



Title	Radiolabeled uracil derivative as a novel SPECT probe for thymidine phosphorylase : suppressed accumulation into tumor cells by target gene knockdown
Author(s)	Li, Hua; Zhao, Songji; Jin, Yongnan; Nishijima, Ken-ichi; Akizawa, Hiromichi; Ohkura, Kazue; Tamaki, Nagara; Kuge, Yuji
Citation	Nuclear Medicine Communications, 32(12), 1211-1215 https://doi.org/10.1097/MNM.0b013e32834b7ea7
Issue Date	2011-12
Doc URL	http://hdl.handle.net/2115/50770
Rights	This is a non-final version of an article published in final form in Nuclear Medicine Communications 32(12), December 2011, pp.1211–1215
Type	article (author version)
File Information	NMC32-12_1211-1215.pdf



[Instructions for use](#)

Radiolabeled uracil derivative as a novel SPECT probe for thymidine phosphorylase: Suppressed accumulation into tumor cells by target gene knockdown

Hua Li^{a,b}, Songji Zhao^{a,b}, Yongnan Jin^{a,b}, Ken-ichi Nishijima^c, Hiromichi Akizawa^d, Kazue Ohkura^d, Nagara Tamaki^{b,e}, Yuji Kuge^e

^aDepartment of Tracer Kinetics & Bioanalysis, Graduate School of Medicine, Hokkaido University, Sapporo, Japan

^bDepartment of Nuclear Medicine, Graduate School of Medicine, Hokkaido University, Sapporo, Japan

^cDepartment of Molecular Imaging, Graduate School of Medicine, Hokkaido University, Sapporo, Japan

^dFaculty of Pharmaceutical Sciences, Health Science University of Hokkaido, Hokkaido, Japan;

^eCentral Institute of Isotope Science, Hokkaido University, Sapporo, Japan

Corresponding author:

Songji Zhao

Department of Nuclear Medicine, Tracer Kinetics & Bioanalysis, Graduate School of Medicine, Hokkaido University

Kita 15 Nishi 7, Kita-ku, Sapporo 060-8638, Japan

Telephone: 81-11-706-5085; Fax: 81-11-706-7155

E-mail: zsj@med.hokudai.ac.jp

First author:

Hua Li

Department of Nuclear Medicine, Tracer Kinetics & Bioanalysis, Graduate School of Medicine, Hokkaido University

Kita 15 Nishi 7, Kita-ku, Sapporo 060-8638, Japan

Telephone: 81-11-706-5085; Fax: 81-11-706-7155

E-mail: ri_ka_@med.hokudai.ac.jp

ABSTRACT

Objects: We developed a radiolabeled uracil derivative, 5-iodo-6-[(2-iminoimidazolidinyl)methyl]uracil (IIMU), as a novel SPECT probe for thymidine phosphorylase (TP). This radioiodinated IIMU has a high affinity for TP, and highly accumulates in the TP-expressing tumor cell line A431 (human epidermoid carcinoma). To evaluate specificity of the cellular uptake of IIMU to TP expression, we examined the effects of TP knockdown on the uptake of ¹²⁵I-labeled IIMU (¹²⁵I-IIMU) in the tumor cells.

Methods: TP-specific siRNA, glyceraldehyde 3-phosphate dehydrogenase (GAPDH)-specific siRNA (positive control), and negative control siRNA were transfected into A431 cells, respectively. Target-mRNA and protein expression levels of TP and GAPDH were examined 48 and 72 hours after transfection, respectively. The cellular uptake level of ¹²⁵I-IIMU was also evaluated 72 hours after transfection. The results were compared after normalization with corresponding negative controls.

Results: After TP- and GAPDH-specific siRNA transfection, the expression levels of TP and GAPDH mRNA significantly decreased to 41% and 29%, respectively, as compared with the negative control ($p < 0.001$ for both). The expression levels of TP and GAPDH protein also significantly decreased to 34% and 30%, respectively ($p < 0.001$ for both). After TP-specific siRNA transfection, the cellular uptake level of ¹²⁵I-IIMU significantly decreased to 66% ($p < 0.001$). In contrast, GAPDH siRNA transfection did not significantly affect the cellular uptake level of

¹²⁵I-IIMU.

Conclusion: siRNA-mediated TP knockdown significantly decreased the cellular uptake level of ¹²⁵I-IIMU. This finding indicates that the uptake of IIMU in tumor cells is TP-specific and directly corresponds to TP expression levels.

Keywords: thymidine phosphorylase, 5-iodo-6-[(2-iminoimidazolidinyl)methyl]uracil (IIMU), small interfering RNA, cellular uptake

INTRODUCTION

Thymidine phosphorylase (TP) is an enzyme that regulates intracellular pyrimidine metabolism through the reversible deoxyribosylation of thymidine to thymine [1]. TP is regarded as an angiogenic factor because it is identical to the platelet-derived endothelial cell growth factor (PD-ECGF) [2,3]. Several experimental data indicate that the enzymatic activity of TP is indispensable for its angiogenic effect [4,5]. Elevated TP levels are associated with an increased microvessel density in various human tumors [6,7]. It is also reported that TP expression well correlates with tumor malignancy, infiltration, metastasis, and overall poor survival [8,9]. Moreover, TP is essential for the bioactivation of 5-fluorouracil (5-FU) and its prodrugs, including tegafur and capecitabine [10,11]. Previous studies using immunohistochemistry indicated that a high expression level of TP is significantly associated with the response to the treatment with 5-FU or its prodrugs [10,11]. Accordingly, *in vivo* imaging of TP activity will contribute not only to the diagnosis of tumor angiogenesis and invasiveness but also to the prognosis and clinical evaluation of cancer chemotherapy using fluoropyrimidine-based anticancer drugs.

To develop a radioprobe for *in vivo* TP imaging, a radiolabeled uracil-based TP inhibitor, 5-iodo-6-[(2-iminoimidazolidinyl)methyl]uracil (IIMU) was designed and synthesized by our group [12]. In our preliminary evaluations, IIMU showed a high TP inhibitory potency and ^{125}I -IIMU exhibited a rapid blood clearance and urinary excretion property in normal mice. We also

demonstrated high and specific accumulation of radioiodinated IIMU in TP-expressing tumors (A431, human epidermoid carcinoma) in *in vitro* and *in vivo* experiments [13]. These findings indicate that radioiodinated IIMU deserves further evaluation as a novel single photon emission computed tomography (SPECT) probe for imaging of TP-expressing tumors. In this study, in order to further evaluate the specificity of the cellular uptake of radioiodinated IIMU to TP expression, we employed the small interfering RNA (siRNA) technology to silence TP expression in A431 cells, then, examined the effects of TP knockdown on the accumulation of ^{125}I -IIMU in the tumor cells.

MATERIALS AND METHODS

Radiosynthesis of ^{125}I -IIMU

^{125}I -NaI (642.8 GBq/mg) was obtained from PerkinElmer (Boston, MA). ^{125}I -IIMU was prepared from its corresponding precursor, 6-(2-iminoimidazolidinyl)methyluracil, by radioiodination at C-5 using the previously described NCS- ^{125}I -NaI method [12]. The chemical and radiochemical purities of ^{125}I -IIMU were both > 99%.

Cell culture and reagents

The human epidermoid carcinoma cell line A431 was obtained from European Collection of Cell Cultures (ECACC). A431 cells were cultured at 37 °C in a fully humidified 5% CO₂ atmosphere in Eagle's minimum essential medium (EMEM, MP Biomedicals, Inc., Irvine, CA) supplemented with 10% fetal bovine serum (FBS), antibiotics (100 units/ml penicillin and 100

mg/ml streptomycin), 2 mM L-glutamine and 1% nonessential amino acids.

RNA interference

siRNA sequences against human TP were designed and synthesized by Applied Biosystems, LLC (Foster City, CA). The siRNA sequences were 5'-CUGCGGACGGAAUCCUAUAtt-3' (sense) and 5'-UAUAGGAUCCGUCCGCAGga-3' (antisense). A non-target siRNA that lacked identity with known gene targets was used as a negative control for non-sequence-specific effects. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) siRNA was used as a positive control. TP siRNA, negative control siRNA, and GAPDH siRNA (final concentration 2 nM) were transiently transfected into A431 cells using siPORT™ *NeoFX*™ Transfection Agent (Applied Biosystems) in according with the manufacturer's instructions. Briefly, siRNA and transfection agent complexes were mixed gently in modified EMEM (Opti-MEM® I reduced serum medium, Life Technologies, Carlsbad, CA) and incubated for 10 minutes at room temperature. Each transfection complex was added into 24-well culture plates (50 µl/well, 3 wells/plate). Then A431 cell suspensions were overlaid onto the transfection complexes at a density of 2.6×10^4 cells/cm² and incubated in a fully humidified 5% CO₂ incubator. Three 24-well culture plates were prepared and used for quantitative reverse transcription-polymerase chain reaction (RT-PCR), western blot analysis, and cellular uptake of ¹²⁵I-IIMU. Forty-eight hours after the transfection, the target messenger RNA (mRNA) was quantified by RT-PCR. Seventy-two hours

after the transfection, protein expression was examined by western blot analysis. The cellular uptake of ^{125}I -IIMU was also evaluated 72 hours after the transfection. All assays were carried out in at least three separate experiments.

Quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Forty-eight hours after the transfection, total RNA was isolated from cell lysates using an RNA isolation kit (RNAqueous®-4PCR, Applied Biosystems) according to the manufacturer's protocol. Then, 200 ng of total RNA was reversely transcribed using a high capacity cDNA reverse transcription kit (Applied Biosystems). Quantitative real-time PCR was performed to measure TP and GAPDH mRNA expression levels using the 7500 Real-Time PCR System (Applied Biosystems). The amounts of TP mRNA or GAPDH mRNA were normalized with respect to those of 18s RNA. Normalized intensity was compared between TP siRNA-transfected cells and negative control siRNA-transfected cells or between GAPDH siRNA-transfected cells and negative control siRNA-transfected cells.

Western immunoblot analysis

Seventy-two hours after the transfection, total protein was extracted using RIPA lysis buffer (20 mM Tris-HCl pH 7.5, 1 mM EDTA, 0.15 M NaCl, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM Na_3VO_4 , 1 $\mu\text{g}/\text{ml}$ leupeptin. Cell Signaling Technology, Inc., Danvers, MA). Protein concentration was quantified

using the Bradford method (Bio-Rad protein assay kit, Bio-Rad Laboratories, Inc., Hercules, CA). The protein extracts (30 ug) were separated on 4-20% SDS-PAGE, then transferred onto a nitrocellulose membrane, and blocked with TBST buffer (20 mmol/L Tris-HCl, 150 mmol/L NaCl, 0.5 ml/L Tween 20) plus 5% bovine serum albumin. The membranes were incubated with a primary antibody: anti-TP (Calbiochem, San Diego, CA) diluted 1:200, or anti-GAPDH (Applied Biosystems) diluted 1:1000 in TBST. After repeated washings, the membranes were incubated with a horseradish peroxidase-conjugated anti-mouse secondary antibody (GE Healthcare UK Ltd., Amersham, UK) diluted 1:2000. Immunopositive bands were detected using a freshly prepared ECL chemiluminescent solution (GE Healthcare) and visualized using luminescent image analyzer, LAS-4000 mini (Fujifilm, Tokyo, Japan). Immunoblotting for β -actin (Sigma-Aldrich, St. Louis, MO) was used as the protein loading control. Densitometric analysis of western blot data was performed using Multi Gauge Ver. 3.X. software (Fujifilm). The densities of TP and GAPDH protein expression, were normalized to β -actin protein expression, and compared between TP siRNA- and negative control siRNA-transfected cells and between GAPDH siRNA- and negative control siRNA-transfected cells.

In vitro cellular uptake experiments

Seventy-two hours after the siRNA transfection, the medium was removed and cells were washed with 1 ml of 0.01 M PBS (-) and incubated at 37 °C in 0.5 ml of 0.01 M PBS (-) for 10

minutes. After preincubation, 0.5 ml of 0.01 M PBS (-) containing 37 kBq ^{125}I -IIMU was added to each well, and the cells were incubated further 120 minutes, according to the methods described previously by Akizawa et al. [13]. At 120 minutes, the incubation solution was removed, and the cells were washed twice with 1 ml of ice-cold 0.01 M PBS (-) and solubilized in 0.5 ml of 0.2% SDS solution (50 mM Tris-HCl pH 7.5, 10 mM EDTA, 100 mM NaCl, 0.2% SDS). Radioactivity in cell lysates was measured using an auto-well gamma counter (1480 WIZARD 3"; Wallac Co., Ltd., Turku, Finland), and the protein content of lysates was measured by BCA protein assay (BCA protein assay kit, Thermo Fisher Scientific, Rockford, IL). ^{125}I -IIMU uptake levels were compared after normalization with that in corresponding negative control. To assess the cell viability, cell death levels were determined with trypan blue staining, before and 120 minutes after the incubation with ^{125}I -IIMU.

Statistical analysis

All data were from three independent experiments and expressed as mean \pm SD. Unpaired Student's *t*-test was used to assess the significance of differences in mRNA expression levels between negative control siRNA- and TP siRNA-transfected cells, or between negative control siRNA- and GAPDH siRNA-transfected cells. One-way analysis of variance (ANOVA) followed by post hoc analysis (Bonferroni/Dunn test) was performed to evaluate the significance of differences in protein expression level and ^{125}I -IIMU uptake level. A value of *p* less than 0.05 was considered

significant.

RESULTS

Gene silencing effect of TP siRNA

The ability of TP and GAPDH siRNAs to decrease target mRNA expression level is shown in Fig.1. Forty-eight hours after the transfection, TP- and GAPDH-specific siRNAs significantly decreased TP and GAPDH mRNA expression levels to 41% and 29%, respectively, compared with negative control siRNA-transfection ($p < 0.001$ for both, Figs. 1a and 1b).

Seventy-two hours after the transfection, the effect of siRNA on protein expression was confirmed by western blot analysis (Fig. 2). TP protein expression level significantly decreased following TP-specific siRNA transfection to 34% that in the negative control ($p < 0.001$, Fig. 2c). TP protein expression was not inhibited by GAPDH-specific siRNA transfection. GAPDH protein expression level was markedly decreased by GAPDH-specific siRNA transfection to 30% that in the negative control ($p < 0.001$, Fig. 2d), and was not altered by TP-specific siRNA transfection.

Cellular accumulation of ^{125}I -IIMU

Figure 3 shows the cellular uptake levels of the radioprobe in the negative control-, TP- and GAPDH-siRNA transfected cells. After TP-specific siRNA transfection, the cellular uptake level of ^{125}I -IIMU significantly decreased to 66% ($p < 0.001$), compared with negative control siRNA transfection. In contrast, GAPDH siRNA transfection did not significantly affect the cellular

uptake of ^{125}I -IIMU. To assess the cell viability, cell death levels were determined with trypan blue staining, before and 120 minutes after the incubation with ^{125}I -IIMU. Before incubation with ^{125}I -IIMU, the percentage of cell death was $15.1\pm 0.7\%$ and $15.8\pm 1.3\%$ in the negative control and TP transfected cells, respectively. At 120 minutes after the incubation with ^{125}I -IIMU, the percentage of cell death was $23.4\pm 9.7\%$ in control cells and $22.6\pm 5.9\%$ in TP transfected cells. At 120 minutes after incubation with ^{125}I -IIMU, the viability of TP siRNA- transfected cells did not show a significant difference compared with that of the control cells.

DISCUSSION

In this study, transfection with TP-specific siRNA decreased TP mRNA expression level to approximately 41% (Fig.1) and TP protein expression level to 34% (Fig. 2), indicating effective gene silencing. Our results revealed a significantly lower uptake level of ^{125}I -IIMU in siRNA-mediated TP-depleted cells than in control cells (Fig. 3). Thus, uptake varied depending on the expression level of TP in A431 tumor cells, indicating the specificity of IIMU uptake to TP expression.

Over the past few years, the utility of RNA interference (RNAi) and post-transcriptional gene silencing has significantly advanced the studies on individual gene functions [14]. Here, we used RNAi technology to directly analyze the TP binding of ^{125}I -IIMU, and demonstrated the effects of post-transcriptional TP silencing on ^{125}I -IIMU uptake. Of note, the radioprobe uptake level significantly decreased in TP-depleted A431 cells, suggesting a reduced binding of ^{125}I -IIMU owing

to the loss of TP.

The selectivity and/or specificity are indispensable prerequisites of radioprobes for *in vivo* imaging. We previously showed, in *in vivo* and *in vitro* experiments, the significantly higher uptake level of ^{125}I -IIMU in high-level TP-expressing A431 cells, being in sharp contrast to its low uptake observed in low-level TP-expressing AZ521 cells (a human gastric cancer cell line) [13]. The selectivity and specificity of ^{125}I -IIMU were also confirmed in our previous ligand competition studies. The uptake level of ^{125}I -IIMU in A431 cells was reduced by about 70% at 400 nM nonlabeled IIMU. There were, however, several limitations in these previous studies. In the experiment using two human cancer cell lines, except for the difference in TP expression level, the difference in cell biology or cell metabolism between the two cell lines (epidermoid carcinoma, A431 and gastric cancer, AZ521) may have also affected the uptake of ^{125}I -IIMU. In the ligand competition studies, the increasing concentrations of nonradioactive drugs compete for the binding of the radioligand to a certain cell component. This is an indirect evidence for the affinity of ^{125}I -IIMU binding to TP, although it has been reported that the three-dimensional structure of TP have some hydrophobic pockets for binding 5-iodouracil, thymine, and thymidine [15], and that IIMU, a 6-substituted uracil analog, binds to the active sites of TP [12]. Therefore, we performed the present study to determine the correlation between ^{125}I -IIMU uptake and TP expression and to confirm the selectivity and specificity of ^{125}I -IIMU binding to TP. The present study provides

additional evidence for the selectivity and/or specificity of ^{125}I -IIMU binding to TP, although RNAi experiments did not provide the evidence of the IIMU binding site on the TP structure. The present study provides practical and beneficial methodology, which is applicable to the studies of a variety of radioactive probes.

In our results, the gene knockdown level did not completely correspond to the ^{125}I -IIMU uptake level. To investigate whether the ^{125}I -IIMU uptake were influenced by cell viability after TP siRNA transfection, we assessed cell viability after incubation with ^{125}I -IIMU. The result revealed that there is no difference in cell viability between TP- and negative control siRNA- transfected cells, indicating that the decreased cell uptake of ^{125}I -IIMU is not influenced by cell viability after TP siRNA transfection. On the other hand, in our ligand competition study [13], the cell uptake of ^{125}I -IIMU was 70% that of the control at the excess dose of non-labeled IIMU (400 nM). Taken together, we guess there may be some non-specific binding of ^{125}I -IIMU, leading to the mismatch between the gene knockdown and declined cell uptake levels. However, further experiments to clarify the reason of the mismatch, are required. Optimization of the incubation conditions, including multi-time point cell uptake assay, may help minimize the mismatch between the gene knockdown and declined cell uptake levels.

The next step for our research is to extend the studies from cell culture to *in vivo* animal models. Particularly, we need *in vivo* investigation on the selectivity and/or specificity of

radiolabeled IIMU using RNAi technology. In this regard, an *in vivo* experiment with a multifunctional envelope-type nano device as a delivery vehicle [16] is ongoing in our laboratory.

CONCLUSION

siRNA-mediated TP knockdown significantly decreased the cellular uptake level of ¹²⁵I-IIMU. This finding indicates that the uptake of IIMU in tumor cells is TP-specific and directly corresponds to TP expression levels.

ACKNOWLEDGMENTS

This study was performed through Special Coordination Funds for Promoting Science and Technology of the Ministry of Education, Culture, Sports, Science and Technology, Japan. This work was also supported in part by grants-in aid for Scientific Research from the Japan Society for the Promotion of Science and from the Japanese Ministry of Education, Culture, Sports, Science and Technology. The authors are grateful to the staff members of the Department of Nuclear Medicine, Department of Molecular Imaging, and Central Institute of Isotope Science, Hokkaido University, for supporting this study.

REFERENCES

1. Iltzsch MH, el Kouni MH, Cha S. Kinetic studies of thymidine phosphorylase from mouse liver. *Biochemistry* 1986; 24:6799-6807.
2. Fukawa T, Yosimura A, Sumizawa T, Haraguchi M, Akiyama S, Fukui K, et al. Angiogenic factor. *Nature* 1992; 356:668.
3. Sumizawa T, Furukawa T, Haraguchi M, Yosimura A, Takeyasu A, Ishizawa M, et al. Thymidine phosphorylase activity associated with platelet-derived endothelial cell growth factor. *J Biochem* 1993; 114: 9-14.
4. Moghaddam A, Zhang HT, Fan TP, Hu DE, Lees VeC, Turley H, et al. Thymidine phosphorylase is angiogenic and promotes tumor growth. *Proc Natl Acad Sci* 1995; 92:998-1002.
5. Miyadera K, Sumizawa T, Haraguchi M, Yoshida H, Konstanty W, Yamada Y, et al. Role of thymidine phosphorylase activity in the angiogenic effect of platelet derived endothelial cell growth factor/thymidine phosphorylase. *Cancer Res* 1995; 55:1687-90.
6. Harino Y, Imura S, Kanemura H, Morine Y, Fujii M, Ikegami T, et al. Role of tumor angiogenesis in gallbladder carcinoma: with special reference to thymidine phosphorylase. *Int J Clin Oncol* 2008; 13:452-7.
7. Han HS, Hwang TS. Angiogenesis in gastric cancer: importance of the thymidine phosphorylase expression of cancer cells as an angiogenic factor. *Oncol Rep* 2007; 17:61-5.
8. Takayama T, Mugiya S, Sugiyama T, Aoki T, Furuse H, Liu H, et al. High levels of thymidine

- phosphorylase as an independent prognostic factor in renal cell carcinoma. *Jpn J Clin Oncol* 2006; 36:564-9.
9. Chen LC, Hsueh C, Tsang NM, Liang Y, Chang KP, Hao SP, et al. Heterogeneous ribonucleoprotein k and thymidine phosphorylase are independent prognostic and therapeutic markers for nasopharyngeal carcinoma. *Clin Cancer Res* 2008; 14:3807-13.
10. Schwartz EL, Baptiste N, Wadler S, Makower D. Thymidine phosphorylase mediates the sensitivity of human colon carcinoma cells to 5-fluorouracil. *J Biol Chem* 1995; 270:19073-7.
11. Tokunaga Y, Hosogi H, Hoppou T, Nakagami M, Tokuka A, Ohsumi K. Prognostic value of thymidine phosphorylase/platelet-derived endothelial cell growth factor in advanced colorectal cancer after surgery: evaluation with a new monoclonal antibody. *Surgery* 2002; 131:541-7.
12. Takahashi M, Seki K, Nishijima K, Zhao S, Kuge Y, Tamaki N, et al. Synthesis of a radioiodinated thymidine phosphorylase inhibitor and its preliminary evaluation as a potential SPECT tracer for angiogenic enzyme expression. *J Label Compd Radiopharm* 2008; 51:384-7.
13. Akizawa H, Songji Z, Masayuki T, Kenichi N, Yuji K, Nagara T, et al. In vitro and in vivo evaluations of a radioiodinated thymidine phosphorylase inhibitor as a tumor diagnostic agent for angiogenic enzyme imaging. *Nu Nucl Med Biol* 2010; 37:427-32.
14. Thanasai J, Limpai boon T, Jearanaikoon P, Sripa B, Pairojkul C, Tantimavanich S, et al. Effects of thymidine phosphorylase on tumor aggressiveness and 5-fluorouracil sensitivity in

- cholangiocarcinoma. *World J Gastroenterol* 2010; 16: 1631-8.
15. Walter MR, Cook WJ, Cole LB, Short SA, Koszalka GW, Krenitsky TA, et al. Three-dimensional structure of thymidine phosphorylase from *Escherichia coli* at 2.8 Å resolution. *J Biol Chem* 1990; 265:14016-22.
16. Hatakeyama H, Akita H, Ito E, Hayashi Y, Oishi M, Nagasaki Y, et al. Systemic delivery of siRNA to tumors using a lipid nanoparticle containing a tumor-specific cleavable PEG-lipid. *Biomaterials* 2011; 32: 4306-16.

FIGURE LEGENDS

FIGURE 1 mRNA expression levels in siRNA-transfected cells. (a) TP mRNA expression. (b) GAPDH mRNA expression.

FIGURE 2 Protein expression levels in siRNA-transfected cells. Western blot analyses of TP (a) and GAPDH (b) with β -actin as a loading control. Quantitative results for TP (c) and GAPDH (d) protein expression levels.

FIGURE 3 Uptake levels of ^{125}I -IIMU in siRNA-transfected cells. The cellular uptake level of ^{125}I -IIMU was significantly decreased by TP-specific siRNA transfection, whereas GAPDH siRNA transfection did not markedly affect ^{125}I -IIMU accumulation.

Fig. 1

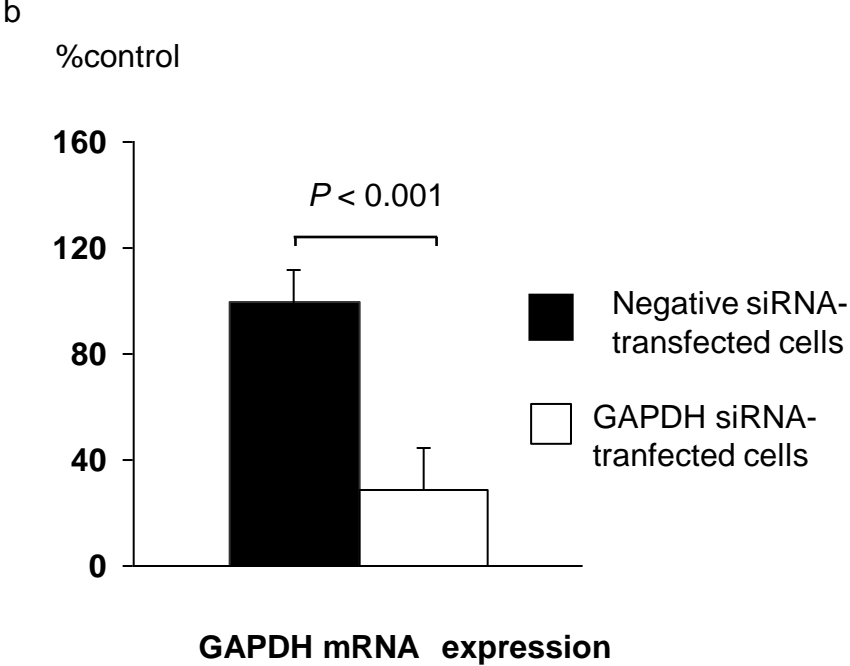
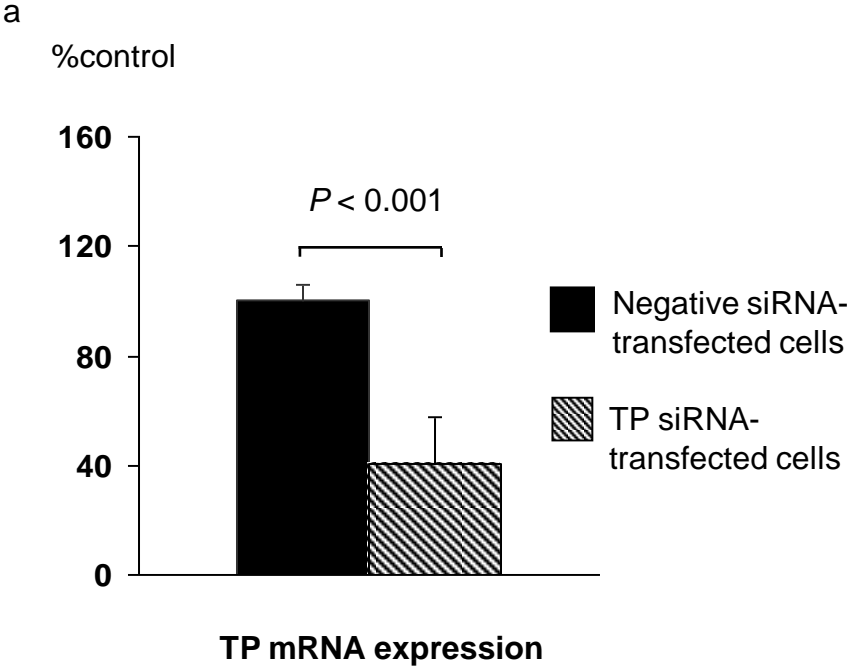


Fig. 2

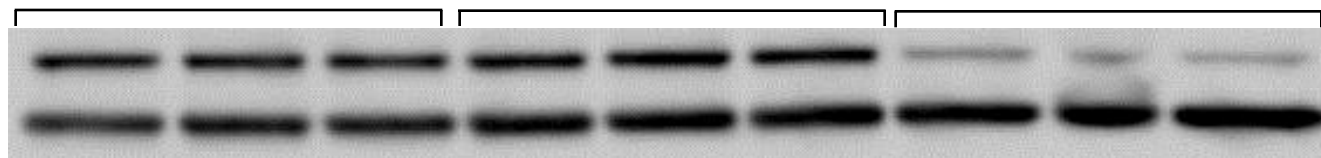
Negative control

GAPDH siRNA

TPsiRNA

a

TP
 β -actin



Negative control

GAPDH siRNA

TPsiRNA

b

GAPDH
 β -actin

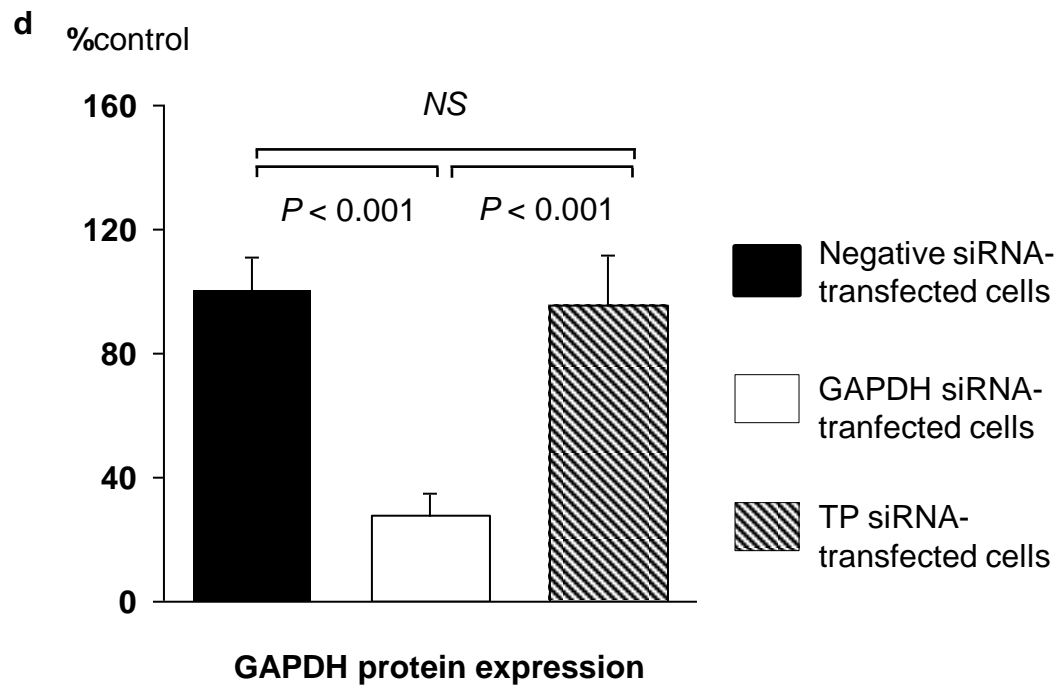
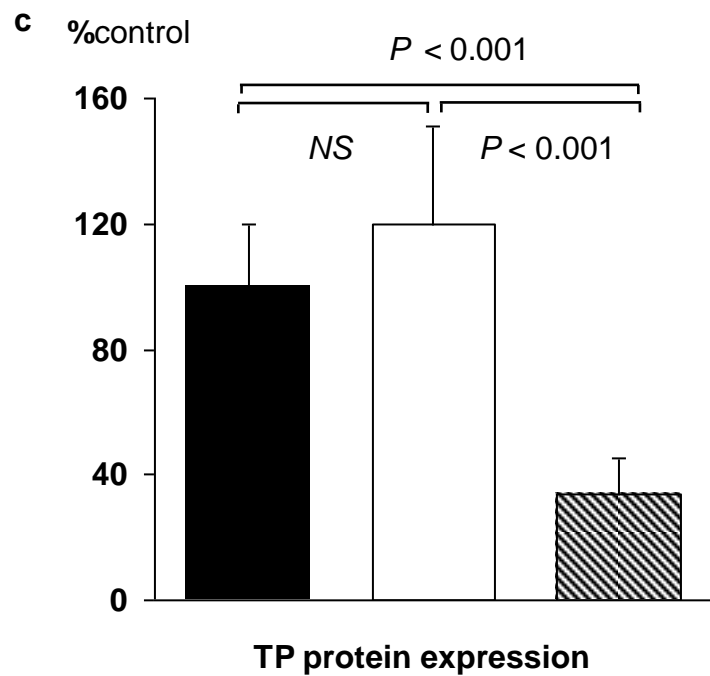
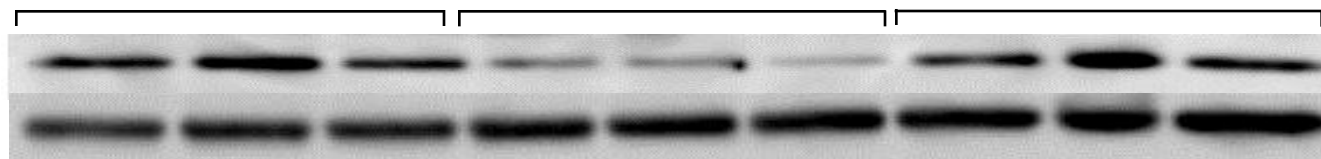


Fig. 3

