Pathophysiological function of Thyroid adenoma associated (THADA) in human cancer cells

Mizuki Katoh [1)], Takuto Fujii [1)], Takahiro Shimizu [1)], Yoshiaki Tabuchi [2)], Hideki Sakai [1)]

1) Department of Pharmaceutical Physiology, Faculty of Pharmaceutical Science & 2) Division of Molecular Genetics Research, Life Science Research Center, University of Toyama, Toyama 930-0194, JAPAN

Background

Cardiac glycosides (CGs) are Na^+,K^+-ATPase inhibitors. It has been reported that CGs have anti-cancer effects such as suppression of cancer cell proliferation and induction of apoptosis. However, anti-cancer mechanisms of CGs have not been fully clarified.

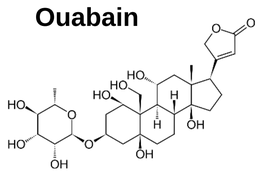
Thyroid adenoma associated (THADA) was identified in benign thyroid tumors. Human THADA is consisted of 1953 amino acid residues, and localized in the cytosolic side of the ER membrane. THADA has been reported to participate in the regulation of organismal metabolism and associated with type 2 diabetes and polycystic ovary syndrome. Very recently, Li *et al*. (J. Immunother. Cancer, 2021) reported that THADA plays a role in upregulating programmed death-ligand 1 expression in cancer.

Here, we studied whether THADA is involved in the anti-cancer mechanism of CGs in human cancer cells.

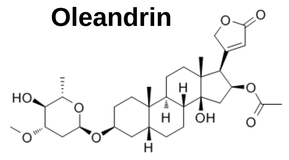
Methods

In this study, cardiac glycosides (ouabain, oleandrin and digoxin) and human oral epidermoid carcinoma KB cells were used. Microarray analysis was performed using KB cells transfected with THADA siRNA. Expression level of protein was assessed by Western blotting and immunostaining. Cell proliferation ability was evaluated by cell counting.

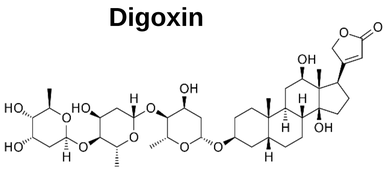
Ouabain



Oleandrin

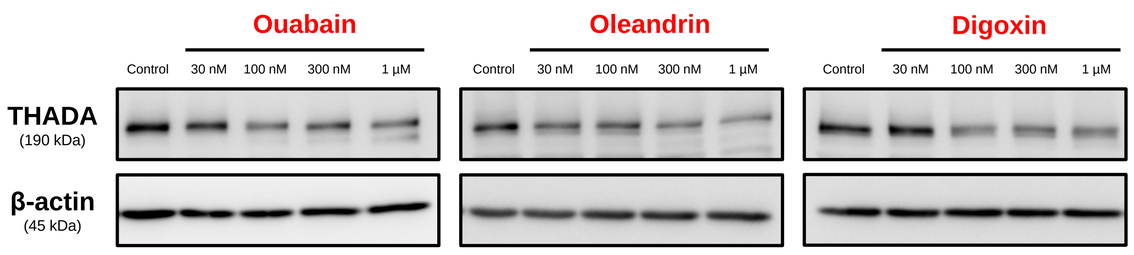


Digoxin



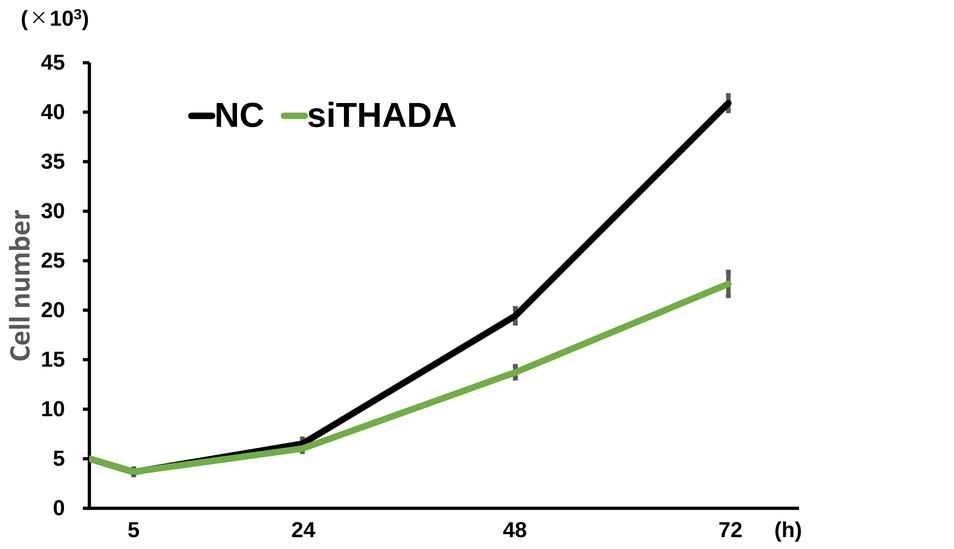
Results

① Expression level of THADA was decreased by CGs.



Effects of CGs (ouabain, oleandrin, and digoxin) on expression of THADA.
KB cells were treated with CGs (30 nM to 1 μM) for 24 h.

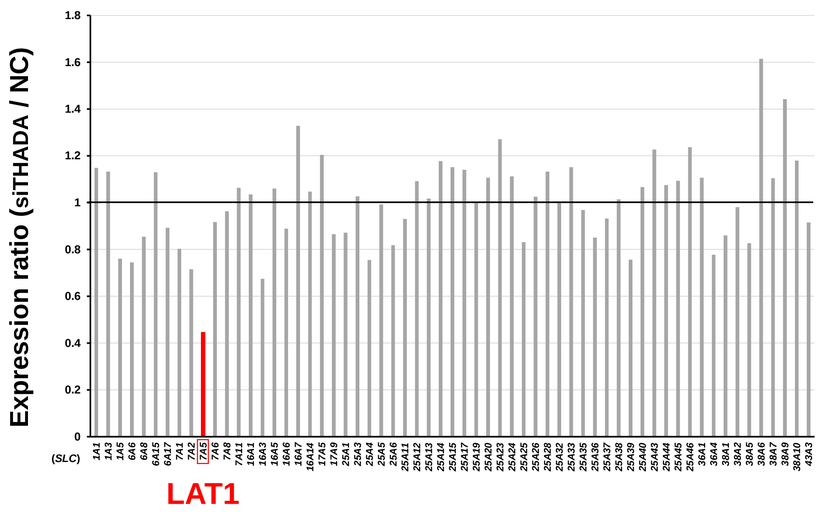
② Cell number was significantly decreased by THADA knockdown.



The number of KB cells was counted 5, 24, 48 and 72 h after transfection with negative control siRNA (NC) or THADA siRNA (siTHADA).

③ *SLC7A5* (LAT1) mRNA was significantly decreased in the THADA-knockdown cells.

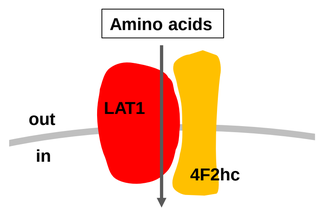
Microarray analysis



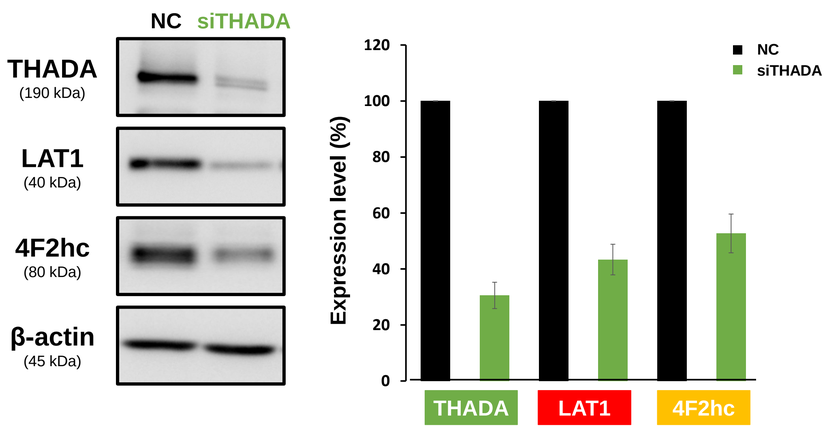
Expression ratio of amino acid transporters in KB cells transfected with THADA siRNA.

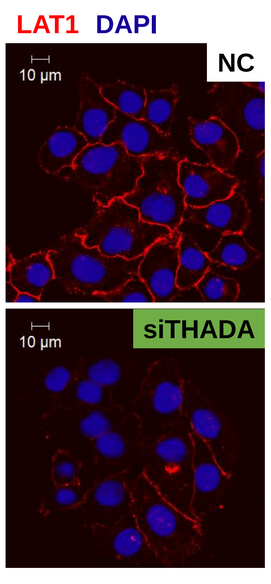
L-type amino acid transporter 1 (LAT1)

- LAT1 is a member of system L-type transporters, and located in the plasma membrane with 4F2hc.
- LAT1 is overexpressed in many malignant tumors, and it stimulates proliferation of cancer cells.



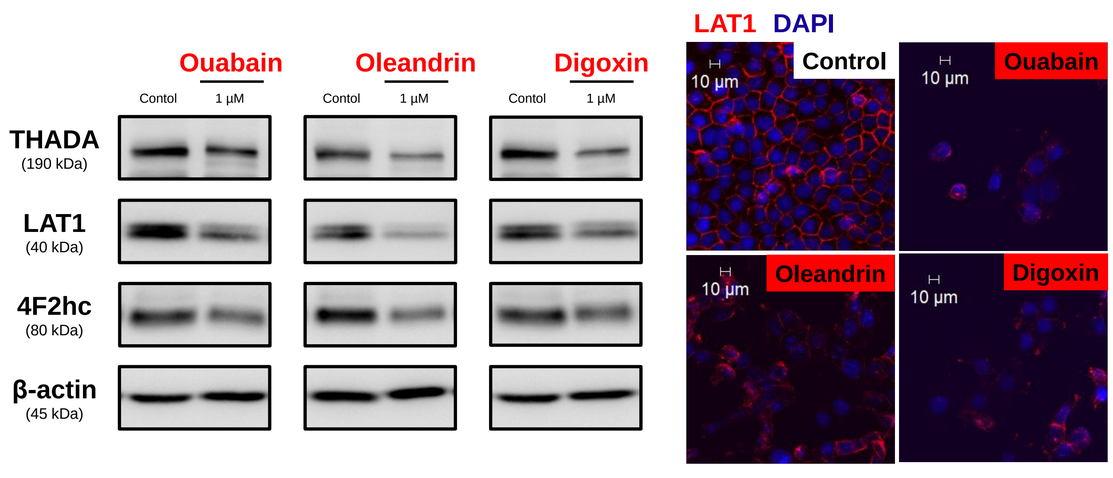
④ Expression level of LAT1 protein was decreased in the plasma membrane of THADA-knockdown cells.





Effects of THADA knockdown on expression level of LAT1 and 4F2hc in KB cells.

⑤ Expression level of LAT1 protein was decreased by CGs.



Effects of CGs (ouabain, oleandrin, and digoxin) on expression level of LAT1 and 4F2hc.
KB cells were treated with 1 μM CGs for 24 h.

Summary

- THADA may be contribute to the proliferation mechanism of cancer cells.
- Decrease in the expression levels of THADA and LAT1 may be involved in the anti-cancer mechanisms of cardiac glycosides.