TREAT-AD
TaRget Enablement to Accelerate Therapy Development for AD

PRDX1 (peroxiredoxin-1)

A Target Enabling Package (TEP)

Gene ID / UniProt ID / EC	5052 / Q06830/-
Target Nominator	AMP-AD, TREAT-AD
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USEFUL LINKS

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Agora	Visit Agora	View Alzheimer's disease target results explorer
AD Knowledge Portal	Visit AD Knowledge Portal	View available target enabling resources

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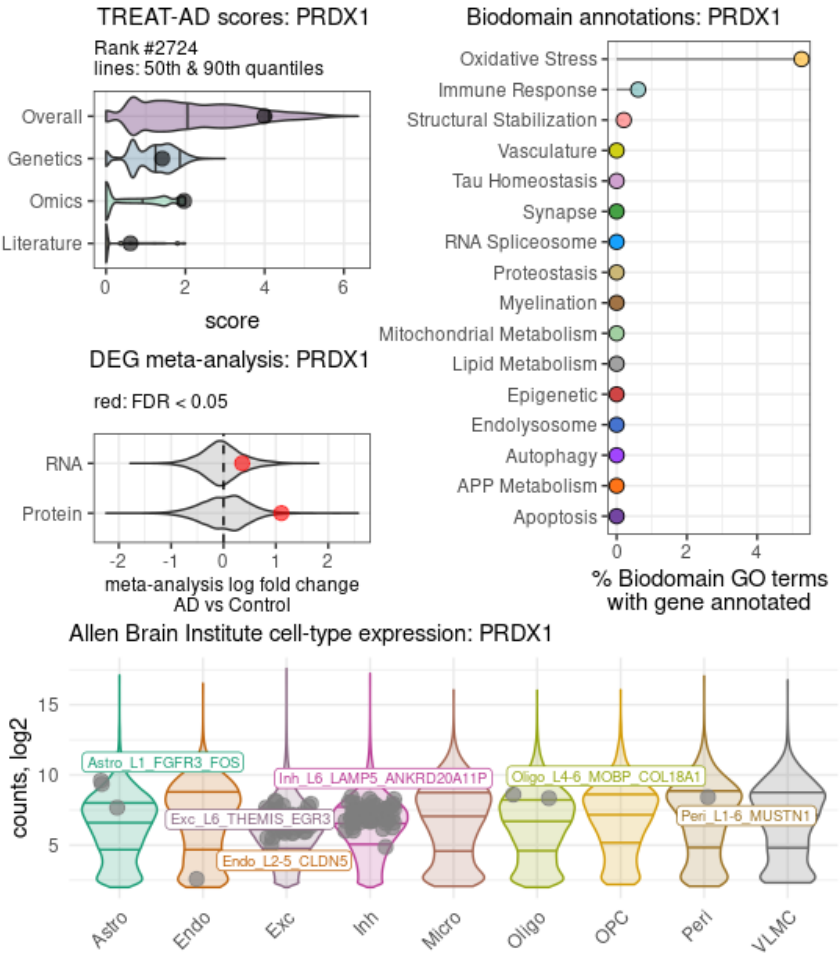
TARGET SOURCE & ANALYSIS

Why was the target selected? This target is found within a LFQ proteomics network module that was highly correlated with cognition. This module (Module 4) contains several novel targets for Alzheimer's Disease (AD), including CAPN2, MSN, and CD44 (1).

TREAT-AD Overall Score: 3.99 (rank #2724)

The Emory-Sage-SGC TREAT-AD Center has developed a target ranking score encompassing genomics, genetics, and literature evidence. The Overall Score represents the gene target's general relevance to Alzheimer's Disease, and is the sum of the target's Genetics Score, Genomics Score, and Literature Score. Overall Score values range from 0 to 7, with 7 being evidence of the strongest association with AD. A complete description of the methodology used to calculate these scores is available here. This score was taken from Agora, Data Version syn13363290-v33.

The TREAT-AD Overall score for this target is 3.99 out of 7 (rank #2724). The individual score components include 1.42 of 3 for genetics, 1.97 out of 2 for genomics, and 0.61 out of 2 for literature recency. The meta-analysis of proteomic and transcriptomic data sources used in the genomics score indicates that the expression of PRDX1, both protein and RNA, is significantly increased in brains from patients with AD.



The TREAT-AD Center has also developed a target categorization system specific to AD relevant processes, termed biological domains (biodomains). These biological domains are defined by constituent Gene Ontology (GO) terms and genes are then annotated to specific biological domains via GO term annotations. The biological domain characterization of the source module (Module 4) indicates that the proteins in this module are enriched for terms annotated to the Structural Stabilization, Lipid Metabolism, and Epigenetic domains. The most significantly enriched terms from this module are "focal adhesion", "cadherin binding", and "membrane raft". PRDX1 is annotated to GO terms primarily from the Oxidative Stress and Immune Response biological domains.

Cell-type specific expression is assessed using single-cell expression data from the Allen Brain Institute. The distribution of expression values for all genes found in each broad cell type are displayed as violins, points indicating the expression of the target in specific sub-types within each broad class including a label for the

highest expressing cell type for each broad class. These data indicate that PRDX1 is expressed in astrocytes, oligodendrocytes, both excitatory and inhibitory neurons, as well as pericytes in healthy adult brains.

SUMMARY OF PROJECT

Peroxiredoxin-1 (PRDX1) is involved in modulating oxidative stress and is expressed in numerous tissues, including brain(2). PRDX family members are thiol-dependent peroxidases which catalyse the reduction of hydrogen peroxide, peroxynitrite and alkyl hydroperoxides(3). Elevated levels of reactive oxygen species (ROS) are commonly observed in Alzheimer's disease (AD), and many other neurodegenerative diseases, and may contribute to neuronal damage initiating apoptotic processes, as noted in recent reviews (4-6). Consequently, PRDX1 may affect signalling pathways involved in neuroprotection and cell death by modulating oxidative stress. **The aim of this project is to produce TEP reagents to help further understand the biology of PRDX1 in Alzheimer's disease.**

SCIENTIFIC BACKGROUND

Cellular metabolism, under normal physiological conditions, produces some reactive oxygen species (ROS) and reactive nitrogen species, both of which are highly regulated by cellular antioxidants and enzymatic elimination by a number of different genes, including catalases, superoxide dismutases and members of the peroxiredoxin family, to give just a partial list (7). During late stage aging, this process becomes vulnerable due to changes in antioxidant enzyme levels, hypometabolic processes leaking ROS and aggregation of ROS catalysts (such as iron), leading to a fragile balance between production and elimination of ROS (8,9). Mitochondrial dysfunction and hypometabolism are commonly observed in AD (6,10,11), and strongly associated with increases in ROS and general oxidative damage in neurodegenerative diseases (12-14). ROS are capable of damaging numerous different biological molecules necessary for viable homeostasis, including DNA, RNA, lipids and proteins--the oxidation of each is implicated in AD (4,6,11). Accordingly, cumulative oxidative damage has been proposed as one mechanism of cellular aging in the brain (4,7). The elevated levels of PRDX1 observed in AD brains, at both the RNA and protein level, as mentioned in the bioinformatics section, may point to increases in oxidative stress and cellular attempts to compensate for elevated basal ROS levels. Also, the regulation of neuronal oxidative damage involves antioxidant mechanism in both neurons and glia (particularly astrocytes) (15), suggesting multiple potential mechanisms by which PRDX1 expression increases may reflect altered neuronal oxidative tone.

Peroxiredoxin-1 (PRDX1) is a member of the peroxiredoxin family of antioxidant enzymes, which reduce hydrogen peroxide and alkyl hydroperoxides (3). PRDX1 is expressed in numerous cell types in the brain in addition to neurons, including astrocytes, oligodendrocytes, and microglia (16). PRDX1 impairment may directly result in altered levels of ROS in brain, as PRDX1 inhibition leads to exacerbation of cellular damage, in diverse neuronal models (14,17,18). Additionally, PRDX1 promotes autophagic mechanisms of cellular clearance (19), which is the primary cellular process for the degradation of dysfunctional mitochondria and pathogenic tau aggregates (20-22), two neurotoxic elements of AD neuropathology. The plausibility of PRDX1 playing a trophic role in neurodegenerative disease is supported by the linkage of PRDX1 with both FGF1 and BDNF mechanisms of neuroprotective signalling (18,23). While circumstantial, the above data suggests a role

for PRDX1 in the regulation of ROS and down-stream signalling events that may contribute to AD pathology and present PRDX1 modulation as an exciting translational target for future investigations.

RESULTS – THE TEP

Validated Antibodies & Generic Knockout Cell Lines

The YCharOS Antibody Characterization Report guides researchers to select the most appropriate antibodies for PRDX1. The YCharOS antibody characterization pipeline uses knockout (KO) cells to perform head-to-head comparisons of available commercial antibodies for PRDX1 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. The cell line background was chosen based on the adequate expression of the target protein.

Complete report: PRDX1 antibody characterization report.

Protein constructs & expression methods

1. PRDX1: Full-length protein expressed in E. Coli: BL21(DE3)-R3-pRARE-pBirA with an N-terminal 6His tag and TEV cleavage site and a C-terminal Avi tag. The protein can be used for crystallography or assays.

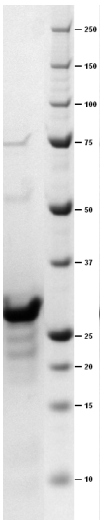


Fig 1. Purified full length PRDX1 with the tag cleaved

CONCLUSION

A number of studies have indicated that PRDX1 is closely correlated with Alzheimer's disease (AD). This TEP focuses on developing tools towards investigating the biology of PDRX1 and its modulation of ROS signalling. The tools presented here provide a foundation for further biological investigation of the role of PRDX1 in AD.

FUNDING INFORMATION

The work performed by the Emory-Sage-SGC TREAT-AD Center has been funded by the National Institute on Aging through grant U54 AG065187.

ADDITIONAL INFORMATION

Materials and Methods

Protein Constructs

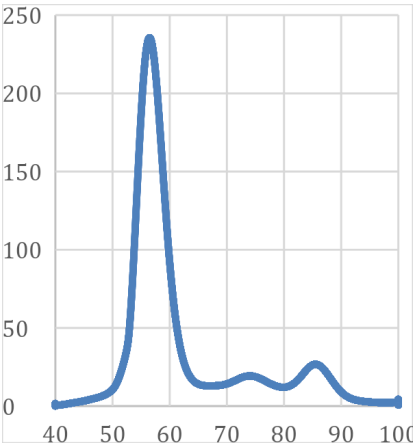
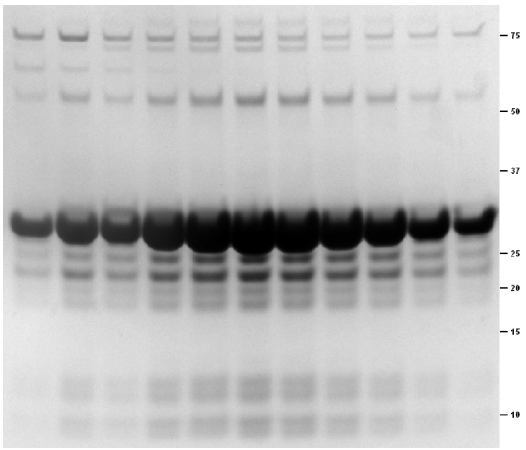
Plasmids are available on addgene: https://www.addgene.org/browse/article/28220285/

Protein Expression and Purification

Gene name	**PRDX1**
Uniprot ID	Q06830
Region	M1-K199

Description	Peroxiredoxin 1
Synonyms	Natural killer cell-enhancing factor A (NKEF-A), Proliferation-associated gene protein (PAG), Thioredoxin peroxidase 2, Thioredoxin-dependent peroxide reductase 2, Thioredoxin-dependent peroxiredoxin 1
Construct ID	PRDX1A-c001
Parental vector	pNIC-Bio3
Tag	N-terminal His6-TEV, C-terminal Avi
Protein mass (with tag)	27053.7
Protein mass (with tag removed)	24588.1
Extinction Coefficient (M^{-1} cm^{-1})	25440
Protein sequence (with tag)	MHHHHHHSSGVDLGTENLYFQSMSSGNAKIGHPAPNFKATAVMPDGQFKDISLSDY KGKYVVFFFYPLDFTFVCPTEIIAFSDRAEEFKKLNCQVIGASVDSHFCHLAWVNTPKK QGGLGPMNIPLVSDPKRTIAQDYGVLKADEGISFRGLFIIDDKGILRQITVNDLPVGRS VDETLRLVQAFQFTDKHGEVCPAGWKPGSDTIKPDVQKSKEYFSKQKSSKGGYGLNDI FEAQKIEWHE
Protein sequence (after tag removal)	SMSSGNAKIGHPAPNFKATAVMPDGQFKDISLSDYKGKYVVFFFYPLDFTFVCPTEIIA FSDRAEEFKKLNCQVIGASVDSHFCHLAWVNTPKKQGGLGPMNIPLVSDPKRTIAQD

	YGVLKADEGISFRGLFIIDDKGILRQITVNDLPVGRSVDETLRLVQAFQFTDKHGEVCPA GWKPGSDTIKPDVQKSKEYFSKQKSSKGGYGLNDIFEAQKIEWHE

Purified Protein	
SEC:	 
Intact Mass Deconvolution:	
Observed Mass:	
Protein yield:	26 mg/L of culture

Expression and Purification Protocol	
Expression host	*E. Coli*: BL21(DE3)-R3-pRARE-pBirA

Expression medium	Terrific broth (TB) with 50 µg/ml kanamycin (kan) and 50 µg/ml streptomycin (Strep). Starter cultures also included 34 µg/ml chloramphenicol (cm).
Transformation and storage	Transform the construct into the *E. coli* strain *E. Coli*: BL21(DE3)-R3-pRARE-pBirA, a phage-resistant variant of Rosetta 2 (MSD). Plate on LB-agar plates containing kan (50 µg/ml), cm (34 µg/ml), and strep (50 µg/ml). Inoculate LB broth containing the same antibiotics with several colonies. After overnight incubation at 37°C, add glycerol to 15% (v/v) final volume, and store at -80°C.
Expression	Inoculate LB + kan + cam +strep media for overnight growth at 37°C. Inoculate 1 L of TB + kan with 10 ml of the overnight culture. Grow cultures at 37°C with vigorous aeration in 2.5 L Tunair flasks until reaching an OD_{600} = 1.5 - 2. Shift the cultures to 18°C for 30 minutes before inducing protein expression with 0.3 mM IPTG. Continue incubation for approximately 16 hours at 18°C before harvesting cells by centrifugation (5000g, 10 min, 4°C). Store pellets and -80°C until needed.
Purification buffers	1. Lysis buffer: 50 mM HEPES (pH 7.5), 500 mM NaCl, 10 mM imidazole, 5% glycerol, 1 mM TCEP 2. Wash Buffer: 50 mM HEPES (pH 7.5), 500 mM NaCl, 30 mM imidazole, 5% glycerol, 1 mM TCEP 3. Elution Buffer: 50 mM HEPES (pH 7.5), 500 mM NaCl, 300 mM imidazole, 5% glycerol, 1 mM TCEP 4. SEC buffer: 50 mM HEPES (pH 7.5), 250 mM NaCl, 5% glycerol, 1 mM TCEP 5. Ni-sepharose beads, equilibrated in Lysis buffer.
Purification step 1: **IMAC**	1. Resuspend thawed pellet in lysis buffer (100 ml/L of original culture). Lyse cells by sonication on ice (20 min, 5 s on, 10 s off, 35% amplitude) with occasional stirring. 2. Centrifuge the lysate (25 min, 67000g, 4°C). Decant the supernatant. 3. Add 2 ml of Ni-sepharose beads per litre culture to lysate in 50 ml falcon tubes. Mix by rotation for 1 hr in a cold room. 4. Spin lysate with Ni-sepharose beads (700g, 5 min, 4°C). Decant lysate and wash beads with 50 ml Lysis buffer. Repeat wash with lysis buffer. Spin again and transfer beads to gravity column in a cold room. 5. Wash column with 10 ml wash buffer. 6. Elute protein with 3x 10 ml elution buffer. Analyse the fractions by SDS-PAGE and determine the protein yield using the Bradford assay.
Purification step 2: **Size-exclusion chromatography**	1. Protein eluted from the nickel beads should already be at a high concentration. 2. Purify the protein further by Size-exclusion chromatography (SEC) on a HiLoad Superdex S200 HR 16/60 column in SEC buffer.

	3. Analyse fractions by SDS-PAGE. Pool fractions containing protein of desired purity and concentrate to 10-20 mg/ml, as measured by UV spectroscopy. 4. Assess quality of protein by LC-MS intact mass analysis. 5. Snap-freeze aliquots in thin-walled PCR tubes in liquid N2, and store at -80°C.

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