The organogermanium compound THGP suppresses norepinephrine-induced EMT in the gastric cancer cell line HGC-27 cells

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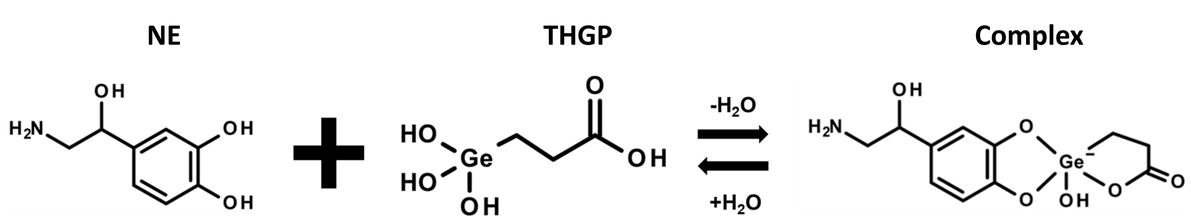
Purpose 3-(Trihydroxygermyl)propanoic acid (THGP) forms complexes with vicinal-diol moiety in the catechol, including norepinephrine (NE). NE, a stress hormone, induces epithelial-mesenchymal transition (EMT) in cancer cells. In this study, the effects of THGP on NE-induced EMT in human gastric cancer HGC-27 cells were evaluated.

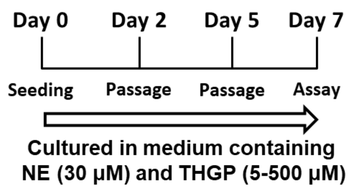
Feature of THGP



Poly-*trans*-[(2-carboxyethyl)germasesquioxane] is a polymeric organogermanium compound that undergoes hydrolysis in aqueous or physiological media to yield the monomer THGP. Additionally, THGP has been shown to form complexes with vicinal-diol structures. Endogenous components containing vicinal-diol structures, such as, adrenaline, L-DOPA, adenosine and ATP, interact with THGP and influence its biological activity.

Method

Protocol for Induction of EMT in HGC-27 Cells (Day 0- Day 7)



Without THGP and NE in assays (Day 8-)

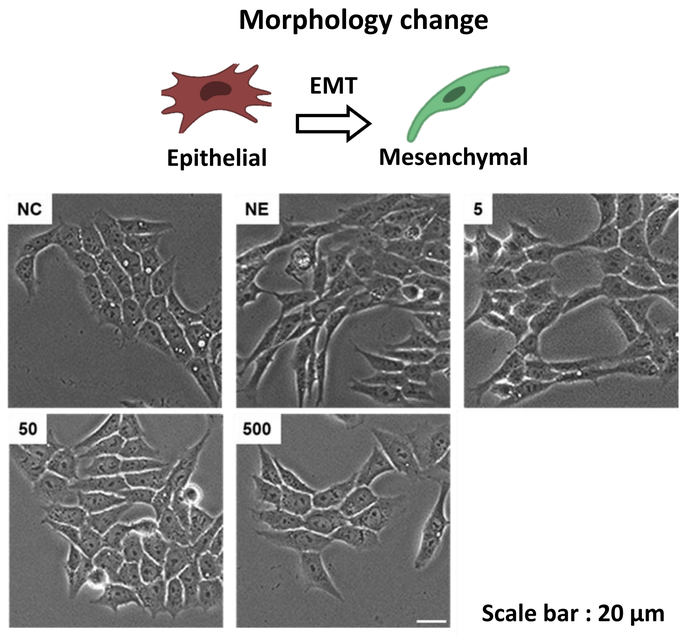
Immunofluorescence
Western blot
Real time RT-PCR
Drug resistance assay
Migration assay
Cell adhesion assay

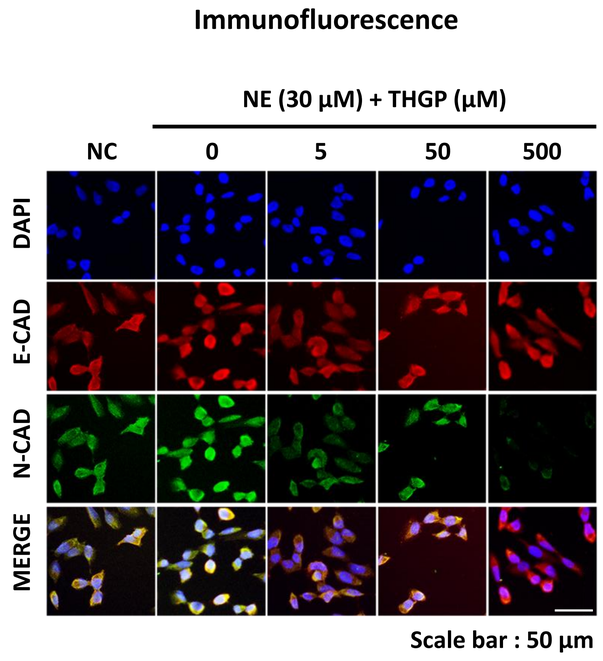
With THGP and NE in assays (Day 8-)

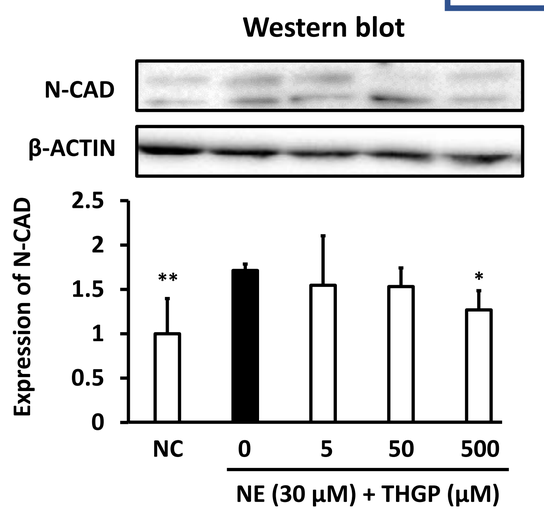
Colony formation assay
Spheroid assay

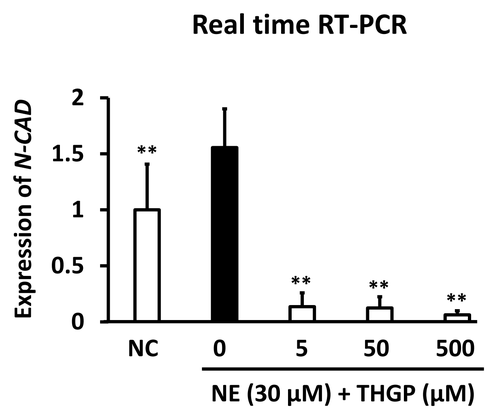
To induce EMT, HGC-27 cells were cultured for one week in medium containing NE and THGP. EMT-induced cells were subsequently used in the experiments described below. The colony-formation and spheroid assays were performed with NE and THGP maintained in the culture medium throughout the assays, whereas all other experiments were conducted in medium without NE and THGP. All experiments (n = 6) were analyzed using Dunnett's test. Significance is indicated as **p < 0.01 and *p < 0.05; all comparisons were made against the black bar in each graph (Ctrl or NE group).

Result 1. Norepinephrine - induced EMT and its inhibition by THGP







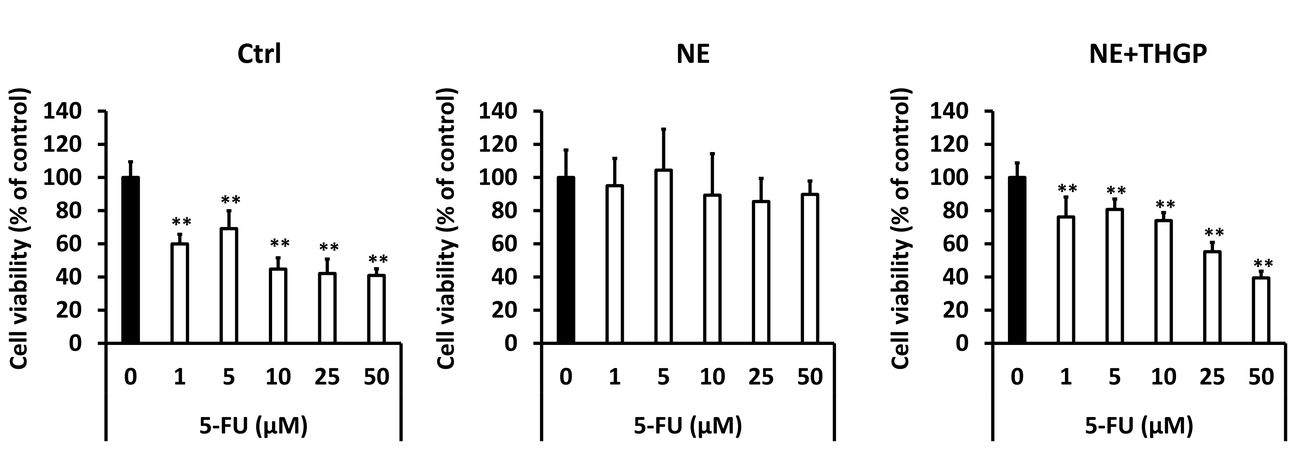


Time course: 0h – 24h (Seeding – Assay)

Seeding density
Western blot : 1×10^6 cells/60 mm dish
Immunofluorescence : 2×10^4 cells/8-well
Real time RT-PCR : 2×10^5 cells/6-well

NE induced a mesenchymal-like morphology in the cells and increased N-cadherin (N-CAD) expression.
Co-treatment with THGP prevented these morphological changes and reduced expression of EMT markers. For all subsequent experiments, cells were treated with 500 μM THGP.

Result 2. Assessment of resistance to anticancer agents



Time course: -24h – 0h – 48h (Seeding – Treated with drugs – Assay)

Seeding density: 5×10^3 cells/96-well

Assay: MTS assay

IC_{50} value (μM)

Drugs	Ctrl	NE	NE+THGP
5-FU	8.6	> 50	31.5
Cisplatin	7.4	> 50	8.9
Doxorubicin	< 0.05	> 2.5	1.4

HGC-27 cells were sensitive to all three drugs, but NE treatment markedly reduced sensitivity. Co-treatment with THGP prevented NE-induced chemoresistance.

Result 3. Evaluation of tumorigenic and metastatic properties *in vitro*

Colony formation assay

Time course: 0d – 7d (Seeding – Assay)

Seeding density: 5×10^3 cells/6-well

1 week

Spheroid assay

Time course: 0d – 28d (Seeding – Assay)

Seeding density: 1×10^4 cells/6-well

4 week

0.3% agar with THGP and NE
0.6% agar

Migration assay

Time course: -48h – -24h – 0h – 48h (Seeding – Scratched – Captured – Captured)

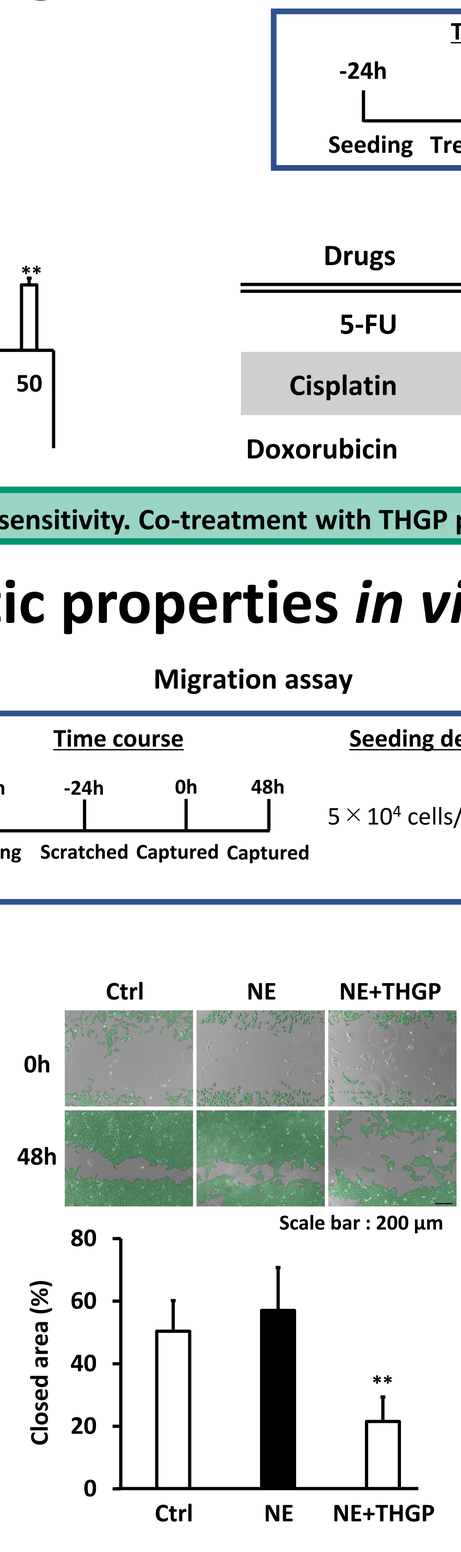
Seeding density: 5×10^4 cells/24-well

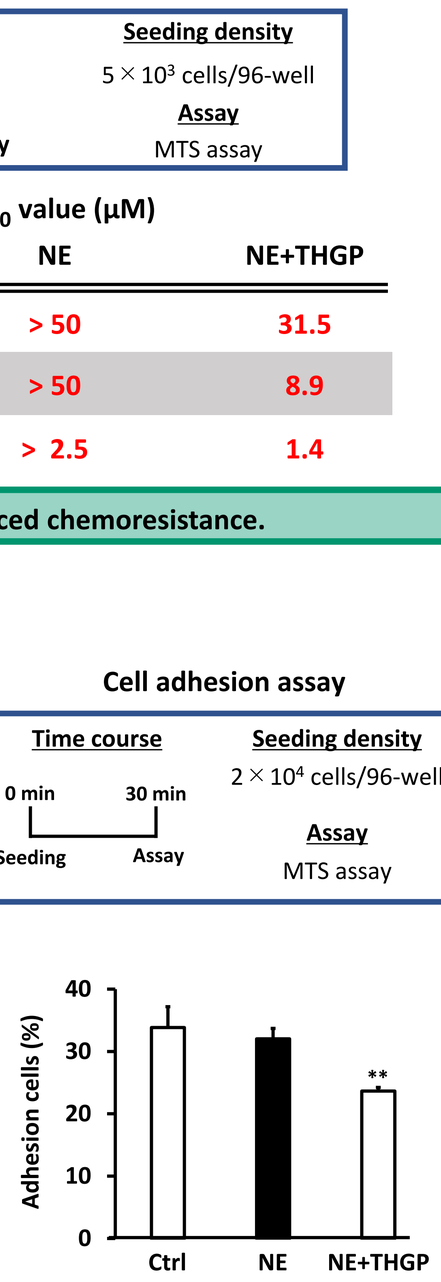
Cell adhesion assay

Time course: 0 min – 30 min (Seeding – Assay)

Seeding density: 2×10^4 cells/96-well

Assay: MTS assay





Treatment with NE increased tumorigenicity and metastatic capacity, and these effects were suppressed by THGP in HGC-27 cells.

Conclusion THGP suppresses NE-induced EMT, potentially enhancing chemotherapy efficacy and preventing metastasis in cancer patients under stress.

COI Disclosure Information

Junya Azumi (Leader presenter, Principal Researcher) is employed by Asai Germanium Research Institute Co., Ltd. Takashi Nakamura (Principal Researcher) is received remuneration from Asai Germanium Research Institute Co., Ltd., as an officer.