Phenotypic screening with TREM2 knockout iCell® Microglia

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Summary

Background

Triggering receptor expressed on myeloid cells 2 (TREM2) is a transmembrane protein expressed in microglia that is known to associate with the signal transduction adaptor protein DAP to activate the Syk pathway, resulting in increased cell signaling for survival, proliferation and phagocytosis. From genome-wide association study, TREM2 has been identified as a risk factor for Alzheimer disease (AD) [1]. Earlier publications found lipoprotein and ApoE as TREM2 ligands and a recent paper identified amyloid-β peptide (Abeta) as a ligand of TREM2 [2].

Recently, it was reported that TREM2 upregulation from mTOR signal enhanced Abeta clearance and improved cognitive function in the AD mouse model [3]. Therefore, upregulation of TREM2 function may improve AD pathology and has emerged as a promising drug target.

1) Guerreiro,R. et al.,N.Engl.J.med.2013; 368(2):117-127. 2) Gratuze, M. et al., Mol. Neurodegener.2018; 13(1):66. 3) Shi,Q. et al., J.Neurosci. 2022; 42(27):5294-5313.

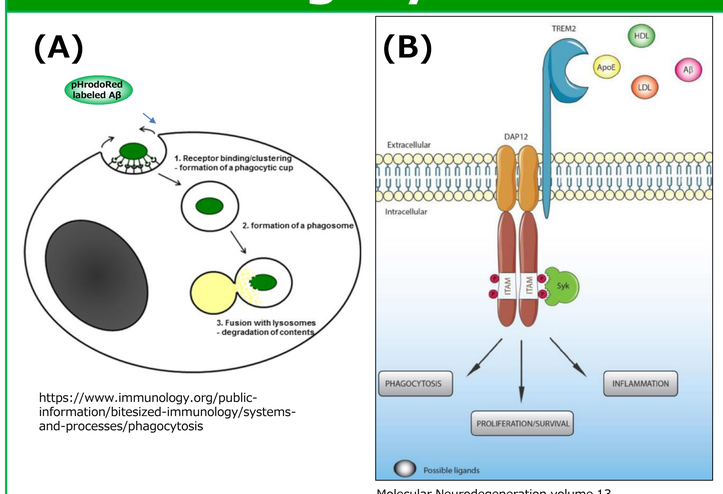
Objective

To discover small-molecule compounds that enhance microglial phagocytosis, we used iCell® Microglia from FUJIFILM CDI that featured a TREM2 variant homozygous (HO) mutant with a frame shift in exon 2 of TREM2 on both alleles, resulting in a functional TREM2 KO cell type.

Result

We successfully set up assay system and performed a high-throughput screening campaign by imaging analysis of phagocytic function using iCell® Microglia, identifying ten (10) compounds from an annotated library (c.a. 4000 cmpds) that enhanced phagocytic clearance of Abeta.

Phagocytic function of microglia



https://www.immunology.org/public-information/bitesized-immunology/systems-and-processes/phagocytosis

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Figure 1. (A) Phagocytosis process and (B) TREM2 signaling pathway

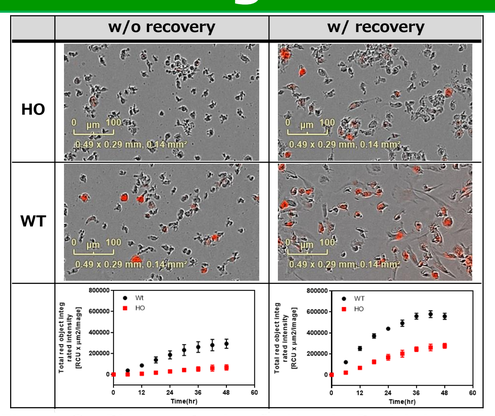


Figure 2. Phagocytosis of pHrodo Red-labeled Abeta in two types of TREM2 variant, a frameshift in TREM2 exon 2 in both alleles (homozygous; P59AfsTer16 and P59AfsTer46; iCell® Microglia TREM2 HO) near amino acid 60 was engineered in iPSC from an otherwise apparently healthy normal donor background, 01279 (WT).

Phagocytic activities for hit compounds

Table 1. Phagocytosis assay data with pHrodoRed-labeled Abeta and ATP cell viability assay with iCell® Microglia.

Compound	Phagocytic function for Abeta						ATP cell viability assay				ED50(-Log(M)) of phagocytic function-IC50(-Log(M)) of ATP assay	
	Maximum activity (% of control_ DMSO of Wt=100)		Fold induction (ratio to control_ DMSO of each cell=1)		ED50(-Log(M))		% Luminescence at concentration of max.phagocytic function, _DMSO of each cell=100		IC50(-Log(M))			
	HO	WT	HO	WT	HO	WT	HO	WT	HO	WT	HO	WT
Compound A	248	246	5.7	2.5	5.9	6.3	120	98	6.3	4.5	-0.4	1.7
Compound B	181	166	2.9	1.7	7.8	7.6	93	81	5.4	5.4	2.5	2.1
Compound C	152	157	2.4	1.6	8.8	8.5	94	91	6.5	6.7	2.3	1.8
Compound D	139	146	2.0	1.5	7.0	6.5	95	90	5.1	5.6	1.8	0.9
Compound E	130	186	1.9	1.9	6.2	5.6	90	84	ND	5.3	ND	0.3
Compound F	130	145	1.9	1.5	6.5	6.1	98	87	ND	4.8	ND	1.3
Compound G	122	99	1.8	1.0	6.6	6.4	111	98	4.7	4.9	1.9	1.4
Compound H	108	132	2.6	1.3	6.8	7.1	93	89	5.1	6.3	1.7	0.8
Compound I	93	131	2.1	1.3	6.8	6.9	97	87	ND	6.0	ND	0.9
Compound J	89	167	1.9	1.7	7.1	6.1	110	115	ND	ND	ND	ND

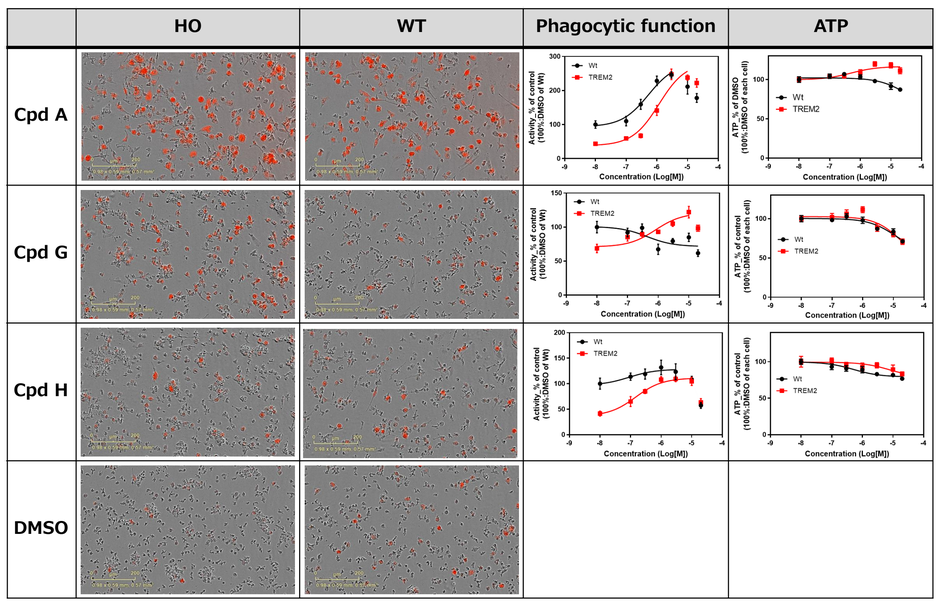
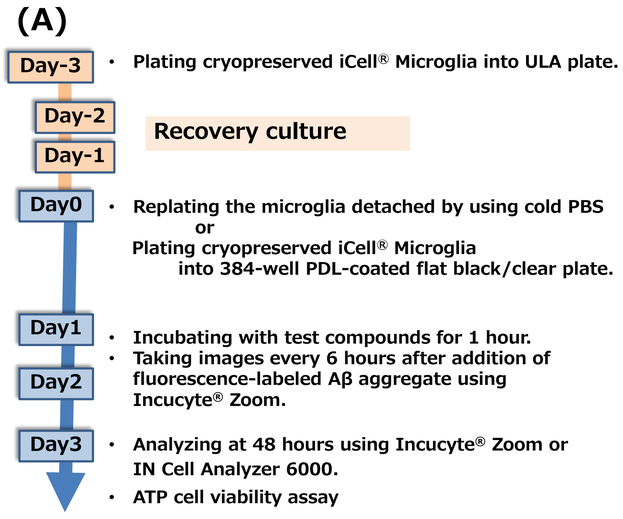


Figure 6. Phagocytosis assay data and ATP cell viability assay with iCell® Microglia for representative three compounds. Phagocytic function was expressed as % of DMSO-treated WT cells in each plate. ATP cell viability assay was expressed as % of each DMSO-treated cell in each plate. Data were Mean±SD, N=4.

Phenotypic screening with phagocytosis assay



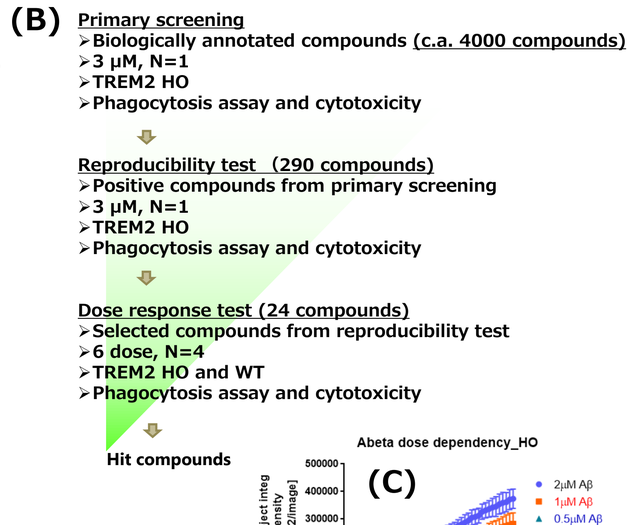


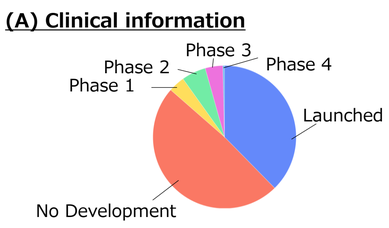
Figure 3. (A) Cell culture method and timeline; (B) Screening cascade; (C) Phagocytic activity with iCell® Microglia TREM2 HO showing dose dependency of Abeta.

Annotation library evaluated on this study

Annotation library

- ✓ Consist of approx. 4000 compounds
- ✓ Cover approx. 2000 genes※1
- ✓ Purchasable compounds
- ✓ Original transcriptome data

※1: The gene annotation data was manually curated from public databases



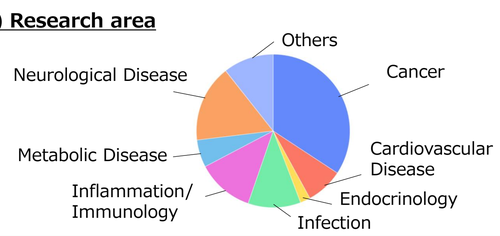


Figure 4. Classification of annotation library based on (A) clinical information and (B) research area.

Primary screening results

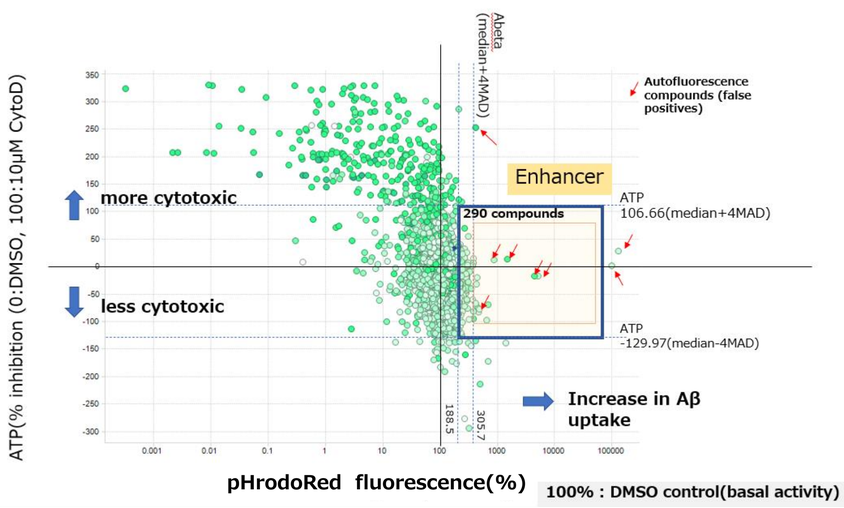


Figure 5. Primary screening data. Plot of ATP (cell viability) vs. Abeta uptake (phagocytosis assay).

Only 21 compounds surrounded by an orange square were low cytotoxic and statistically significantly enhanced pHrodoRed fluorescence. For reproducibility test, 290 compounds, maximum number of assay capacity, surrounded by a blue square were selected. Autofluorescence compounds featured with red arrow were excluded from hit candidate compounds.

Classification of hit compounds based on the MOA

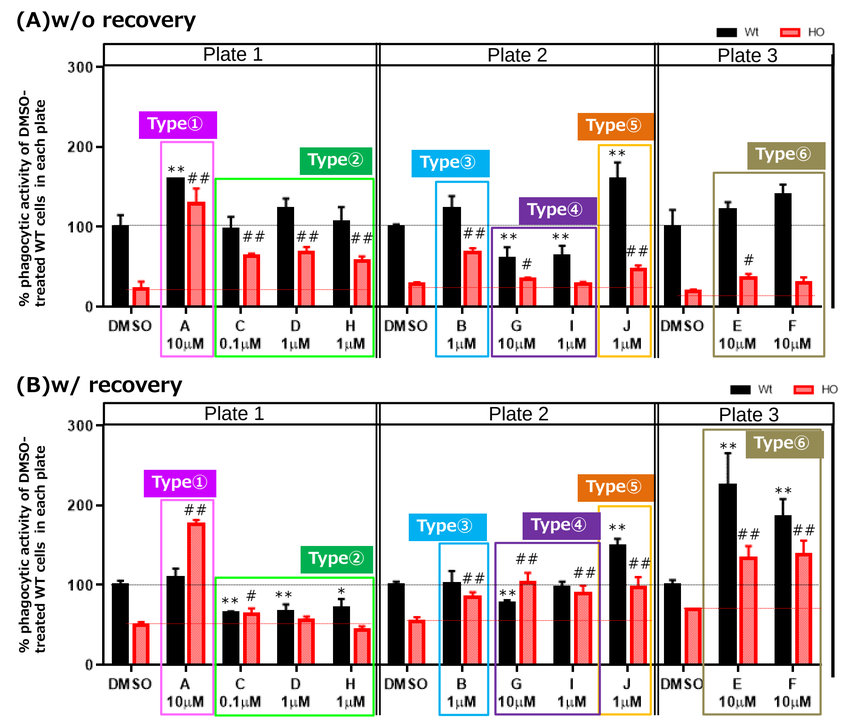


Figure 7. The effects of hit compounds on phagocytosis at 48hr in iCell® Microglia without recovery (A) and with recovery (B).
Each data was expressed as % of DMSO-treated WT cells in each plate.
A statistical test was performed by unpaired two-tailed t-test with the aid of GraphPad Prism version 6.07. **p<0.01, *p<0.05 as compared with data from DMSO-treated WT in each plate. ##p<0.01, #p<0.05 as compared with data from DMSO-treated HO in each plate.

Type	w/o recovery		w/ recovery	
	WT	HO	WT	HO
Type①	↑↑	↑↑	→	↑↑
Type②	→	↑↑	↓	→/↑
Type③	→	↑↑	→	↑↑
Type④	↓↓	→/↑	→/↓↓	↑↑
Type⑤	↑↑	↑↑	↑↑	↑↑
Type⑥	→	→/↑	↑↑	↑↑

Table 2. Classification of 10 hit compounds into six types based on the pattern of their effects on phagocytic function.
↑↑:Increasing strongly, ↑:increasing weakly, →:no change, ↓:decreasing weakly, ↓↓:decreasing strongly.

■ **TREM2 HO vs WT**

- ✓ Type ①, ⑤ and ⑥ statistically significantly enhanced phagocytic activity in both WT and TREM2 HO cells. Type ② and ④ enhanced phagocytic activity in TREM2 HO cells, but moderately lowered phagocytic activity in WT cells. These results suggest that they do not act directly on TREM2 protein but may act on the molecules which work downstream TREM2 signaling pathway, or which are involved in other phagocytic signaling pathway.
- ✓ The effects of type ③ were observed only in TREM2 HO cells. It also suggests that they do not act directly on TREM2, but act on the molecules which play a critical compensatory function in TREM2 HO cells.

■ **Without recover vs with recovery**

- ✓ Our data suggested that the effects on phagocytosis activity of hit compounds could alter depending on cell culture conditions (w/o recovery and w/ recovery), possibly reflecting their MOAs. It is worth characterizing the mechanisms laying behind the differences.

◆Further MOA analysis is ongoing. We will evaluate the effects of gene knockdown/knockout on the compound activity and will also test other functional assay systems, such as cytokine secretion and migration.

Acknowledgment

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