Targeted manipulation of the sortilin-progranulin axis rescues progranulin haploinsufficiency

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Abstract

Progranulin (*GRN*) mutations causing haploinsufficiency are a major cause of frontotemporal lobar degeneration (FTLD-TDP). Recent discoveries demonstrating sortilin (SORT1) is a neuronal receptor for PGRN endocytosis and a determinant of plasma PGRN levels portend the development of enhancers targeting the SORT1/PGRN axis. We demonstrate the efficacy of several approaches through which impairing PGRN's interaction with SORT1 restores extracellular PGRN levels. Our report is the first to demonstrate the efficacy of enhancing PGRN levels in iPSC-neurons derived from FTD patients with PGRN deficiency. We validate a small molecule preferentially increases extracellular PGRN by reducing SORT1 levels in various mammalian cell lines and patient-derived iPSC-neurons and lymphocytes. We further demonstrate that SORT1 antagonists and a small molecule binder of PGRN588-593, residues critical for PGRN-SORT1 binding, inhibit SORT1-mediated PGRN endocytosis. Collectively, our data demonstrate that the SORT1/PGRN axis is a viable target for PGRN-based therapy, particularly in FTD-*GRN* patients.

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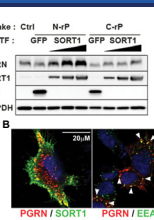
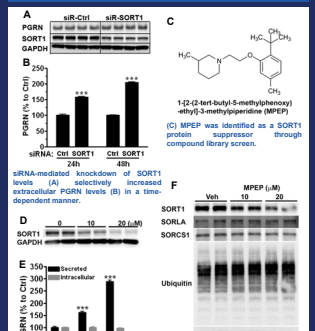
The authors declare no conflict of interest.

1. SORT1-mediated endocytosis in M17 neuroblastoma cells



(A) Dose-dependent uptake of PGRN (N-rP) by SORT1 over-expression in M17 cells as analyzed by western blot. As a negative control, C-terminal tagged PGRN (C-rP) was not endocytosed due to blocking of SORT1 binding motif. (B) Upon endocytosis, PGRN is localized to endo-lysosomal compartments as analyzed by double-immunocytofluorescence staining.

2. MPEP decreases SORT1 expression and increases extracellular PGRN

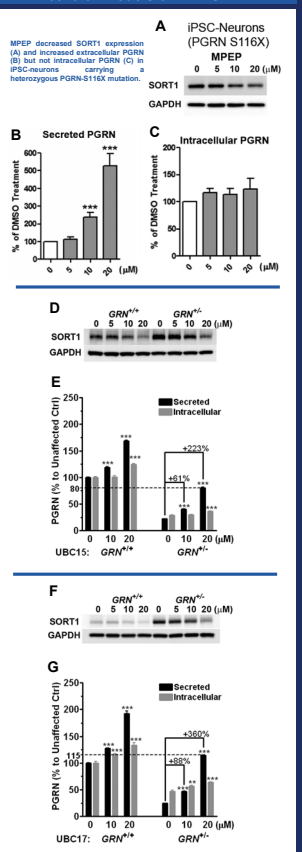


MPEP decreased SORT1 expression (D) and increased extracellular PGRN (E) in the M17 cells and others (not shown).

(F) To demonstrate compound specificity, MPEP reduced SORT1 level but not affecting levels of SORLA, SORCS1 and ubiquitinated proteins in M17 cells upon 24 hours treatment.

(G-H) MPEP increased PGRN and reduced SORT1 levels independent of transcriptional regulation. (I) MPEP suppressed SORT1 protein beginning at 2 hours of treatment. (J) MPEP-induced SORT1 downregulation was independent of autocrine regulation exerted by increased extracellular PGRN level.

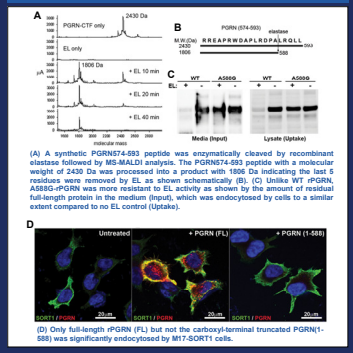
3. MPEP decreases SORT1 expression and increases extracellular PGRN in cellular models of FTD-*GRN*



MPEP decreased SORT1 expression (A) and increased extracellular PGRN (B) but not intracellular PGRN (C) in iPSC-neurons carrying a heterozygous PGRN-S116X mutation.

MPEP reduced intracellular SORT1 levels (D,F) and preferentially increased extracellular PGRN levels (E,G) in lymphoblastoid cell lines (LCLs) from 2 FTD-*GRN* families, UBC15 (D-E) and UBC17 (F-G). MPEP at 20 μM restored extracellular PGRN to near normal level in mutation carrier (*GRN*+/-) compared to non-carrier control (*GRN*+/+). UBC15 carries a p.R418X mutation. UBC17 carries a p.C31Lfs*34 mutation.

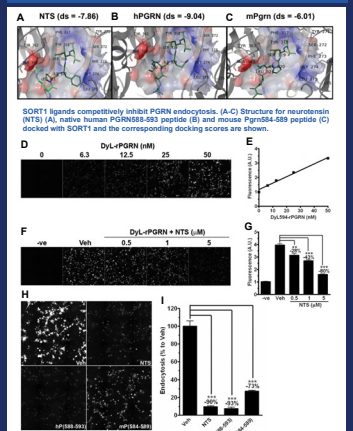
4. Elastase-mediated removal of C-terminal motif of PGRN blocks PGRN endocytosis by SORT1



(A) A synthetic PGRN574-593 peptide was enzymatically cleaved by recombinant elastase followed by MS-MALDI analysis. The PGRN674-693 peptide with a molecular weight of 2430 Da was processed into a product with 1606 Da indicating the last 5 residues were removed by EL as shown schematically (B). (C) Unlike WT rPGRN, A586G-rPGRN was more resistant to EL activity as shown by the amount of residual full-length protein in the medium (Input), which was endocytosed by cells to a similar extent compared to no EL control (Uptake).

(D) Only full-length rPGRN (FL) but not the carboxyl-terminal truncated PGRN(1-588) was significantly endocytosed by M17-SORT1 cells.

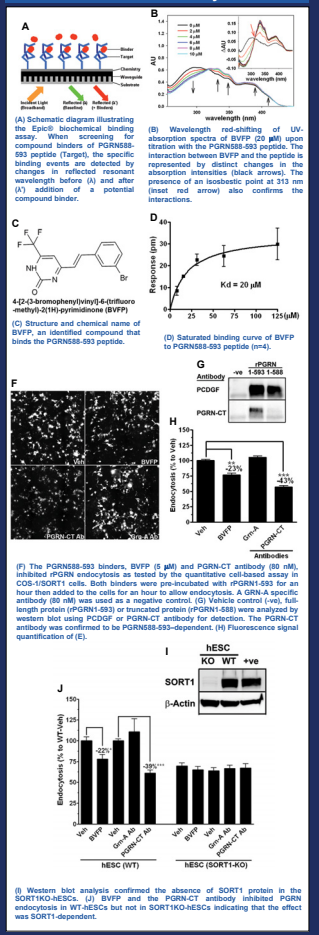
5. SORT1 ligands competitively inhibit PGRN endocytosis.



SORT1 ligands competitively inhibit PGRN endocytosis. (A-C) Structure for neurotensin (NTS) (A), native human PGRN588-593 peptide (B) and mouse Pgrn584-589 peptide (C) docked with SORT1 and the corresponding docking scores are shown.

DyLight™ 594-labeled rPGRN was endocytosed dose-dependently in COS-1SORT1 cells. (D) Images were captured by BD-pathway system. (E) Quantitative cellular endocytosis of DyL-rPGRN was measured by fluorescence signal from treated cells. (F) NTS at 0.1, 1 and 5 μM dose-dependently inhibited PGRN endocytosis. Untreated COS-1SORT1 cells were included as negative control (-ve). (G) Quantification of signal from (F). (H-I) SORT1 ligands at 10 μM. NTS, human PGRN588-593 peptide and mouse PGRN584-589 peptide competitively inhibited DyL-rPGRN endocytosis as compared to vehicle control, respectively.

6. Small molecule and antibody binders of the PGRN588-593 motif inhibit SORT1-mediated PGRN endocytosis.



(A) Schematic diagram illustrating the Epic® biochemical binding assay. When screening for compound binders of PGRN588-593 peptide (Target), the specific binding events are detected by changes in reflected resonant wavelength before (A) and after (A') addition of a potential compound binder.

(B) Wavelength red-shifting of UV-absorption spectra of BVFP (20 μM) upon titration with the PGRN588-593 peptide. The interaction between BVFP and the peptide is represented by distinct changes in the absorption intensities (black arrows). The presence of an isosbestic point at 313 nm (inset red arrow) also confirms the interactions.

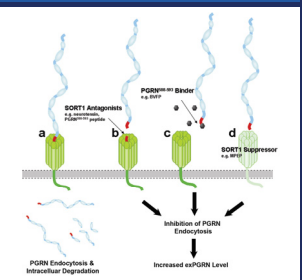
(C) Structure and chemical name of BVFP, an identified compound that binds the PGRN588-593 peptide.

(D) Saturated binding curve of BVFP to PGRN588-593 peptide (n=4).

(F) The PGRN588-593 binders, BVFP (5 μM) and PGRN-CT antibody (80 nM), inhibited rPGRN endocytosis as tested by the quantitative cell-based assay in COS-1/SORT1 cells. Both binders were pre-incubated with rPGRN1-593 for an hour then added to the cells for an hour to allow endocytosis. A GRN-A specific antibody (80 nM) was used as a negative control. (G) Vehicle control (-ve), full-length protein (rPGRN1-593) or truncated protein (rPGRN1-588) were analyzed by western blot using PCDGF or PGRN-CT antibody for detection. The PGRN-CT antibody was confirmed to be PGRN588-593–dependent. (H) Fluorescence signal quantification of (E).

(I) Western blot analysis confirmed the absence of SORT1 protein in the SORT1KO-hESCs. (J) BVFP and the PGRN-CT antibody inhibited PGRN endocytosis in WT-hESCs but not in SORT1KO-hESCs indicating that the effect was SORT1-dependent.

Summary



Schematic diagram summarizing the strategies applied to inhibit SORT1-mediated endocytosis in the current study. (a) Under normal conditions, extracellular PGRN interacts with the β-propeller tunnel structure of SORT1 using its C-terminal end binding motif. SORT1 facilitates endocytosis of exPGRN and directs it to the endolysosomal pathway for degradation. (b) High-affinity SORT1 ligands such as neurotensin (NTS) or the PGRN$_{583-593}$ peptide competitively limits the access of exPGRN to SORT1 binding sites, thereby inhibiting PGRN endocytosis. (c) To improve target specificity, we have also identified a small molecule binder, BVFP, targeting the PGRN$_{588-593}$ motif which is essential for PGRN-SORT1 interaction. We demonstrated that BVFP significantly reduced the amount of rPGRN captured by SORT1 *in vitro* and inhibited rPGRN endocytosis. (d) Suppressors of SORT1 expression, such as MPEP, reduce SORT1-mediated endocytosis, thereby increasing extracellular PGRN levels.

Conclusions

1. We have identified a compound termed MPEP that can selectively increase extracellular PGRN through reducing intracellular SORT1 in various models including iPSC-neurons from FTLD patient.

2. SORT1 ligands including neurotensin and PGRN588-593, a carboxyl-terminal peptide, block SORT1-mediated PGRN endocytosis in a quantitative cell-based endocytosis assay.

3. We have identified a compound termed BVFP that binds PGRN588-593 through compound library screen. As demonstrated in a human ES-SORT1 knockout cell model, BVFP inhibits PGRN endocytosis in a SORT1-dependent manner.

4. Hence we have demonstrated multiple strategies applied to inhibit SORT1-mediated PGRN endocytosis which support SORT1/PGRN axis as a target to develop PGRN enhancers for FTLD therapeutics.