THGP stimulation promotes M1 macrophage differentiation and antitumor activity via the SIRP-α/CD47 axis in THP-1 cells

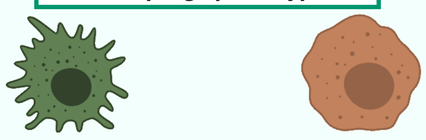
Junya Azumi[1], Tomoya Takeda[1], Yasuhiro Shimada[1], Hisashi Aso[1,2], and Takashi Nakamura[1]

[1]Research Division, Asai Germanium Research Institute Co., Ltd. [2]Laboratory of Animal Health Science, Graduate School of Agricultural Science, Tohoku University

The 85th Annual Meeting of the Japanese Cancer Association | Poster presentation

ASAI Germanium

Macrophage phenotypes



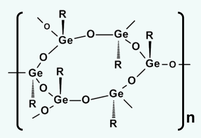
M1-like, pro-inflammatory/antitumor phenotype

M2-like, tumor-supportive phenotype

Shifting macrophages toward an M1-like state is important for cancer therapy

Reagent

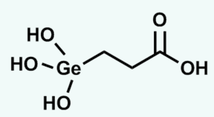
Polymer



Asaigermanium

Hydrolysis

Monomer



THGP

3-(trihydroxygermyl)propanoic acid

Asaigermanium is reported biological activities include immunostimulatory, anti-inflammatory, and analgesic effects.

Macrophage origins

Tissue-resident macrophages (TRMs)

Long-lived macrophages residing in tissues.



Langerhans cells



Alveolar macrophages



Microglia



Osteoclasts

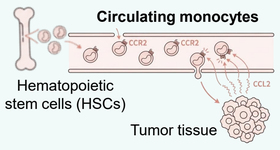


Kupffer cells

Involved in cancer initiation and early-stage progression

Monocyte-derived macrophages (MDMs)

Macrophages derived from recruited circulating monocytes.



Involved in later-stage cancer progression

Objective

Previous study



THGP addition to macrophage culture promoted M1-like differentiation

Macrophage-stage treatment in the previous study

Target of this study

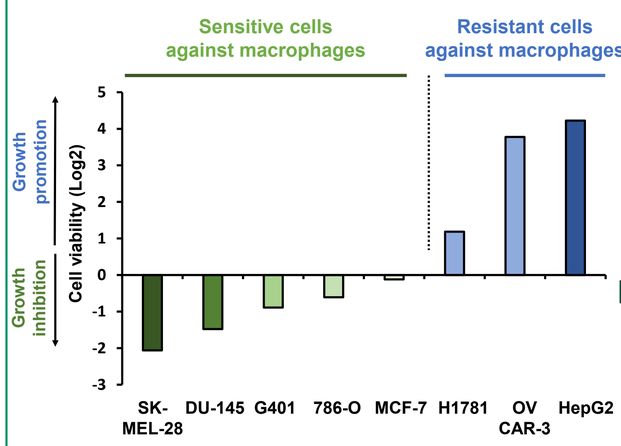


This study tested the effect of THGP addition to monocyte culture

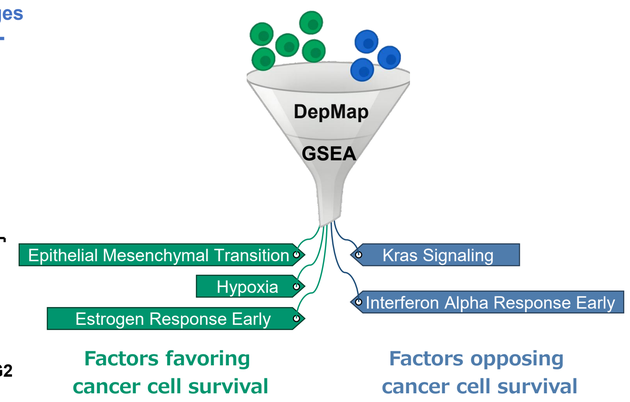
Monocyte-stage treatment in the present study

Result 3-1. EMT-related gene sets are enriched in resistant cancer cells

To identify factors that determine differences in the antitumor effects of THGP-treated macrophages, we obtained RNA sequencing data from various cancer cell lines from DepMap and performed database analyses.



Sensitive vs resistant cell lines



EMT-related gene sets were enriched in resistant cells.
This suggests that EMT may reduce sensitivity to THGP-conditioned macrophages.

Background and strategy

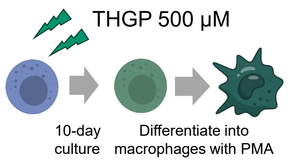
Background

Tumor-associated macrophages can suppress or support tumor growth. This study tested whether monocyte-stage THGP exposure programs THP-1 cells toward an M1-like state.

Strategy

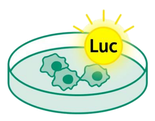
Phenotype, phagocytosis, coculture assays, DepMap expression data, Gene Set Enrichment Analysis, and CD47 blockade were used to define response mechanisms.

Step 1 — Differentiation assessment



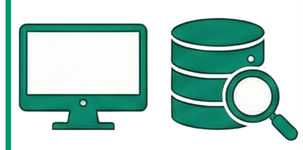
After THGP-conditioned monocytes were differentiated into macrophages, M1 markers were assessed

Step 2 — Antitumor activity assay



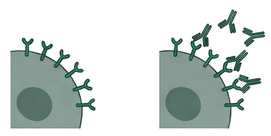
Assess macrophage antitumor activity against multiple cancer cell lines

Step 3 — Candidate factor analysis



Use database analysis to identify factors linked to antitumor activity

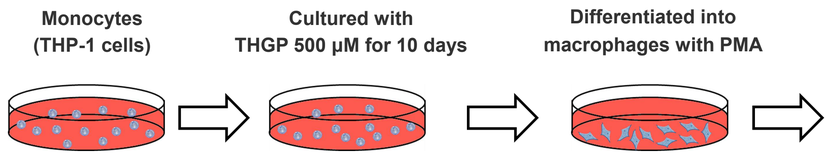
Step 4 — Functional validation



Test the effect of blocking candidate determinants with neutralizing antibodies

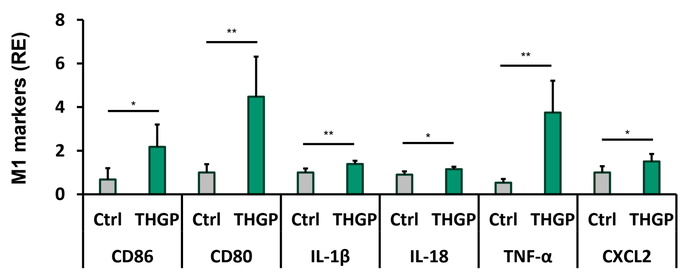
Result 1. THGP induces an M1-like macrophage phenotype

Methods



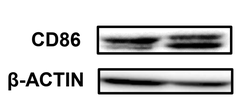
(A) Gene expression (qPCR)
(B) Protein expression (Western blot)
(C) Morphology
(D) Nuclear NF-κB localization (IF)
(E) Phagocytosis assay

(A)

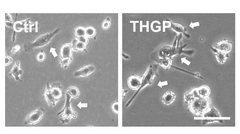


1.1-4.2 × Expression of M1 markers

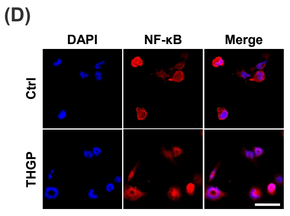
(B)



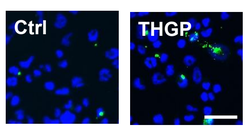
(C)

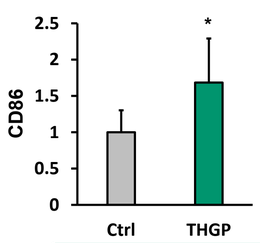


(D)

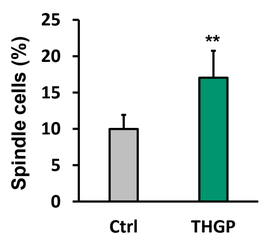


(E)

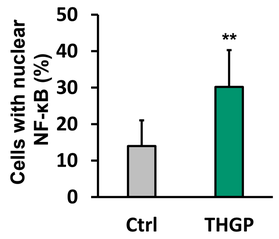




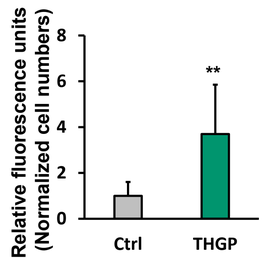
1.7× CD86 protein



1.7× dendritic morphology



2.3× nuclear NF-κB

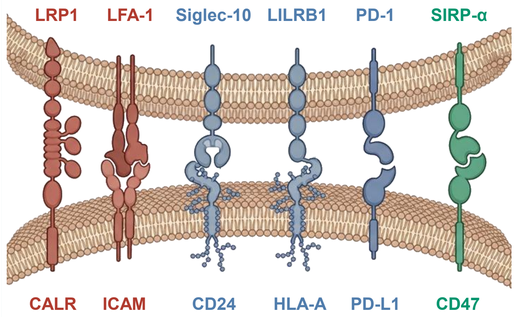


3.7× phagocytosis

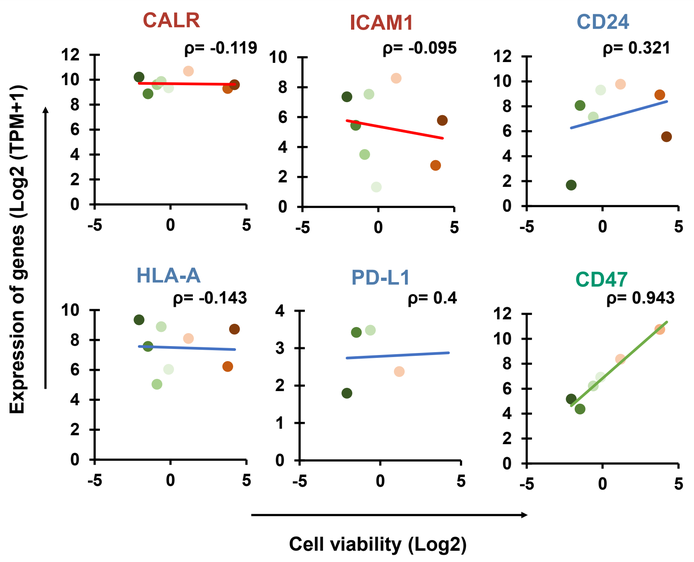
THGP increased M1-like morphology, M1 markers and phagocytosis.

Result 3-2. CD47 expression marks resistant cancer cells

We evaluated the correlations between the expression levels of cell-surface proteins involved in macrophage recognition (CALR and ICAM1) or inhibition of macrophage recognition (CD24, HLA-A, PD-L1, and CD47) and cancer cell viability in the presence of THGP-treated macrophages.



Red: recognition receptor
Blue/green: inhibitory recognition receptor



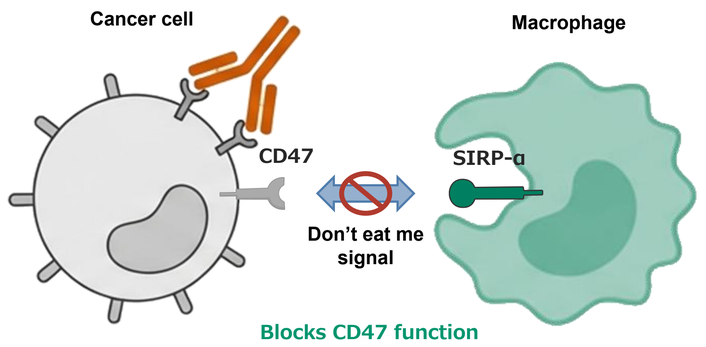
Plots compare gene expression with cancer cell viability after coculture with THGP-conditioned macrophages.

CD47 showed the strongest positive correlation with viability.

Result 4. CD47 blockade supports the mechanism

Mechanistic model

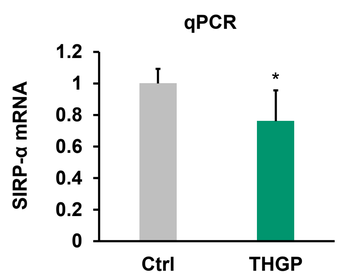
CD47-neutralizing antibody

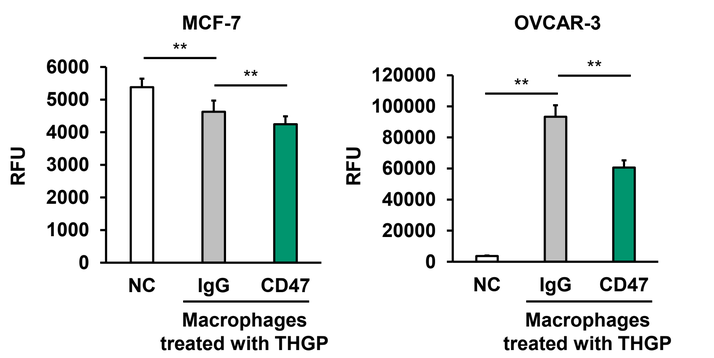


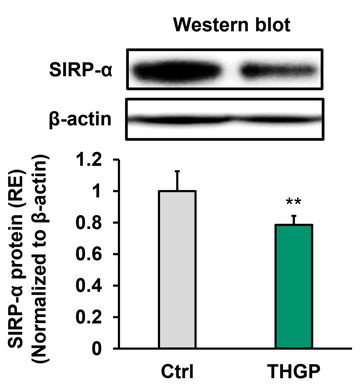
Blocks CD47 function

Functional validation

CD47 neutralization was tested in coculture. SIRP-α expression was measured by quantitative polymerase chain reaction and western blot.

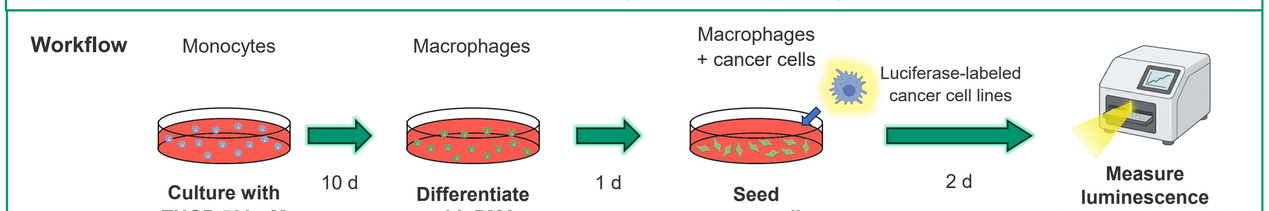


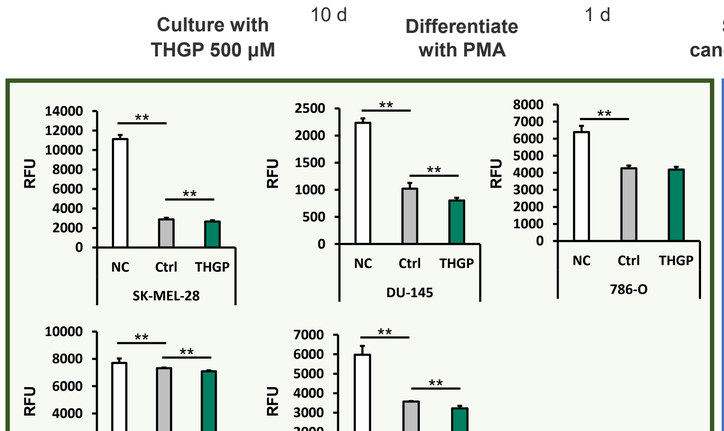




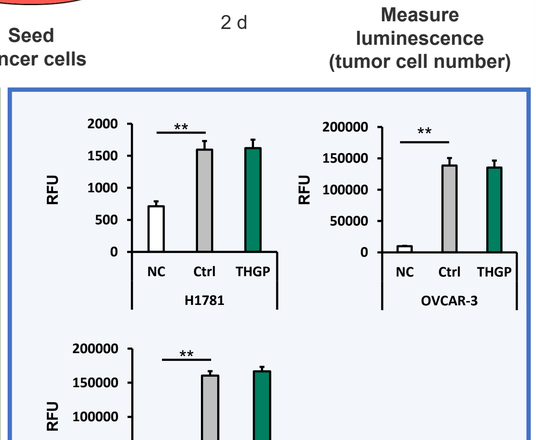
Pretreatment of monocytes with THGP was suggested to attenuate the SIRP-α/CD47 axis, thereby enhancing antitumor activity.

Result 2. THGP enhances antitumor activity in macrophage-sensitive cancer cell lines





Macrophage-sensitive cell lines

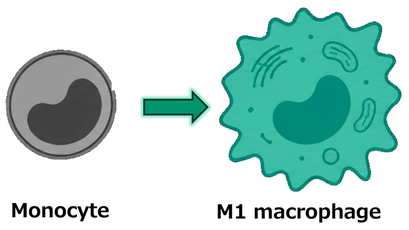


Macrophage-resistant or growth-promoted cell lines

THGP-conditioned macrophages showed enhanced antitumor activity in 4 of the 5 macrophage-sensitive cancer cell lines.

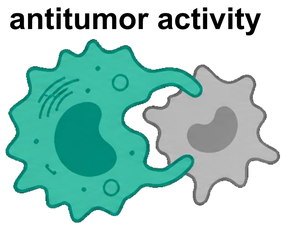
Conclusion

① M1 differentiation



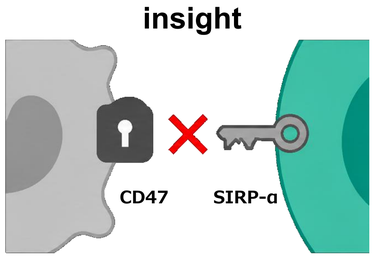
THGP promotes monocyte to M1-like macrophage differentiation.

② Enhanced antitumor activity



Differentiated macrophages by THGP are up-regulated antitumor activity.

③ Mechanistic insight



Reduced SIRP-α may attenuate the cancer-cell "don't eat me" signal.

Overall interpretation: Long-term THGP conditioning promotes M1-like macrophage differentiation and increases CD47-sensitive antitumor activity in THP-1 cells.

COI Disclosure Information Junya Azumi is employed by Asai Germanium Research Institute Co., Ltd.

Abbreviations: NF-κB, nuclear factor kappa B; PMA, phorbol 12-myristate 13-acetate; RFU, relative fluorescence units; EMT, epithelial-mesenchymal transition; GSEA, Gene Set Enrichment Analysis.