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An assessment of the human Sortilin1 protein network, its expression and targetability using small molecules

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Abstract

Background Sortilin1 (SORT1) is a ubiquitously expressed transporter involved in sorting or clearing proteins and is pathologically linked to tissue fibrosis and calcification. Targeting SORT1 may have potential clinical efficacy in controlling or reversing cardiovascular fibrosis and/or calcification. Hence this study assessed the protein-protein network of human SORT1 and its targetability using small molecule nutra/pharmaceuticals.

Material and methods Network proteins of SORT1 in homo sapiens was identified using the String database, and the affinity of the protein-protein interaction of this network was analysed using Chimera software. The tissue specific expression profile of SORT1 was evaluated and assessed for enrichment in different cell types including the immune cells. A library of in-house small molecules and currently used therapeutics for cardiovascular diseases were screened using AutoDock vina to assess targetability of human SORT1. Concentration affinity (CA) ratio of the small molecules was estimated to assess the clinical feasibility of targeting SORT1.

Results IGF2R, NTRK2, GRN and GGA1 were identified as high affinity interaction networks of SORT1. Of these high affinity interactions, IGF2R and GRN can be considered as relevant networks in regulating tissue fibrosis or microcalcification process due to their influence on T-cell activation, inflammation, wound repair, and tissue remodelling process. The tissue cell type enrichment indicated major

expression of SORT1 in adipocytes, specialised epithelial cells, monocytes, cardiomyocytes, and thyroid glandular cells. The binding pocket analysis of human SORT1 showed twelve potential drug interaction sites with varying binding score (0.86 to 5.83) and probability of interaction (0.004 to 0.304). Five of the drug interaction sites were observed to be targetable at therapeutically feasible concentration of the small molecules evaluated. Empagliflozin, sitagliptin and lycopene showed superior affinity and CA ratio compared to established inhibitors of SORT1.

Conclusion IGF2R and GRN are relevant networks of SORT1 regulating tissue fibrosis or microcalcification process. SORT1 can be targeted using currently approved small molecule therapeutics (empagliflozin and sitagliptin) or widely used nutraceutical (Lycopene) which should be evaluated in a randomised clinical trial to assess the efficacy to reduce cardiac/vascular microcalcification process.

Introduction

Sortilin1 (SORT1) is a ubiquitously expressed membrane glycoprotein which functions as sorting receptor in the Golgi apparatus and a clearance receptor on the cells membrane which is vital to functional regulation of cell homeostasis.[1-4] In its role as a sorting receptor, it transports proteins from Golgi apparatus to their intended target site using the endosomes/lysosomes as a cargo, based on a pH based dissociation kinetics.[3, 4] Among the various transporter functions of SORT1, its ability to scavenge extracellular lipoprotein lipase[5] and promote mineralisation of extracellular matrix,[6] contribute to tissue fibrosis, calcification, and foam cell formation.[7, 8] SORT1 seems to favour intracellular accumulation of lipids and triglycerides by suppression and/or degradation of adipogenesis factors and lipid efflux transporters.[3, 7] The expression of SORT1 is highly enriched in adipocytes[9, 10] and smooth muscle cells,[7, 11] which together with its contribution to tissue fibrosis and calcification makes it a vital target in the development of unstable atherosclerotic plaques (UAP) and vascular calcification.

Increased expression of SORT1 is observed in calcified arteries of humans.[7, 8] Consistent with this SORT1 knockout mice show reduced arterial calcification without impacting bone mineral density.[7, 8] This suggest a specific role of SORT1 in pathological microcalcification process without disturbing the physiological bone mineralisation. SORT1 is reported to transport alkaline phosphatase (ALP) to extracellular vesicles (EV)[12] and trigger mineralisation of extracellular matrix (specifically collagen), which leads to functional compromise of otherwise an elastic tissue.[6, 7] The role of SORT1 in microcalcification can collaterally contribute to formation of unstable atherosclerotic plaques (UAP) through the synergistic effects of SORT1 on secreting proinflammatory cytokines (IL6 and IFN gamma) from macrophages.[13, 14] Inflammation and microcalcification are hallmarks of UAP to which SORT1 seems to have a direct contributory role, independent from its effects on lipoprotein metabolism.[7, 13] Interestingly the contribution of SORT1 to UAP is also independent of plasma cholesterol levels.[7, 13]

The microcalcification promotion role of SORT1 is not limited to vascular smooth muscles, as recently SORT1 was reported to promote aortic valve fibrosis and calcification following mechanical injury.[8] It appears to me that following tissue injury the transporter function of SORT1 facilitates extracellular matrix deposition (EV dependent and independent mechanisms) which is further reinforced by ALP induced calcification to minimise the impact of the tissue injury. During this calcification process if the endogenous pool of progenitor cells are not efficient to replace the damaged tissue, then the calcified tissue will persist by further mineralisation. There seems to be a transient window during which the activity of SORT1 can be pharmacologically interfered to promote tissue regeneration by either endogenous or exogenous progenitor cells. Hence to address the pharmacological interference of SORT1, this study assessed its protein network, expression profile in human tissues, and its targetability using small molecules.

Materials and Methods

The network protein analysis of human SORT1 was conducted as reported before using the STRING Database (https://string-db.org), to observe its functional protein-protein interactions.[15, 16] The protein networks were identified and reviewed in the UniProt database and the most resolved protein structure based on the full length of protein and atomic resolution was selected for analysis in this study. The PDB code or AlphaFold structure of the selected protein was used to import the protein structure onto the Chimera software and the number of hydrogen bonds (H-bond) formed between SORT1 and its associated network proteins at 10 Armstrong (10A) distance was evaluated. A heatmap of the number of H-bonds formed between SORT1 and its associated network proteins was generated to identify the high affinity interactions.

The protein expression profile of SORT1 in various human tissues were text mined from the Human Protein Atlas (https://www.proteinatlas.org) database together with the data on enrichment of SORT1 in various tissue cell types. The SORT1 mRNA expression pattern in human immune cells was also assessed from this database. The binding sites of human SORT1 were identified using the PrankWeb: Ligand Binding Site Prediction tool (https://prankweb.cz/). To assess the targetability of SORT1 by small molecules, in this study molecular docking of selected drugs approved for therapeutics of cardiovascular disease were evaluated against AlphaFold structure of human SORT1 using AutoDock vina 1.2.0. Briefly, the AlphaFold structure of SORT1 was optimised for molecular docking in the AutoDock MGL tools. The isomeric SMILES sequence of the selected small molecules were obtained from the PubChem database and were converted into the PDB format using the Chimera software.[17, 18] The PDB structures of small molecules were further processed in the AutoDock MGL tools for molecular docking as reported previously.[15-19] SORT1 AlphaFold structure was also screened against our in-house compound library to identify a high affinity inhibitor. AF38469 and SB203580 which are reported to be orally bioavailable SORT1 inhibitor were used as reference compounds.[3, 7-9] To assess the

targetability potential of SORT1 by the small molecules, the concentration affinity ratio (CA ratio) for each of the small molecules was calculated as described before.[17, 19]

Results

The network protein analysis of SORT1 in humans showed high affinity interactions with IGF2R, NTRK2, GRN and GGA1 (**Figure 1**). IGF2R is a cation-independent mannose-6-phosphate receptor, which facilitates transport of phosphorylated lysosomal enzymes from the Golgi complex/cell surface to lysosomes and also positively regulated T-cell coactivation.[20, 21] NTRK2 is a neurotrophic factor receptor tyrosine kinase which supports development and maturation of nervous system, including establishing nerve-nerve or nerve-glial cell synapses.[22, 23] GRN is a secreted protein which modulates inflammation, wound repair, and tissue remodelling through cytokine like mechanisms influencing fibroblasts and endothelial cells.[24, 25] GGA1 is a binding protein which facilitates a two-way protein sorting and trafficking between the trans-Golgi network (TGN) and endosomes.[26]

Figure 1:

Affinity of human Sortilin1 (SORT1; UniProt ID Q99523) to its network proteins. Network proteins of SORT1 from the string database is shown. The network proteins are represented using their gene code and UniProt ID. Heatmap showing the degree of interaction (number of hydrogen bonds (H Bonds)) between each of the network protein and SORT1 at 10 Armstrong (10A) bond distance. Scale: green to red = high to low. The high affinity network proteins (IGF2R, NTRK2, GRN, and GGA1) in cyan are show interacting (yellow lines represent the H Bonds) with human SORT1 (brown).

The analysis of SORT1 protein expression in human tissues suggested major presence in brain, kidneys, skin, colon, placenta, endocrine glands and testis (epididymis) (**figure 2**). The tissue cell type enrichment of this data suggested major expression in adipocytes, specialised epithelial cells, cardiomyocytes and thyroid glandular cells (**figure 2**). The analysis of SORT1 mRNA expression in immune cells indicated significantly higher expression in monocyte subsets (especially nonclassical monocytes) (**figure 2**).

Figure 2:

Expression profile of Sortilin1 (SORT1) in human tissues. The quantitative protein expression of SORT1 in various human tissue and cell types is shown as a heat map (Scale: green to red = high to low). The mRNA expression of SORT1 in human immune cells is shown as bar graph (The expression profile is categorised based on the nTPM ranging from 0 to 12 and colour coded based on cell category type). The cell type enrichment (Scale 0 to 1) of SORT1 protein in human tissues is shown as a vertical line graph and colour coded based on cell category type. The gender specific protein expression of SORT1 in various human tissue is shown qualitatively using images.

The binding pocket analysis of human SORT1 showed twelve potential drug interaction sites with varying binding score (0.86 to 5.83) and probability of interaction (0.004 to 0.304) (**figure 3**). The top binding pocket consisted of 11 amino acids (A432 A631 A634 A635 A636 A637 A667 A669 A671 A672 A718), 48 SAS points and 25 surface atoms with following dimensions (x, y, and z: -29.6892, 3.1138,

and -12.6381). The second binding pocket had a score of 4.6 and consisted of 17 amino acids (A341 A377 A386 A387 A388 A435 A674 A675 A676 A677 A678 A679 A680 A681 A691 A697 A698), 66 SAS points and 33 surface atoms with following dimensions (x, y, and z: -31.2511, -5.5778, and 0.9165). The rest of the binding pockets had a score of <3 and probability of interaction of < 0.092 and hence are not described.

Figure 3:

Targetability of human Sortilin1 (SORT1). The twelve binding pockets of SORT1 identified are colour coded and represented in 2D (the relative area of the colour indicates the binding score which ranged from 0.86 to 5.83). The molecular docking of lycopene (ball and stick model) with human SORT1 with its five interaction sites are shown. The bottom left panel shows the highest affinity interaction between lycopene (stick model) with human SORT1. The bottom right panel shows the interaction sites of lycopene (purple stick model) between human SORT1 (brown colour) and yes-associated protein 1 (YAP; cyan colour).

The screening of in-house ligand library against human SORT1 showed high affinity of lycopene against five of the SORT1 binding pockets (**Table 1**, **figure 3**). In a recent study SORT1 was reported to contribute to calcification of human aortic valves involving MAPK and YAP pathways which drive trans differentiation of functional cells to myofibroblastic-osteogenic phenotype.[8] Lycopene was observed to bind at several SORT1-YAP interaction sites (**figure 3**), suggesting its potential to interfere with SORT1-YAP signalling. The potential of various cardiovascular therapeutics to target SORT1 was also analysed (**figure 4**). Empagliflozin and sitagliptin showed therapeutically feasible affinity and CA ratio against SORT1 similar to that of lycopene (**figure 4**). Among the small molecules tested bisoprolol had the highest affinity against SORT1, however considering its very low CA ratio, the feasibility to achieve therapeutic safety is unlikely. Two of the orally bioavailable SORT1 inhibitors reported in the literature[3, 8] i.e., SB203580 (3.24 µM) and AF38469 (638.76 µM) showed affinity against SORT1 which was inferior to lycopene (1.23 µM) (**figure 4**).

Table 1:

Molecular docking of Lycopene with human SORT1

Figure 4:

Affinity of small molecules with human Sortilin1 (SORT1). The affinity and concentration-affinity (CA) ratio value of various small molecules against SORT1 is summarised in the table. The images show the degree of interaction (number of hydrogen bonds; yellow lines) and the interaction sites between the selected small molecules and SORT1 (brown colour).

Discussion

SORT1 is reported to be the driver of microcalcification process associated with cardio-valvular and cardiovascular diseases independent of abnormalities with lipid metabolism and plasma cholesterol levels.[1, 7, 8, 27] Hence this study analysed targetability of SORT1 and has identified new/therapeutic

small molecules (Lycopene, empagliflozin and sitagliptin) with affinity to SORT1 at therapeutically feasible range which can be tested for efficacy in a clinical trial.

The major network proteins of SORT1 identified in this study seem to vastly support the transporter function of SORT1. However the SORT1-GRN network is of specific interest within the context of microcalcification process. The GRN dependent modulation of inflammation, wound repair, and tissue remodelling process is synergistic with the SORT1 facilitated extracellular matrix deposition reinforced by ALP induced tissue calcification.[24, 25, 28] A recent study has reported the role of MAPK and YAP pathways in SORT1 induced aortic valve calcification following mechanical injury.[8] While it is possible that biological response to acutely induced mechanical injury may differ from subacute/chronic injury classically seen with cardio-valvular and cardiovascular diseases in humans. The increased expression of SORT1 observed in calcified arteries of humans[7, 29, 30] does suggest that some overlap exists in biological response to acute, subacute and chronic injury to tissues. This concept was validated by a preclinical study simulating acute mechanical tissue injury[8] and pharmacological interventions demonstrating the efficacy of orally bioavailable SORT1 inhibitors in reducing the incidence of microcalcification process.[7, 8, 31, 32] The comparison of the affinity of these SORT1 inhibitors with lycopene, empagliflozin and sitagliptin in this study suggest the potential superior efficacy outcomes from using these small molecules, which will be interesting to be evaluated in randomised clinical trials. The therapeutic use of empagliflozin[33, 34] and sitagliptin[35, 36] in patients who are at higher risk of developing tissue calcification should allow rapid testing of its clinical efficacy in reducing the incidence of calcified aortic valves and/or unstable plaques. Further as lycopene is a generally regarded as safe nutraceutical,[37, 38] a coformulation of lycopene/empagliflozin or lycopene/sitagliptin can also be evaluated. Concerns are also expressed over achieving therapeutic concentration of lycopene in circulation.[39, 40] However the large volume of distribution of lycopene (150 to 1400 litres or 2-19 l/kg) together with its high lipo-solubility suggests that it is extensively distributed into peripheral tissues.[39, 40] Additionally population studies have shown serum lycopene concentrations of >0.7 μM, suggesting SORT1 targetable concentration (CA ratio ~ = 1) is achievable. In addition to the SORT1 inhibitors other allosteric approachs such as small molecules which can interfere with SORT1-YAP or SORT1-GRN signalling may also be developed as therapeutics for clinical management of calcified valves/arteries.

The expression of SORT1 in cardiomyocytes although is consistent with its pathological role in valvular calcification,[8, 41] its lower expression in vascular smooth muscles contradicts its reported role in arterial calcification. In this context the contributory role of non-classical monocytes (myeloid cells) to formation of viable vascular smooth muscles can be a mechanism by which SORT1 leads to vascular classification.[42, 43] The constant recycling of vascular smooth muscle cells by myeloid cells is an essential process in retaining optimal vascular physiology and the role of SORT1 in this process is unclear. However following mechanical injury to arteries, the myeloid cells promote remodelling of vascular smooth muscles under an inflammatory milieu and paraphs SORT1 plays an active role in this process.[42, 43] The highest expression profile of SORT1 mRNA in the monocyte subpopulation among all

immune cells together with its expression in calcified vascular tissue supports its role in pathological vascular remodelling process eventually leading to microcalcification. SORT1 is highly expressed in adipocytes which is consistent with several studies linking the disturbed lipid metabolism with valvular and arterial calcification process.[3, 44, 45] However some preclinical studies have also reported lipid and cholesterol independent mechanisms of SORT1 in inducing tissue calcification.[41, 46-49] The lipid independent role of SORT1 on tissue calcification is of specific interest in the context of UAP, as none of the therapeutics currently used for lipid management are shown to reduce the risk associated with UAP vascular pathology.[50-52] Hence, targeting of SORT1 together with or without lipid management therapeutics can prove to be beneficial in improving vascular physiology by reducing microcalcification process. The potential to target SORT1 using currently approved small molecule therapeutics (empagliflozin and sitagliptin) or widely used nutraceutical (Lycopene) identified in this study is particularly promising and should be evaluated in a randomised clinical trial to assess the efficacy to reduce cardiac/vascular microcalcification process.

Declaration of interest statement

none

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