXP (PPII) loop which links α1 and α2 helices. In addition, SNX10 contains five cysteine resides, where Cys39 and Cys49 in β2 and β3 strands form a disulfide bond which is unique compared with other homologues, such as SNX3, SNX11 and SNX12 (Xu, Xu et al. 2014).
The SNX10 gene is expressed in various cells and tissues, including neurons (Zhou, Li et al. 2011), osteoclasts, bone and teeth (Zhu, Morse et al. 2012). Utilising Genevisible® analyses on mouse transcripts (Hruz, Laule et al. 2008), SNX10 is most abundantly expressed in mouse osteoclasts, bone marrow macrophage and lumbar spinal cord motor neurons (Fig. 3A). SNX10 is expressed in many cell lines of various origin, as shown in Figure 3B, including RAW 264.7, macrophage like cells, derived from neoplastic cell lines of the hematopoietic, supportive of a unique functional requirement for SNX10 in these cell types.

The role of SNX10 in skeletal biology

A missense mutation in the human SNX10 gene, R51Q was first identified in patients with MIOP in distantly related consanguineous pedigrees (Aker, Rouvinski et al. 2012) (Fig. 4). Subsequently, a homozygous mutation, R16X (c.46C>T, p.Arg16X) in SNX10 gene was reported in a patient born from a consanguineous family (Megarbane, Pangrazio et al. 2013). Since then, several mutations in the sorting nexin 10 (SNX10) gene have been identified in families with ARO (Pangrazio, Fasth et al. 2013) (Figure 4).

Recently, individuals in Northern Sweden with ARO were identified with a premature stop (p.S66Nfs*15) of SNX10 caused by a splice site mutation in the SNX10 gene c.212+1 G>T, resulting from activation of a cryptic slice site, with defective ruffled border formation of osteoclast and impaired osteoclast function (Stattin, Henning et al. 2017) (Figure 4). More recently, ARO patients with polymorphisms of c.24+36T>A and c.112-84G>A in the intronic region of SNX10 gene were also reported (Kocak, Guzel et al. 2019). Thus, SNX10 is now firmly recognised as a candidate gene for ARO or MIOP (Shamriz, Shaag et al. 2017, Zhang, He et al. 2017), with mutations in SNX10 account for approximately 4% of affected infants.
among the known genes implicated in all reported ARO cases (Pangrazio, Fasth et al. 2013, Amirfiroozy, Hamidieh et al. 2017, Kocak, Guzel et al. 2019).

Hematopoietic stem cell transplantation treatment has been employed clinically to correct ARO phenotypes (Stepensky, Grisariu et al. 2019). Recently, the generation of iPS cells from an SNX10-deficient ARO patient has been attempted (Xu, Stattin et al. 2017). Using a retroviral-based reprogramming protocol, an induced pluripotent stem cell (iPSC) line (ARO-iPSC1-11) was generated from an ARO patient carrying the homozygous c.212+1G>T mutation (Xu, Stattin et al. 2017). The generated iPSCs were found to express pluripotency markers, and display normal karyotype and pluripotent differentiation capacity (Xu, Stattin et al. 2017).

Consistently, the osteopetrotic phenotype is recapitulated in mice lacking SNX10 (SNX10 -/-) owing to the defect of osteoclast bone resorption function (Ye, Morse et al. 2015, Zhou, You et al. 2016). Similarly, mimicking the phenotype of human mutation R51Q of SNX10 with ARO (Aker, Rouvinski et al. 2012), SNX10 R51Q knock-in mouse model manifest severe osteopetrosis with lack of bone resorption capability of mutant osteoclasts and impaired proton pump activity (Stein, Barnea-Zohar et al. 2020).

SNX10 global knockout mice exhibit osteopetrosis with defective bone resorption accompanied with impaired extracellular acidification, ruffled border formation in osteoclasts (Ye, Morse et al. 2015). In addition, the osteopetrotic phenotype of SNX10 knockout mice is superimposed with rickets (osteopetrorickets) owing to impaired bone mineralization. This impaired calcium-phosphorus homeostasis was attributed to increased stomach pH and low serum calcium in SNX10-deficient mice (Ye, Morse et al. 2015). In keeping with this position,
calcium supplementation was able to rescue rachitic phenotype of global SNX10-deficient mice. Interestingly, osteoclast-specific SNX10 knockout only developed severe osteopetrosis without rickets without affecting calcium homeostasis, indicating that osteopetrorickets is likely a result of dysfunctional gastrointestinal calcium absorption (Ye, Morse et al. 2015).

Further, SNX10 deficiency was resilient to collagen-induced arthritis (CIA)-induced bone loss and joint destruction partly owing to impaired osteoclast maturation and bone-resorption function (Zhou, You et al. 2016). In addition, in SNX10 knockout mice, the serum levels of TNF-alpha, interleukin 1 beta and anti-collagen IgG 2 alpha antibody were attenuated compared to those of (CIA)-induced wild type mice. Mechanistically, SNX10 (-/-) osteoclasts showed impaired integrin beta3-Src-PYK2 signalling that is required for osteoclastic bone resorption signalling. Consistently, adenovirus–based overexpression of SNX10 was able to rescue the osteoclast function, accompanied with the rescue of osteoclast signalling defects and specific protein expressions (Zhou, You et al. 2016).

At the molecular level, SNX10 is highly expressed in femur and calvaria, and developing teeth of mouse embryo (Zhu, Morse et al. 2012). Interestingly, the expression of SNX10 is up-regulated during RANKL-induced osteoclast formation (Zhu, Morse et al. 2012), whereas SNX10 knockdown expression by siRNA inhibits osteoclast formation and resorptive function (Zhu, Morse et al. 2012). Using immunostaining and co-immunoprecipitation, SNX10 was found to co-localize with MMP9 in osteoclasts, and to mediate the secretion and activity of MMP9, key enzyme for osteoclast function (Zhou, Wang et al. 2017). SNX10 was also found to coimmunoprecipitate and colocalize with FKBP12 and early endosome antigen 1 (EEA1) in osteoclasts, suggesting that FKBP12 are involved in SNX10 mediated regulation of endosome/lysosome pathway, critical for osteoclast function (Battaglino, Jha et
SNX10 co-localizes with phosphoinositide(3)phosphate (PI3P) in osteoclast early endosomes, and coimmunoprecipitate with vesicle fractions (Sultana, Morse et al. 2020). Inhibition of Phosphoinositide Kinase, FYVE-Type Zinc Finger Containing (PIKfyve) using small molecule compound apilimod or knockdown expression of PIKfyve in cells lead to the accumulation of early endosomes, and inhibition of lysosome formation, and osteoclast tartrate-resistant acid phosphatase enzyme secretion (Sultana, Morse et al. 2020). Further, signalling studies revealed that SNX10 positively regulates JNK, p38, and ERK phosphorylation in osteoclast signalling pathway (Zhou, Wang et al. 2017).

The loss of function of SNX10 is also involved in the dysregulation of the small GTPase Rab8 and the endosomal pathway (Chen, Wu et al. 2012), the latter essential for osteoclast function. Consistently, ruffled border assembly/maturation is impaired in SNX10 mutant osteoclasts (Stattein, Henning et al. 2017, Battaglino, Jha et al. 2019). While the exact role of SNX10 in ruffled border formation requires further study, the localization of SNX10 in the endosome-lysosome compartment is in keeping with the widely recognised importance of endolysosomal and autophagic pathways in ruffled formation and function (DeSelm, Miller et al. 2011, Ng, Ribet et al. 2019).

The potential role of SNX10 in cancers

While SNX10 is best recognised for its homeostatic role in the skeletal system, emerging evidence now suggests that it may have an equally important role in the regulation of cancer development (Galvan, Frullanti et al. 2013, Warnecke-Eberz, Metzger et al. 2016, Cervantes-Anaya, Ponciano-Gomez et al. 2017, Zhang, Hu et al. 2018), metabolic disorders (Lind 2019, You, Bao et al. 2020), and inflammation (Lou, Li et al. 2017, Armstrong, Chen et al. 2019).
By microarray analysis, SNX10 was identified as one of six candidate tumor markers of a rat model of liver cancer with characteristics resembling a putative suppressor protein (Cervantes-Anaya, Ponciano-Gomez et al. 2017). By clusters of co-expressed genes associated with stomach adenocarcinoma (SA), underexpression of SNX10 was linked to poor prognosis, and suggested to be a putative prognostic biomarker of SA (Zhang, Wu et al. 2018). Galvan et al identify snx10 as involved in the gene expression signature of the non tumoral tissue associated with better prognosis: specifically, low snx10 expression is associated with better prognosis (Galvan, Frullanti et al. 2013). Further, snx10, as well as other genes, is overexpressed in the tumoral tissue as compared to the healthy one not only in advanced stages of esophageous cancer, but also in early stages, thus can be proposed as prognostic marker (Warnecke-Eberz, Metzger et al. 2016).

Compared to our current understanding of SNX10 in bone homeostasis and disease, the mechanism of SNX10 in cancer biology remains poorly understood. One study identified that SNX10 is an important gene regulated by apilimod, a phosphatidylinositol phosphate kinase PIP5KIII inhibitor which displays selective cytotoxic activity in B-cell non-Hodgkin lymphoma (B-NHL) (Gayle, Landrette et al. 2017, Gayle, Landrette et al. 2017). It was suggested that an anti-proliferative effect of apilimod might be attributed to the disruption of SNX10 mediated lysosome homeostasis (Gayle, Landrette et al. 2017, Gayle, Landrette et al. 2017).

Recently, a role from SNX10 in the regulation of autophagy processes has been reported in cancer cell lines (Gayle, Landrette et al. 2017, Le, Zhang et al. 2018, Zhang, Yang et al. 2020). Using HCT116 cells model of colorectal cancer (CRC), SNX10 knockout resulted in
Increased chaperone-mediated autophagy (CMA) dependent amino-acid metabolism and mTOR activation (Le, Zhang et al. 2018). SNX10 knockdown promoted whereas SNX10 over-expression decreased tumorigenesis in the mouse colorectum (Zhang, Hu et al. 2018). Consistently, injection of neoplastic colorectal epithelial cells with SNX10 under-expression showed increased initiation and progression of CRC in mice (Zhang, Yang et al. 2020). It was revealed that SNX10 regulated the SRC levels by mediating autophagosome-lysosome fusion and non-receptor tyrosine kinase (SRC) recruitment for autophagic degradation, as well as the SRC-STAT3 and SRC-CTNNB1 signalling pathways. These studies suggest that injection with SRC-S1161 adenovirus significantly increased SRC activation and promoted tumorigenesis in CRC xenograft models in mice (Zhang, Yang et al. 2020). Interestingly, increased expression of SNX10 was found in human atherosclerotic lesions of aortic sections in a preclinical study in animal models (Padilla, Jenkins et al. 2014). SNX10 has been associated with the metabolic syndrome with cardio-metabolic risk factors in individuals (Lind 2019). Using genome-wide association study, annotated variants at the SNX10 locus were found involved in influencing adipose tissue function (Cannon, Curnin et al. 2019). Further, increased expression of SNX10 was reported in one of the 15 genes that were among the less known roles.

The role of SNX10 in other pathological conditions

SNX10 mediates SRC signalling pathway is important for cellular pro-metaboligenic signals in CRC (Zhang, Yang et al. 2020).
that SNX10 deficiency inhibited foam cell formation through interrupting the internalization of CD36. Further, Lyn-AKT activation in SNX10 KO cells is attenuated, leading to increased nuclear translocation of transcription factor EB (TFEB), a master regulator of lysosomal biogenesis, which results in increased lysosomal biogenesis and Lysosomal Acid Lipase (LAL) enzyme activity, free fatty acids accumulation and mitochondrial fatty acid oxidation (You, Bao et al. 2020).

In a mouse model of alcoholic liver disease (ALD), SNX10 deficiency increases the stability of LAMP-2A by inhibiting cathepsin A maturation, resulting in the increase of chaperone-mediated autophagy (CMA) activity and, thus, alleviates alcohol-induced liver injury and steatosis (You, Li et al. 2018).

More recently, SNX10 was implicated in immune-related biological processes in lung macrophages associated with metabolic differences in healthy human subjects (Armstrong, Chen et al. 2019). In this study, they observed 95 CpG loci with significant differential methylation in lung macrophages, and identified SNX10 as a potential gene loci in lung macrophages that may influence metabolic differences in various regions of lung (Armstrong, Chen et al. 2019). Consistent with its role in immune system, SNX10 expression was found upregulated in macrophages infected with L. monocytogenes and it promoted phagosome maturation by recruiting the Mon1-Ccz1 complex to endosomes and phagosomes. SNX10 deficiency decreased the bacterial killing ability of macrophages, and SNX10-deficient mice showed increased susceptibility to L. monocytogenes infection in vivo (Lou, Li et al. 2017).

Conclusions
In summary, this review encapsulates the structure, expression and function of SNX10, with a focus on in skeletal system. In addition, the emerging roles of SNX10 in cancers, metabolic disorders and inflammation are also discussed. There is an increasing interest in unlocking the precise regulatory role of SNX10 in endolysosomal and autophagic pathways (Fig. 5). There are remaining issues as to how SNX10 regulates various cell types and disease processes. In addition, it is important to identify small-molecule agonists or antagonises of SNX10 to modify its activity and localization. Thus, further deciphering the mechanism by which SNX10 mediates various pathological conditions will pave the way for developing SNX10 as a therapeutic and diagnostic target in translational medicine.
References


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Author contribution

Jiake Xu conducted research and drafted the manuscript. Heng Qiu and Jinmin Zhao provided evaluation and assistance in figure conformation and protein structural analysis. Nathan Pavlos contributed to the art work of Figure 5. Nathan Pavlos and Jiake Xu supervised the study and revised the manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest: All authors declare that they have no conflict of interest.
Figure legends

Figure 1. A. Multiple sequence alignment of amino acid sequences of SNX10 in of human, mouse, bovine, danre and rat, with conservative nature. B. A family tree of SNX10 proteins is shown.

Figure 2. Molecular structural views of human SNX10. It contains a functional domain carrying out two main purposes: phosphoinositide-binding (PX domain) and ATP6V1D interaction (ATP6V1D interaction domain). This functional domain also includes three phosphatidylinositol 3-phosphate binding sites required for protein sorting and membrane trafficking. Differ from the canonical isoform, SNX10 isoform 2 where 1-84AA region is missing was indicated. B-D. Tertiary structure analysis of SNX10. Two human SNX10 crystal molecules in different colors were lined up in the asymmetry unit. B-factor refers to the certainty of analytical atoms in which Blue indicates high confidence while Red is low confidence. This figure was generated by Pymol. Source: PDB 4PZG.

Figure 3. Human mutations of SNX10. Naturally occurring mutations have been identified with autosomal recessive osteopetrosis (ARO) in human: R16X, R16L, Y32S, R51P, R51Q and S66Nfs*15.

Figure 4. Expression analyses showing the SNX101 genes in 10 most highly mouse tissues (A) and cell lines (B). The analyses were performed based on Genevisible data (http://genevisible.com).

Figure 5. Hypothetical working model of SNX10 in osteoclast function via regulating endolysosomal and autophagic pathways.
Fig. 1A

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Fig. 1B

![Diagram of SNX10 Proteins](image)
Figure 2

A. Schematic representation of SNX10 domain structure.

- **PX Domain**: 10aa-127aa
- **ATP6V1D Interaction Domain**: 79aa
- **Putative Phosphatidylinositol 3-Phosphate Binding site**: 53aa-94aa

B. Molecular modeling of SNX10 domains.

- **Front view**
- **Side view**
- **Vertical view**

C. Structural components labeled:
- **α1, α2, α3, α4, α5**
- **β1, β2, β3**
- **PPII loop**

D. 3D model of SNX10.
Mutant R16L

Mutant R16X

Mutant Y32S

Mutant R51P

Mutant R51Q

Mutant S66Nfs*15