受賞講演
2026. 8. 28

カドミウムの毒性発現における細胞内転写経路の関与

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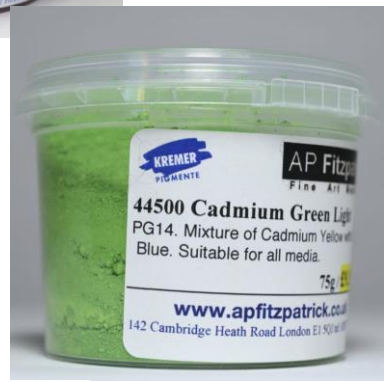
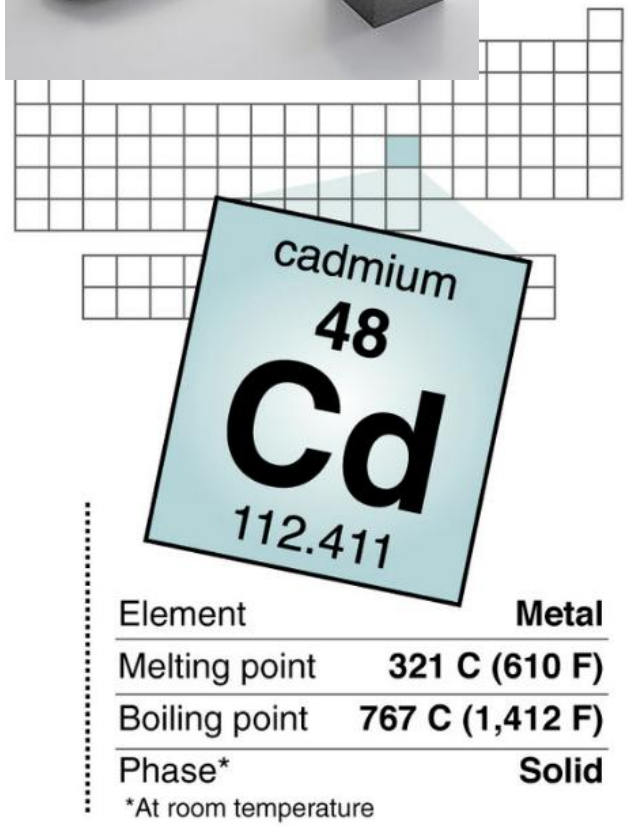
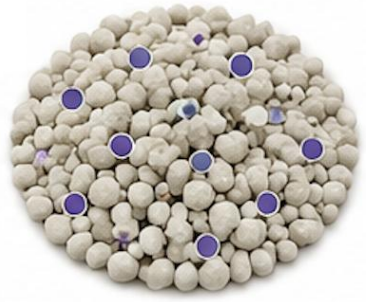
- 2019. 4. 愛知学院大学薬学部 准教授（現在迄）

Cadmium



Cadmium plating





It's cheap, shiny, strong and malleable in room temperature.

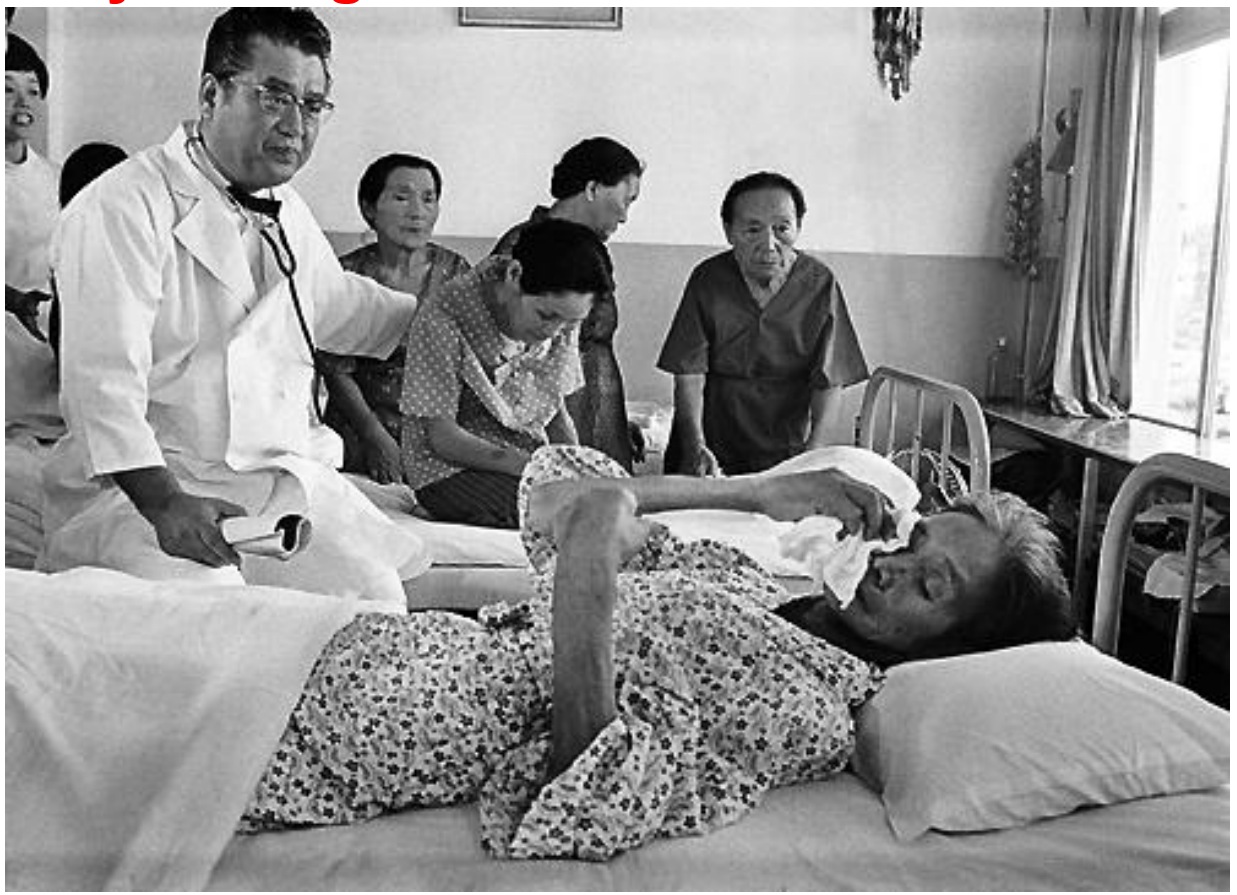
Cadmium in phosphate rock

Cadmium as impurity in phosphate fertilizer

Itai-itai Disease

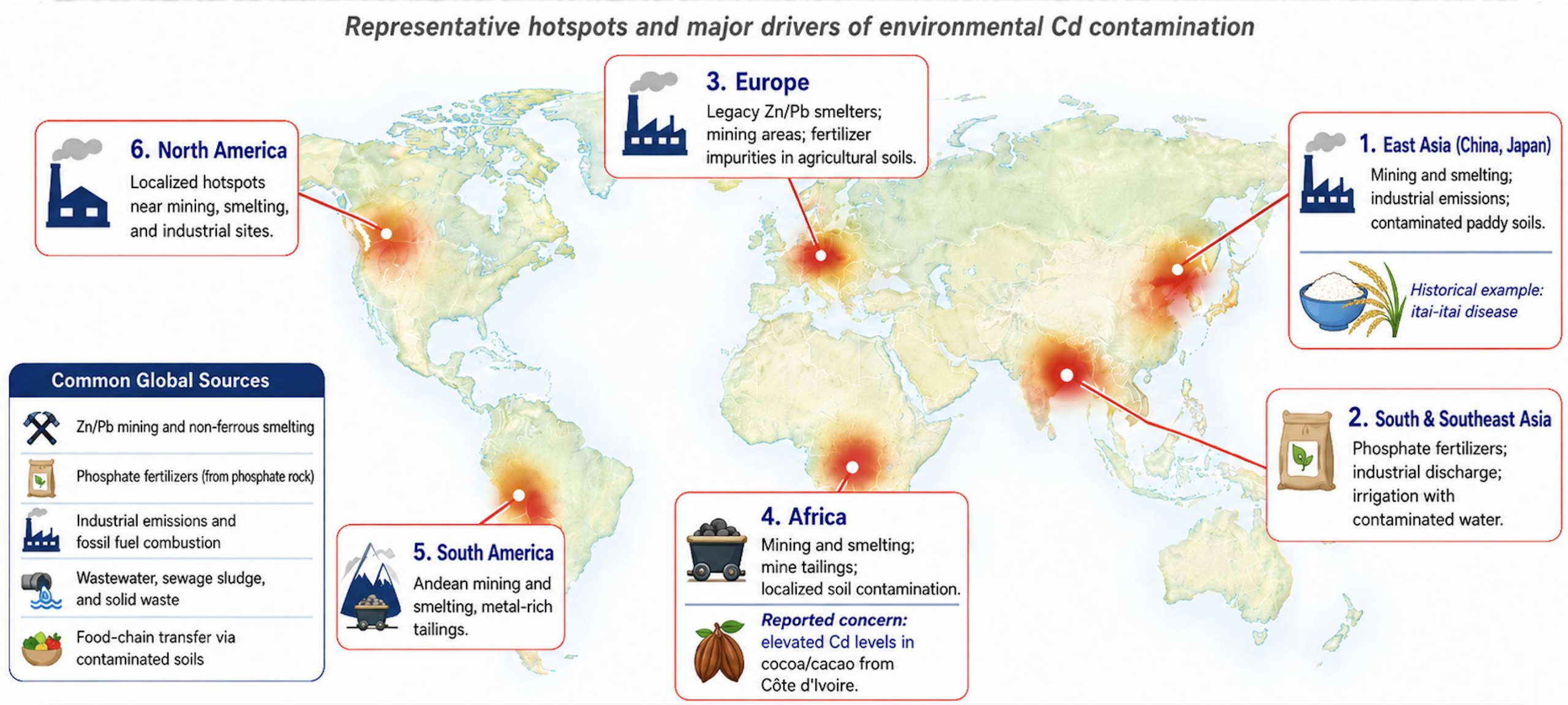
***Itai-itai* disease was officially recognized in Japan in 1968.**

Main Symptom: Kidney damage and Osteomalacia



Causing Material : Cadmium

Global Cadmium Pollution Today



Cd pollution is not only a historical problem; it remains a current global food and public health issue

Human Exposure to Cadmium

Cadmium exposure occurs through multiple routes, but food is the dominant source for most non-smoking adults.

1 Food

Main route for the general population

Rice, cereals, vegetables
Shellfish and seafood
Cocoa/chocolate in some regions

2 Tobacco smoke

Important additional exposure

Active smoking increases Cd burden
Passive smoke can contribute
Kidney Cd accumulates over time

3 Occupational exposure

High-risk groups

Mining and smelting
Battery manufacturing/recycling
Pigments, plating, industrial dust

Exposure pathway

Environment → **Food / Air / Work** → **Body burden** → **Kidney accumulation** → **Health risk**

Human Cd exposure occurs mainly through food, with additional contributions from tobacco smoke and occupational settings; long-term kidney accumulation makes route-specific exposure control essential.

Sources: WHO Cadmium factsheet; European Commission food contaminant information; UNEP cadmium overview.

Cadmium Safety Assessment

International guidance value vs Japan domestic reference

International: FAO/WHO JECFA

PTMI

25 µg/kg bw/month

Provisional Tolerable Monthly Intake

≈5.8 µg/kg bw/week

Japan: Food Safety Commission / MHLW

TWI

7 µg/kg bw/week

Tolerable Weekly Intake used in Japan

≈28 µg/kg bw/month

Different time units; similar purpose

Why JECFA changed to PTMI

Cd accumulates slowly; kidney steady state may require decades

Monthly intake better reflects long-term body burden

Why Japan differs

Japan retained a weekly TWI based on domestic dietary and epidemiological data

Rice-centered exposure history shaped national risk management

These values differ in unit and assessment basis, but both are designed to protect against long-term renal effects of dietary cadmium exposure.

Sources: WHO/JECFA; Food Safety Commission of Japan; MAFF Japan

Maximum Levels of Cadmium in Foods

Maximum levels for Cd in selected food categories as established by the Codex Alimentarius Commission.

Commodity/ Product Name	ML (mg/kg)	Commodity/ Product NAME	ML (mg/kg)	Commodity/Product Name	ML (mg/kg)
Brassica vegetables	0.05	Stalk and stem vegetables	0.1	Natural mineral waters	0.003 *
Bulb vegetables	0.05	Cereal grains	0.1	Salt, food grade	0.5
Fruiting vegetables	0.05	Rice, polished	0.4	Chocolates containing or declaring <30% total cocoa solids on a dry matter basis	0.3
Leafy vegetables	0.2	Wheat	0.2	Chocolate containing or declaring ≥30% to <50% total cocoa solids on a dry matter basis	0.7
Legume vegetables	0.1	Quinoa	0.15	Chocolate containing or declaring ≥50% to <70% total cocoa solids on a dry matter basis	0.8
Pulses	0.1	Marine bivalve molluscs	2	Chocolate containing or declaring ≥70% total cocoa solids on a dry matter basis	0.9
Root and tuber vegetables	0.1	Cephalopods	2	Cocoa powder (100% total cocoa solids on a dry matter basis) ready for consumption	2.0

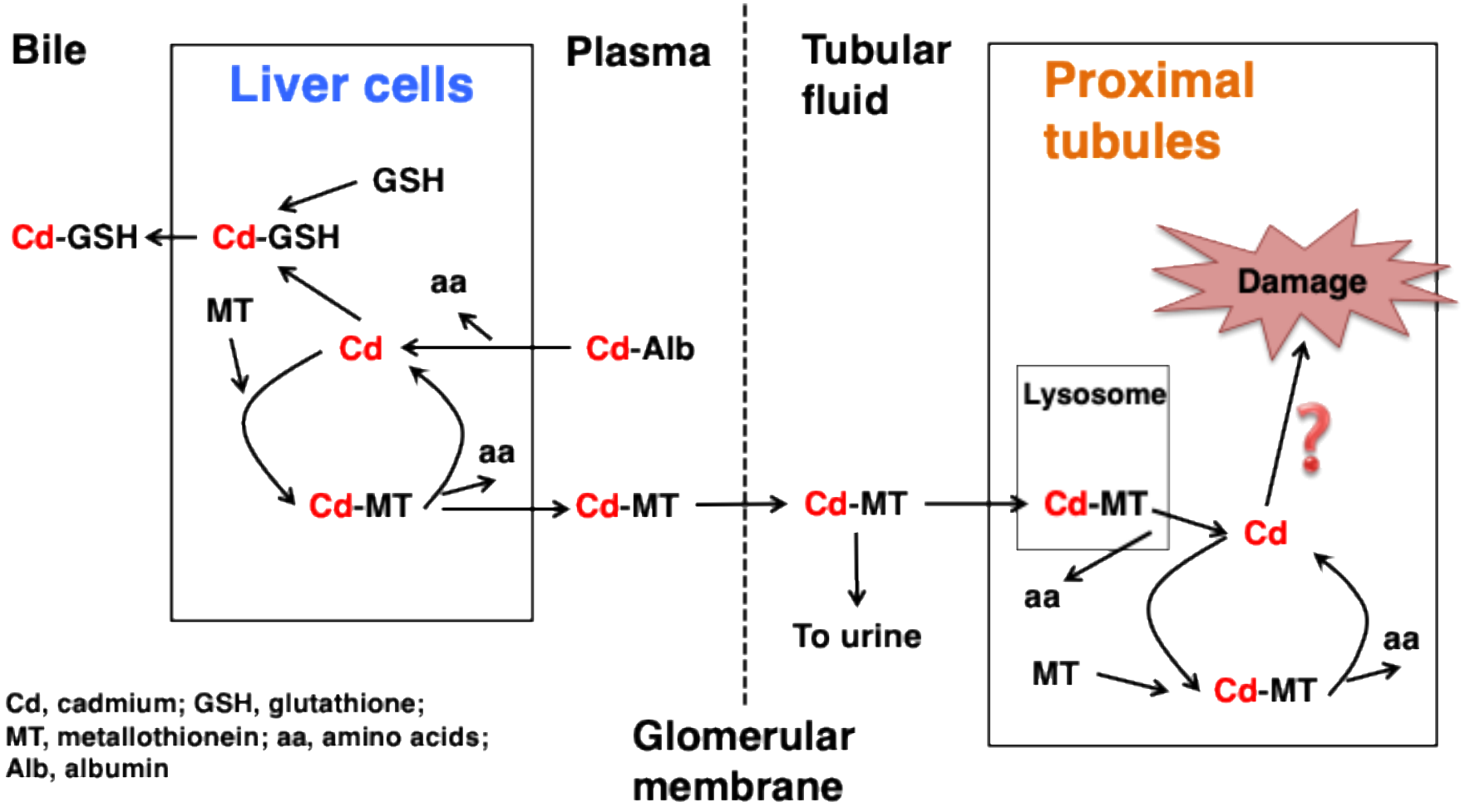
ML, maximum level; * the ML is expressed in mg/L.

Sources: Codex CXS 193-1995 (examples for staple foods); EU Commission Regulation No 488/2014 (examples for cocoa/chocolate).

To reduce dietary exposure to cadmium
To protect consumers
To support international food trade

Food standards translate toxicological risk assessment into practical public health measures.

Flow scheme of Cd in the body



Modified from Satoh, M. Integration of modern life science into preventive and environmental medicine. p 667. 2010

Main concerns on Cd toxicity

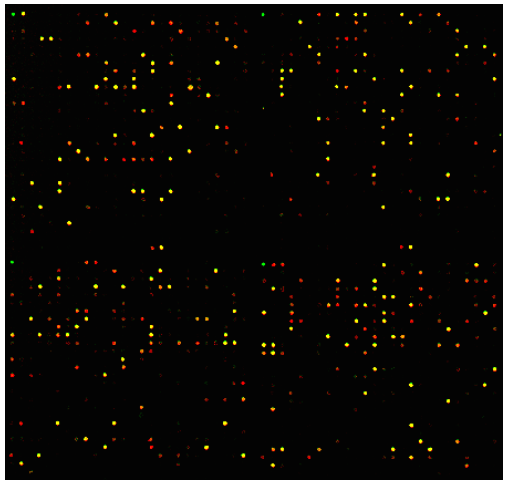
- The chronic toxic effects of Cd are clearly a much greater concern than the rare acute toxic exposures
- Major tissue of Cd chronic toxicity: Kidney
- Main target of renal toxicity : Proximal tubular cells

The underlying precise molecular mechanism of Cd renal toxicity is still remaining to elucidate.

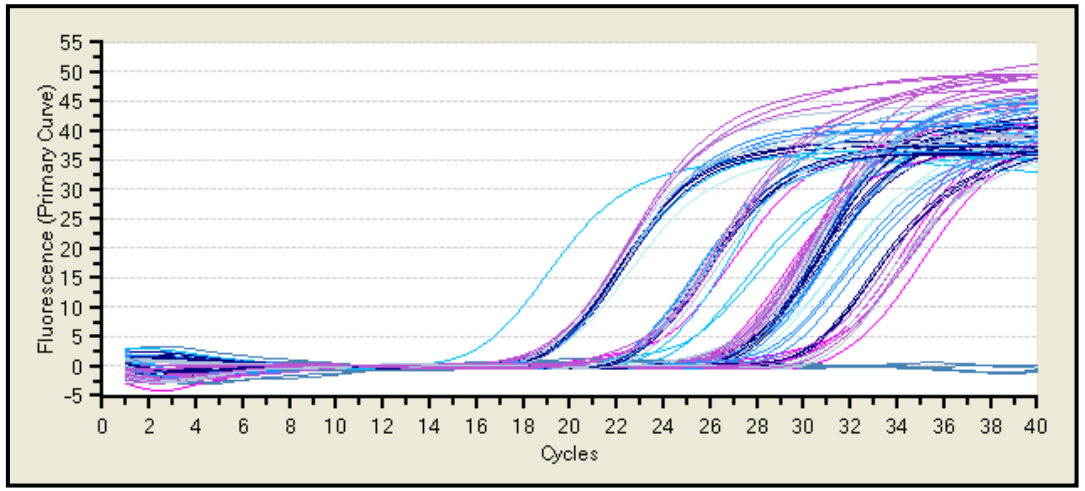
Screening of target factors in Cd renal toxicity

TOXICOGENOMICS

HK-2 cell: Human proximal tubular cell



DNA microarray



Real-time RT-PCR

Identification of the genes involved in Cd renal toxicity

The Journal of Toxicological Sciences (J. Toxicol. Sci.)
Vol.38, No.6, 959-962, 2013

Toxicomics Report

Gene expression analysis using DNA microarray in HK-2 human proximal tubular cells treated with cadmium

Jin-Yong Lee[1], Maki Tokumoto[1,2], Yasuyuki Fujiwara[1] and Masahiko Satoh[1]

[1]Laboratory of Pharmaceutical Health Sciences, School of Pharmacy, Aichi Gakuin University, 1-100 Kusumoto-cho, Chikusa-ku, Nagoya 464-8650, Japan
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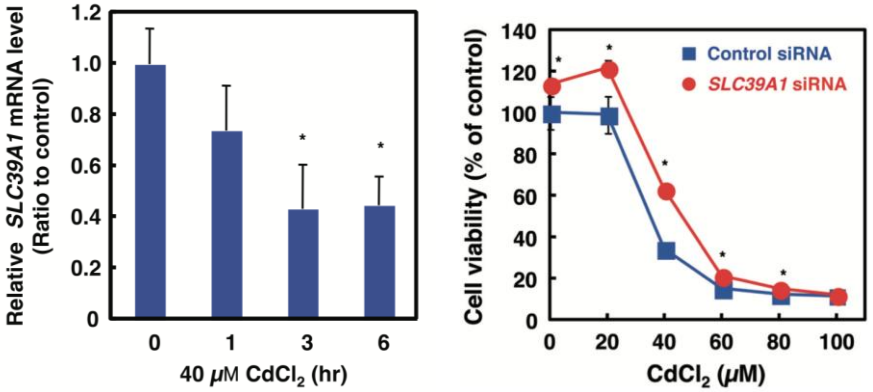
- **30 Genes whose Expression Increased by Cd**
- **21 Genes whose Expression Decreased by Cd**

Lee *et al.*, *J. Toxicol., Sci.*, 2013

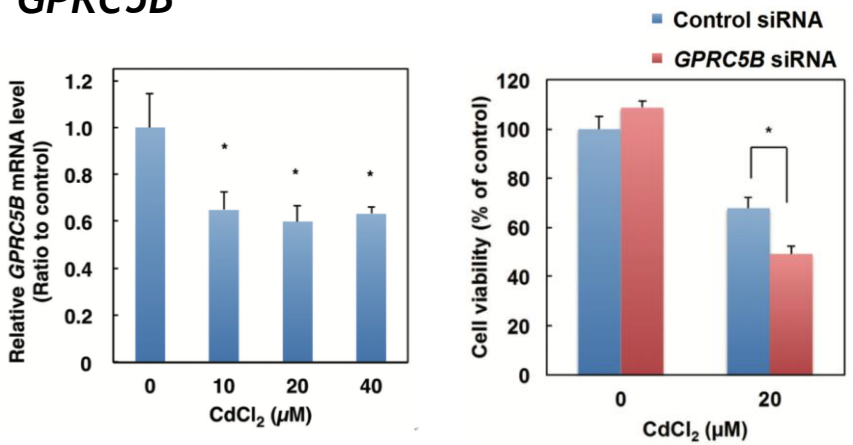
Identification of the genes involved in Cd renal toxicity

SLC39A1



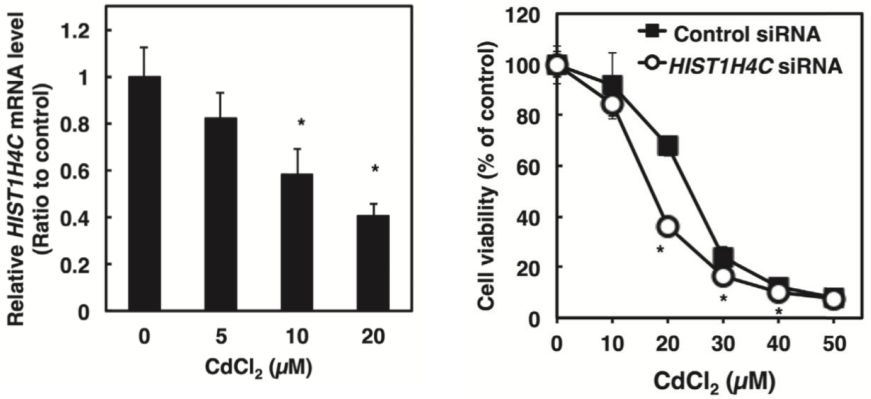
Lee *et al.*, *Fundam. Toxicol., Sci.*, 2014a

GPRC5B



Lee *et al.*, *Fundam. Toxicol., Sci.*, 2014b

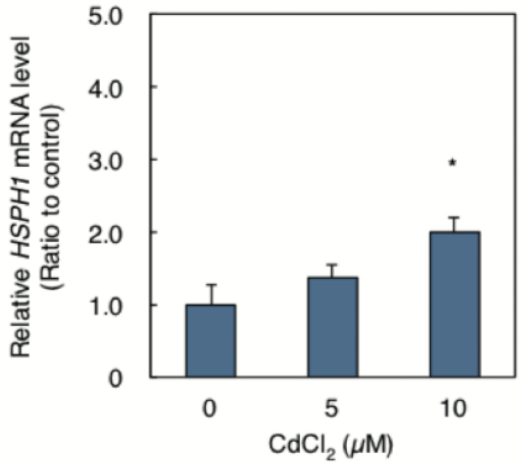
HIST1H4C

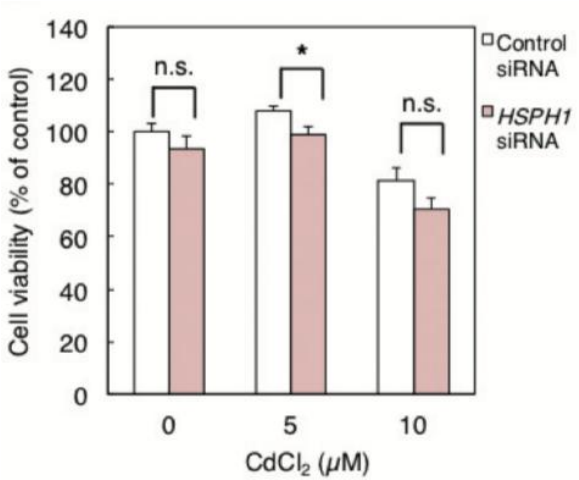


Lee *et al.*, *Fundam. Toxicol., Sci.*, 2015

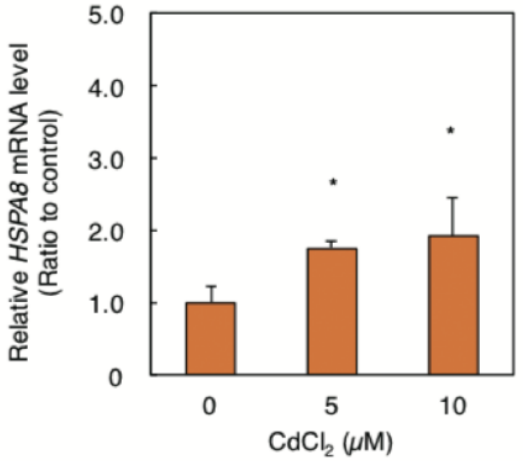
Identification of the genes involved in Cd renal toxicity

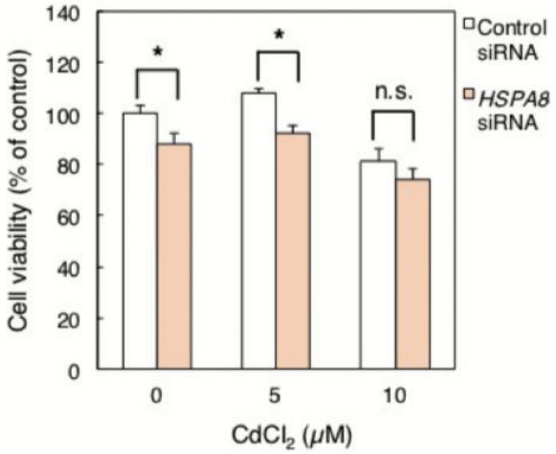
HSPH1





HSPA8





Lee *et al.*, *Fundam. Toxicol., Sci.*, 2018

Purpose

- To elucidate the target transcription factors responsible for Cd renal toxicity

- To elucidate the downstream gene of target transcription

Protein/DNA binding assay with nuclear extraction from Cd-treated HK-2 cells

HK-2 cells:
Human proximal tubular cells

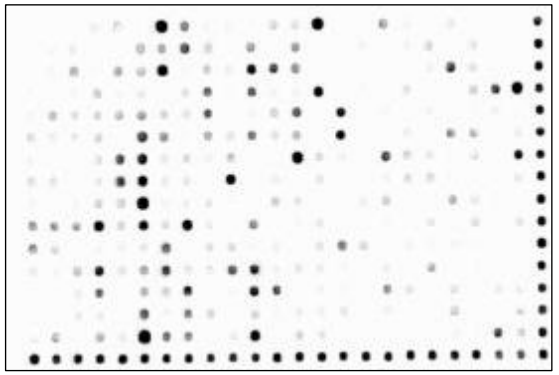
HK-2 cells

↓ 40 μM Cd 3 h

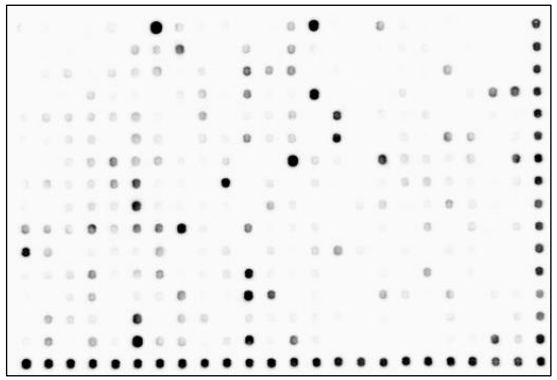
Nuclear extraction

↓

Protein/DNA binding assay



Control



Cd

Protein/DNA binding assay Combo;
The screening method for binding activities of 345 transcription factors to DNA elements in the promoters

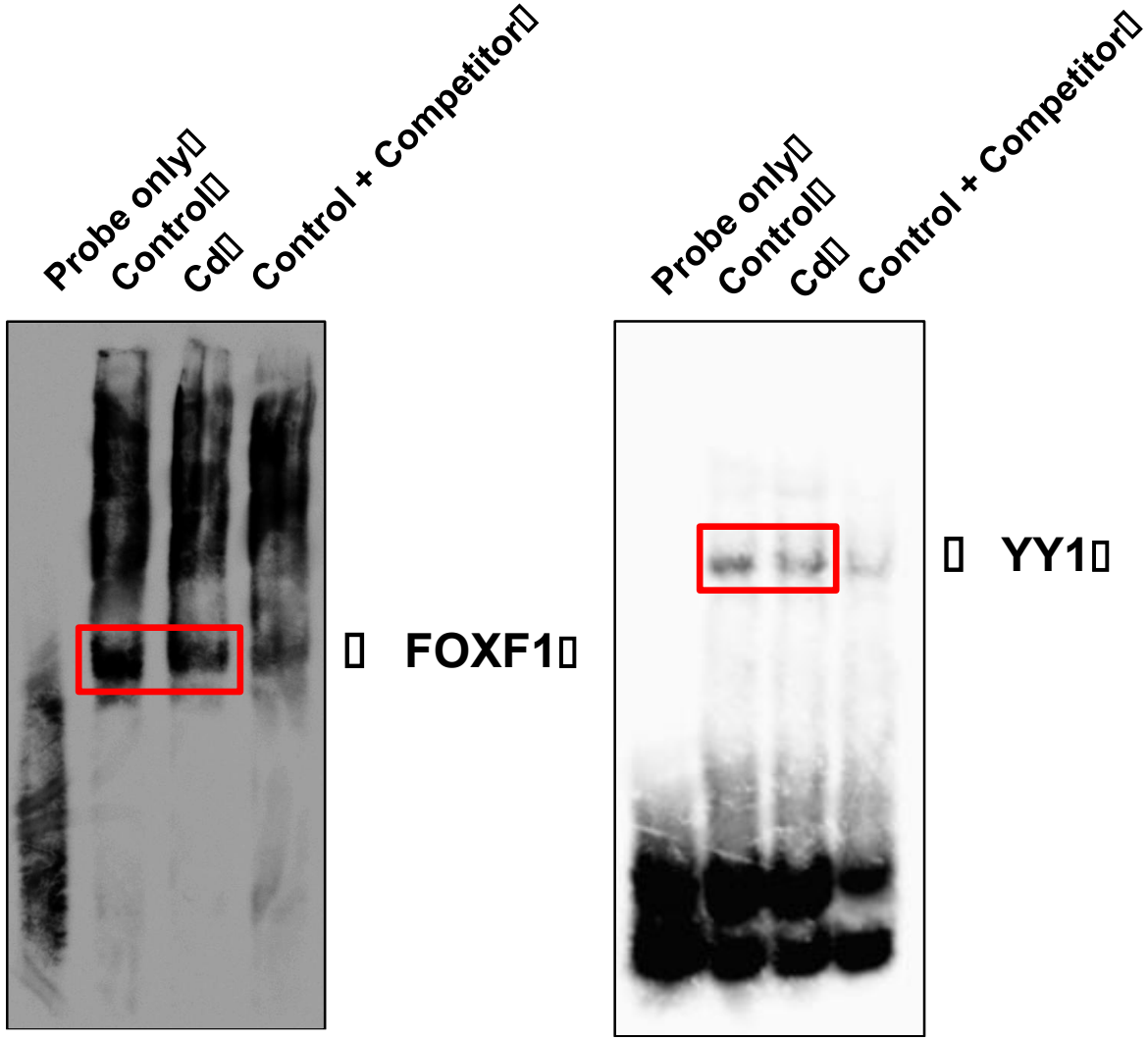
- 20 Transcription Factors Activated by Cd
- 28 Transcription Factors Deactivated by Cd

Lee *et al.*, Sci. Rep. 2017

Effects of Cd on DNA binding activity of FOXF1 and YY1 in HK-2 cells

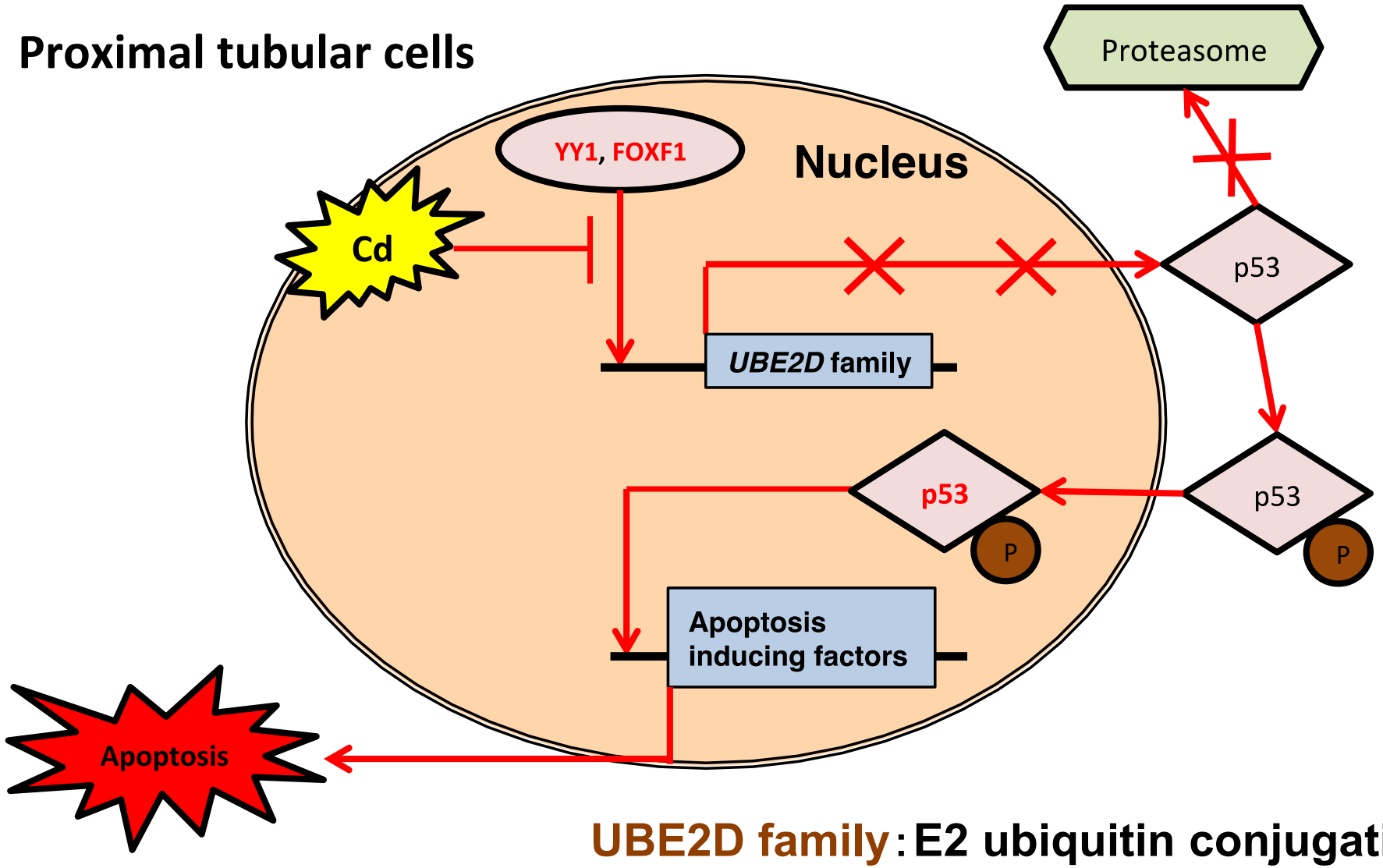
Gel Shift Assay

HK-2 cells
↓Cd 40 μM for 3 h
Nuclear extracts
↓Incubation with DNA probe
Separated on a polyacrylamide gel
↓
Transfer to a nylon membrane
↓
Detection



Lee *et al.*, Sci. Rep. 2016

Scheme of Proximal Tubule Toxicity of Cd through Overaccumulation of p53



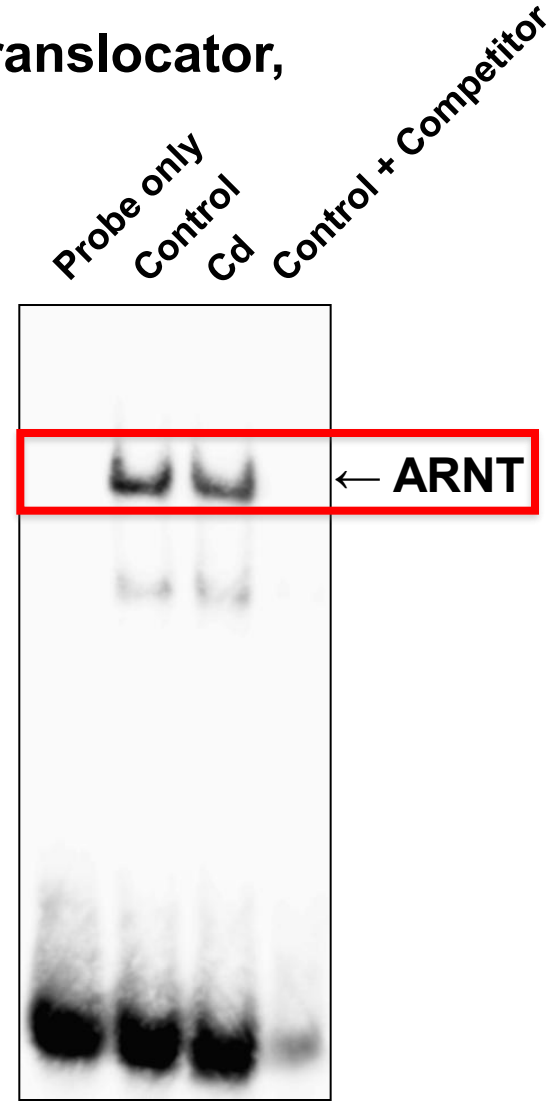
UBE2D family : E2 ubiquitin conjugating enzymes

p53 : Tumor suppressor, apoptosis inducer, a target of Ube2d family

DNA binding activity of ARNT in HK-2 cells treated with Cd

ARNT: aryl hydrocarbon receptor nuclear translocator, known as HIF-1β

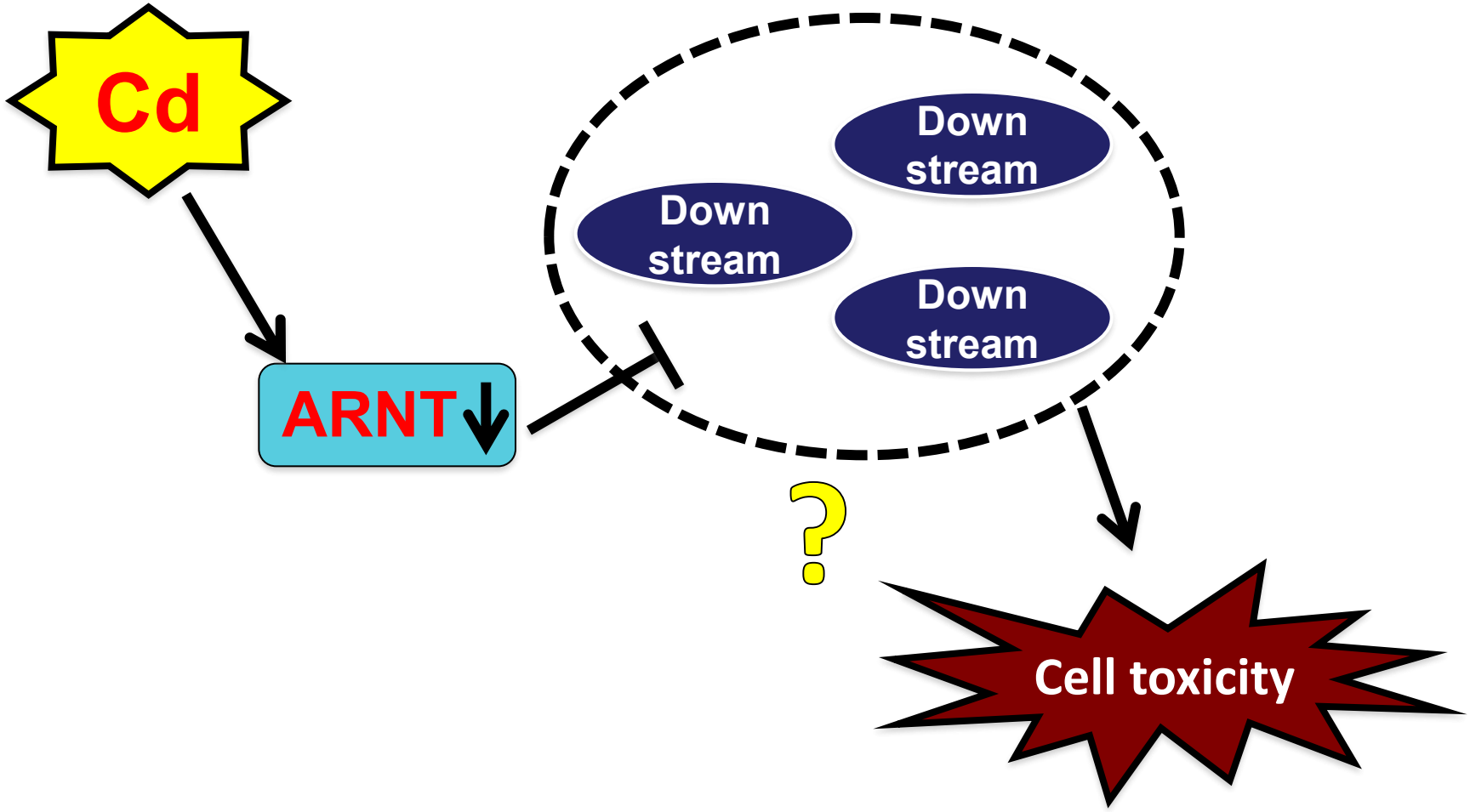
HK-2 cells
↓Cd 40 μM for 3 h
Nuclear extracts
↓Incubation with DNA probe
Separated on a polyacrylamide gel
↓
Transfer to a nylon membrane
↓
Detection



Cd decreased transcription activity of ARNT in HK-2 cells

Lee *et al.*, Sci. Rep. 2017

Downstream factor of ARNT may regulate Cd toxicity



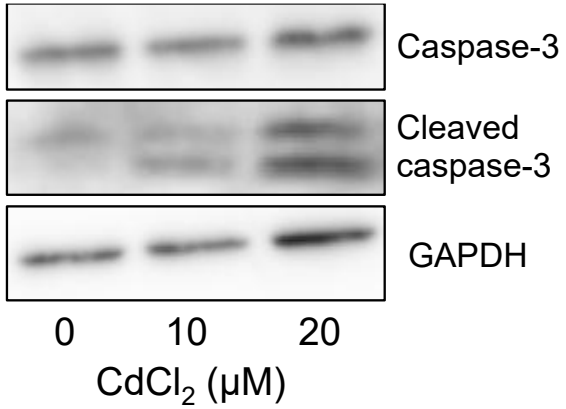
Down-regulated genes by knockdown of *ARNT* in HK-2 cells

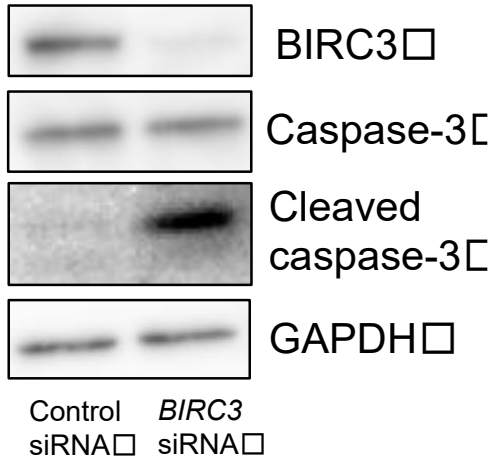
Accession Number	Gene Name	Description	Ratio
NM_152417	*TMEM68*	Transmembrane protein 68	0.219
NM_006243	*PPP2R5A*	Protein phosphatase 2, regulatory subunit B', alpha	0.314
NM_031885	*BBS2*	Bardet-Biedl syndrome 2	0.373
NM_003003	*SEC14L1*	SEC14-like 1 (S. cerevisiae)	0.376
NM_080605	*B3GALT6*	UDP-Gal:betaGal beta 1,3-galactosyltransferase polypeptide 6	0.393
NM_017901	*TPCN1*	Two pore segment channel 1	0.398
NM_020422	*TMEM159*	Transmembrane protein 159	0.421
NM_021158	*TRIB3*	Tribbles pseudokinase 3	0.421
NM_033308	*ABCA7*	ATP-binding cassette, sub-family A (ABC1), member 7	0.423
NM_004199	*P4HA2*	Prolyl 4-hydroxylase, alpha polypeptide II	0.432
NM_005178	*BCL3*	B-cell CLL/lymphoma 3	0.433
NM_145753	*PHLDB2*	Pleckstrin homology-like domain, family B, member 2	0.443
NM_006688	*C1QL1*	Complement component 1, q subcomponent-like 1	0.443
NM_006013	*RPL10*	Ribosomal protein L10	0.452
NM_016374	*ARID4B*	AT rich interactive domain 4B (RBP1-like)	0.454
NM_016096	*ZNF706*	Zinc finger protein 706	0.459
NM_018354	*TMEM74B*	Transmembrane protein 74B	0.467
NM_001541	*HSPB2*	Heat shock 27kDa protein 2	0.480
NM_006896	*HOXA7*	Homeobox A7	0.482
NM_018155	*SLC25A36*	Solute carrier family 25 (pyrimidine nucleotide carrier), member 36	0.485
NM_001707	*BCL7B*	B-cell CLL/lymphoma 7B	0.487
NM_015062	*PPRC1*	Peroxisome proliferator-activated receptor gamma, coactivator-related 1	0.488
NM_016076	*DESI2*	Desumoylating isopeptidase 2	0.489
NM_018132	*CENPQ*	Centromere protein Q	0.492

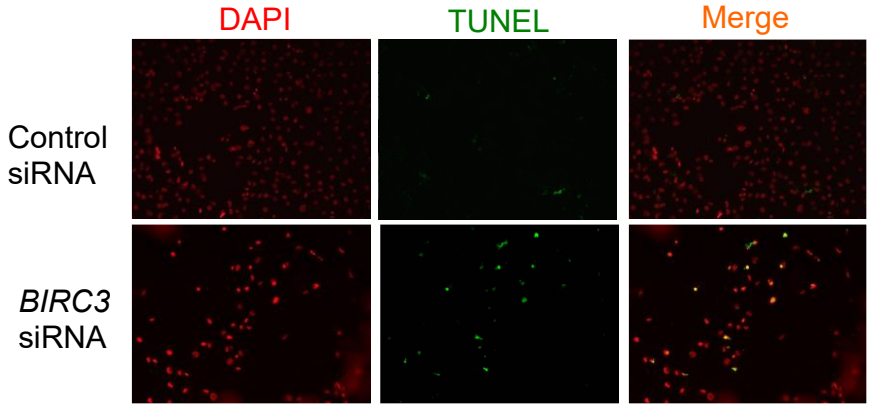
⇒ BIRC3 : An apoptosis suppressor *via* caspase inhibition

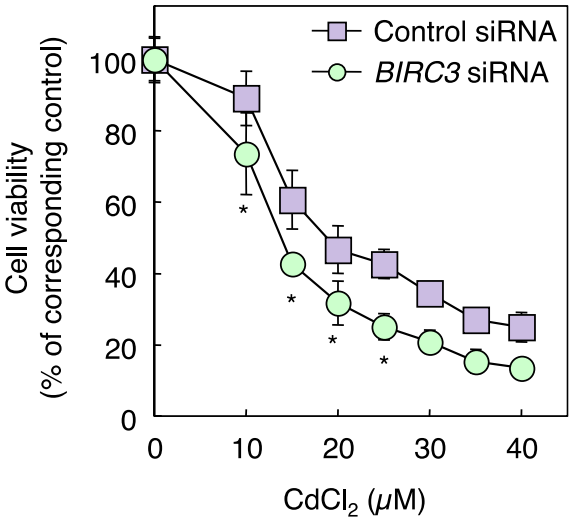
DNA microarray in ARNT-depleted cells revealed a decrease in the expression of 27 genes less than a half including *BIRC3*

Apoptosis in *BIRC3* knockdown HK-2 cells







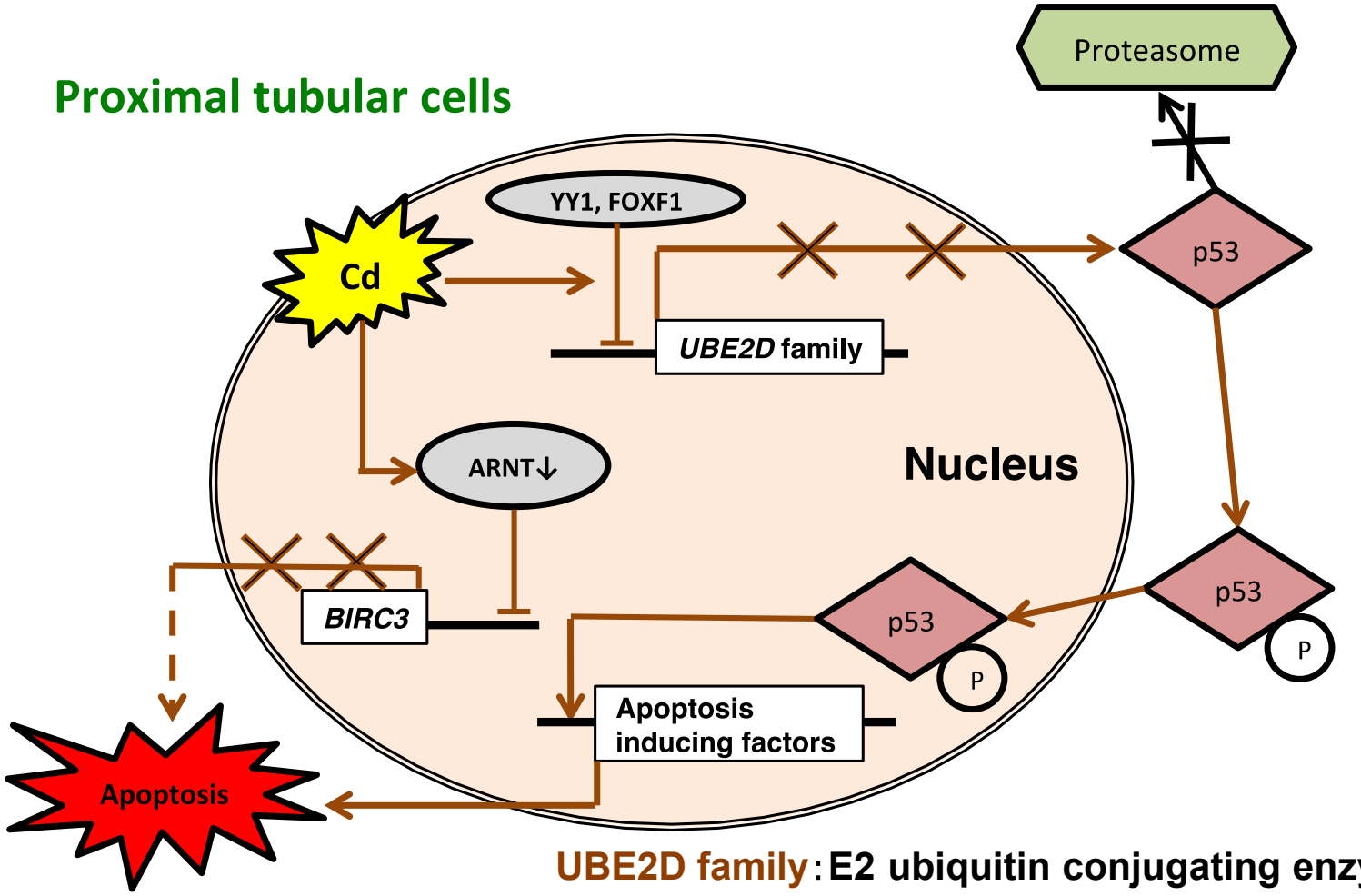


mRNA levels were normalized with *GAPDH*. Values are means ± S.D. (n=3). *Significantly different from control, $p < 0.05$.

Decrease of BIRC3 was involved in Cd-induced apoptosis in HK-2 cells

Lee *et al.*, Sci. Rep. 2017

The pathway of Cd toxicity through the inhibition of transcription factors in proximal tubular cells



UBE2D family: E2 ubiquitin conjugating enzymes

p53 : Tumor suppressor, apoptosis inducer, a target of Ube2d family

BIRC3 : Apoptosis inhibitor

Transcription factors altered by Cd

Cd-altered activities of transcription factors

HK-2 cells

Cd 40 μM 3 h

Nuclear extraction

Protein/DNA-binding assay

SCIENTIFIC REPORTS

OPEN

Identification of ARNT-regulated BIRC3 as the target factor in cadmium renal toxicity

Received: 5 July 2017
Accepted: 27 November 2017
Published online: 11 December 2017

Jin-Yong Lee[1], Maki Tokumoto[1], Gi-Wook Hwang[2], Moo-Yeol Lee[3] & Masahiko Satoh[1]

Cadmium (Cd) is an environmental contaminant that exhibits renal toxicity. The target transcription factors involved in Cd renal toxicity are still unknown. In this study, we demonstrated that Cd decrease

SCIENTIFIC REPORTS | 7: 17287 | DOI:10.1038/s41598-017-17494-9

[1]Laboratory of Pharmaceutical Health Sciences, School of Pharmacy, Aichi Gakuin University, 1–100 Kusumoto-cho, Chikusa-ku, Nagoya, Aichi, 464–8650, Japan. [2]Laboratory of Molecular and Biochemical Toxicology, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, 980-8578, Japan. [3]College of Pharmacy, Dongguk University, Goyang, Gyeonggi-do, 410-820, Republic of Korea. Correspondence and requests for materials should be addressed to M.S. (email: masahiko@dpc.agu.ac.jp)

The transcription factors whose activities were decreased by Cd

Name	Description	Ratio
CEA	Carcinoembryonic antigen gene promoter	0.09
HIF-1	Hypoxia-inducible factor 1	0.12
GATA-1	GATA binding protein-1	0.19
EGR	Early growth response element	0.21
MT-Box	Tentative new binding domain, located in hTERT promotoer	0.21
MZF1	Myeloid zinc finger 1	0.22
POU2F3	Octamer-binding site in epidermis (POU domain factor)	0.23
PPAR	Peroxisome proliferator activated receptor alpha	0.27
CEF-2	cTnC (Slow/Cardiac Troponin C)	0.27
MEF2A	MADS box transcription enhancer factor 2	0.29
TEF1	Transcription enhancer factor-1	0.29
MSP-1	SAA with SP1 binding site removed	0.31
Sp-1	Sp1 transcription factor	0.32
ARNT	Aryl hydrocarbon receptor nuclear translocator binding element	0.34
Myb	Myb proto-oncogene protein	0.34
GATA-3	GATA binding protein-3	0.36
HOXD-9	Homeobox D9	0.36

Table 2. Transcription factors with decreased binding activities in response to Cd treatment. HK-2 cells were treated with 40 μM Cd in serum-free culture medium for 3 h. The cells were separated into nuclear and post-nuclear fractions. The protein/DNA binding array was performed using the Combo Protein/DNA Array. Spot density was evaluated using ImageQuantTL software. Transcription factors whose activities were decreased 0.5-fold or less are listed.

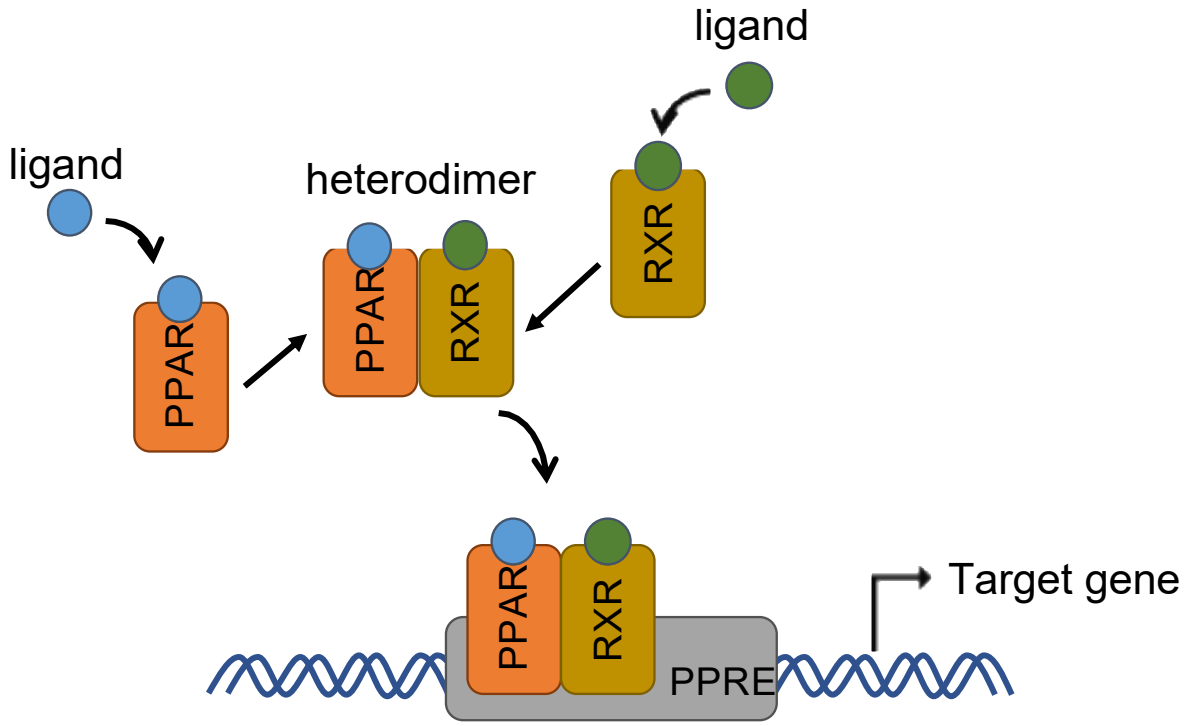
Lee *et al.*, *Sci. Rep.*, 2017

PPAR

- Peroxisome Proliferator-Activated Receptor
- The nuclear receptors expressed in many tissues such as kidneys, livers, and adipose tissue, and are involved in lipid metabolism and cell differentiation

- Sub-types

PPARα (gene name: *PPARA*)
PPARδ (gene name: *PPARD*)
PPARγ (gene name: *PPARG*)

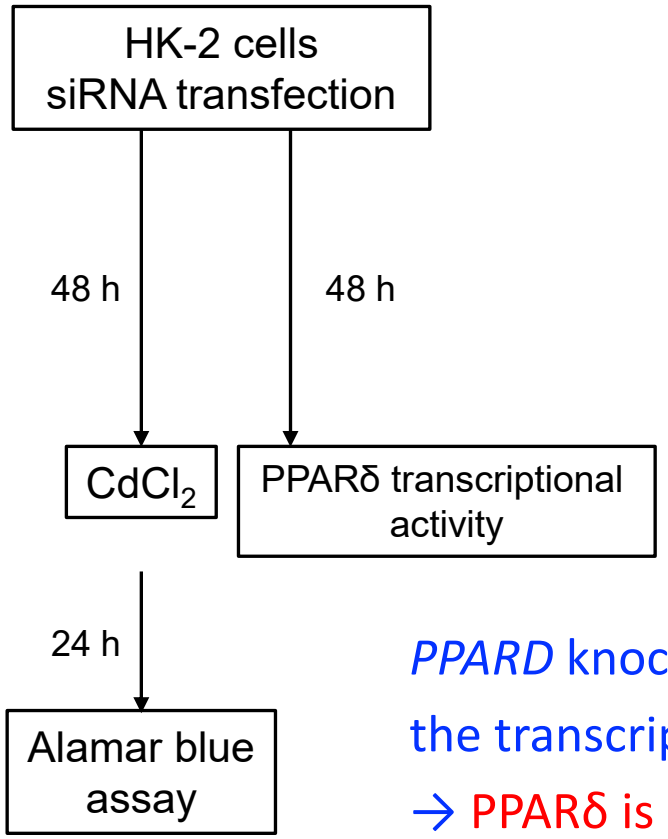


Effect of *PPARD* knockdown on the Cd toxicity and the activity of PPARδ in HK-2 cells

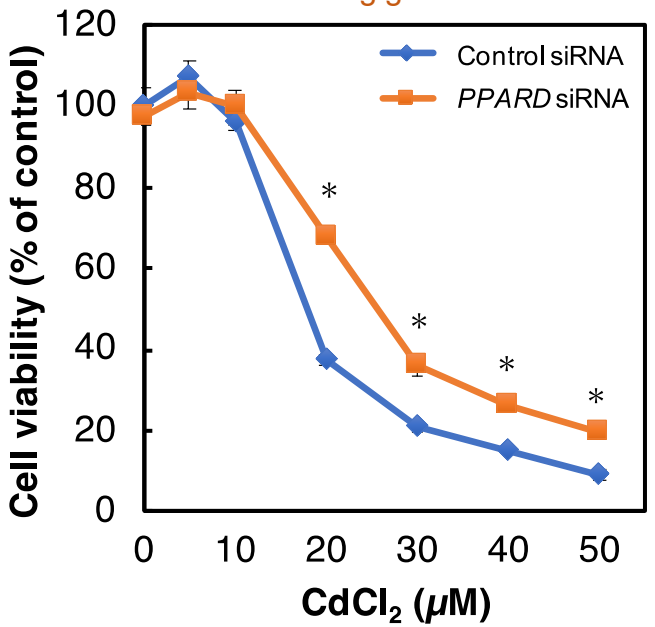
HK-2 cells:

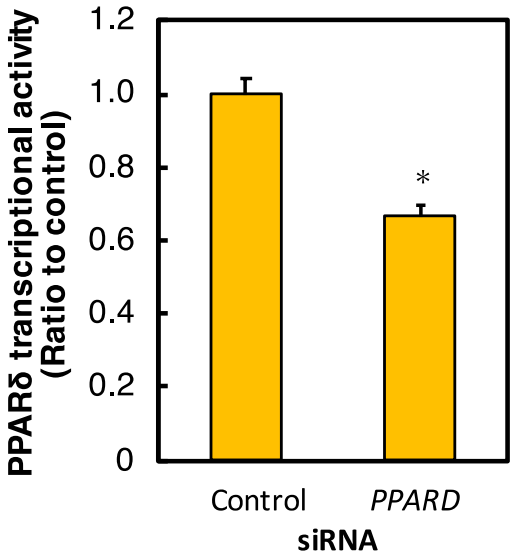
Human proximal tubular cells

Methods



PPARD :
PPARδ coding gene





Values are means ± S.D. (n = 3).
*Significantly different from control group , $P < 0.05$.

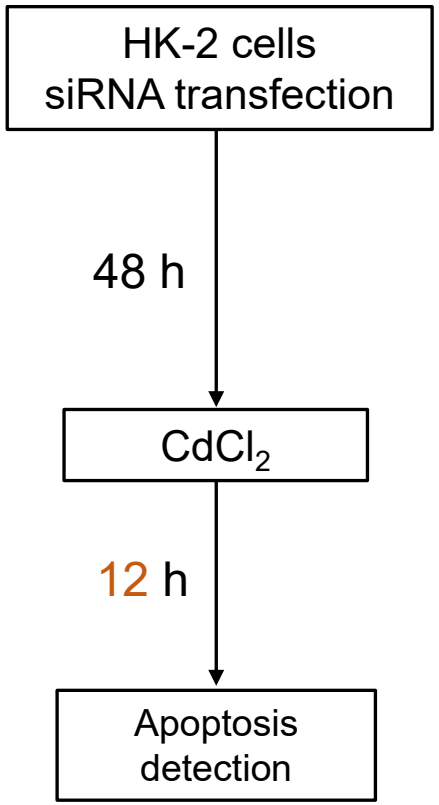
PPARD knockdown showed the resistance to Cd and significantly reduced the transcriptional activity of PPARδ.

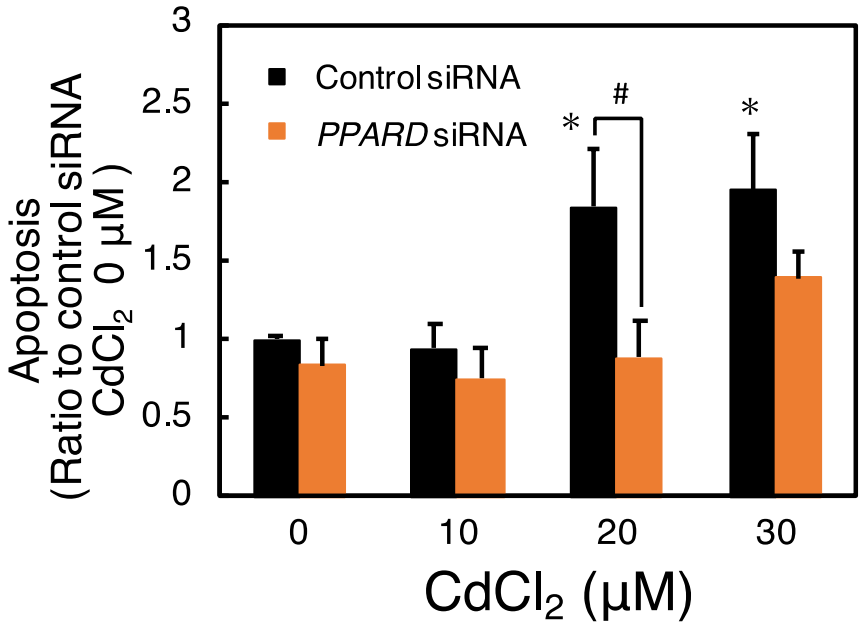
→ PPARδ is a modifying factor of Cd toxicity, and reduced transcriptional activity of PPARδ may provide the resistance to Cd toxicity.

Mori *et al.*, *Int. J. Mol. Sci.*, 2022

Effect of *PPARD* knockdown on the Cd-induced apoptosis

Methods



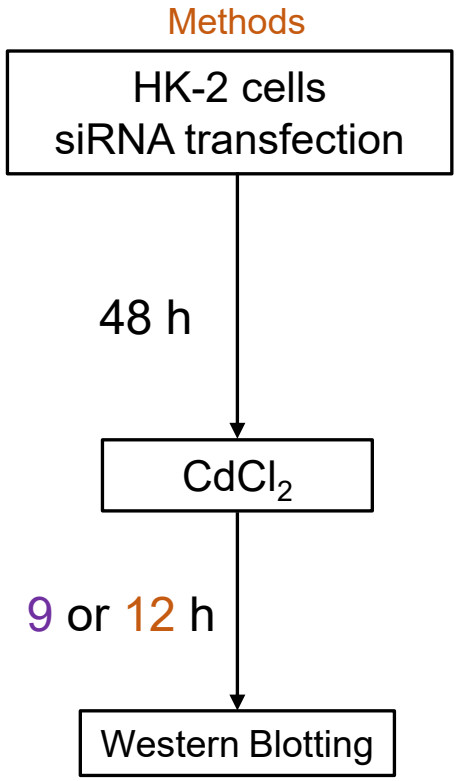


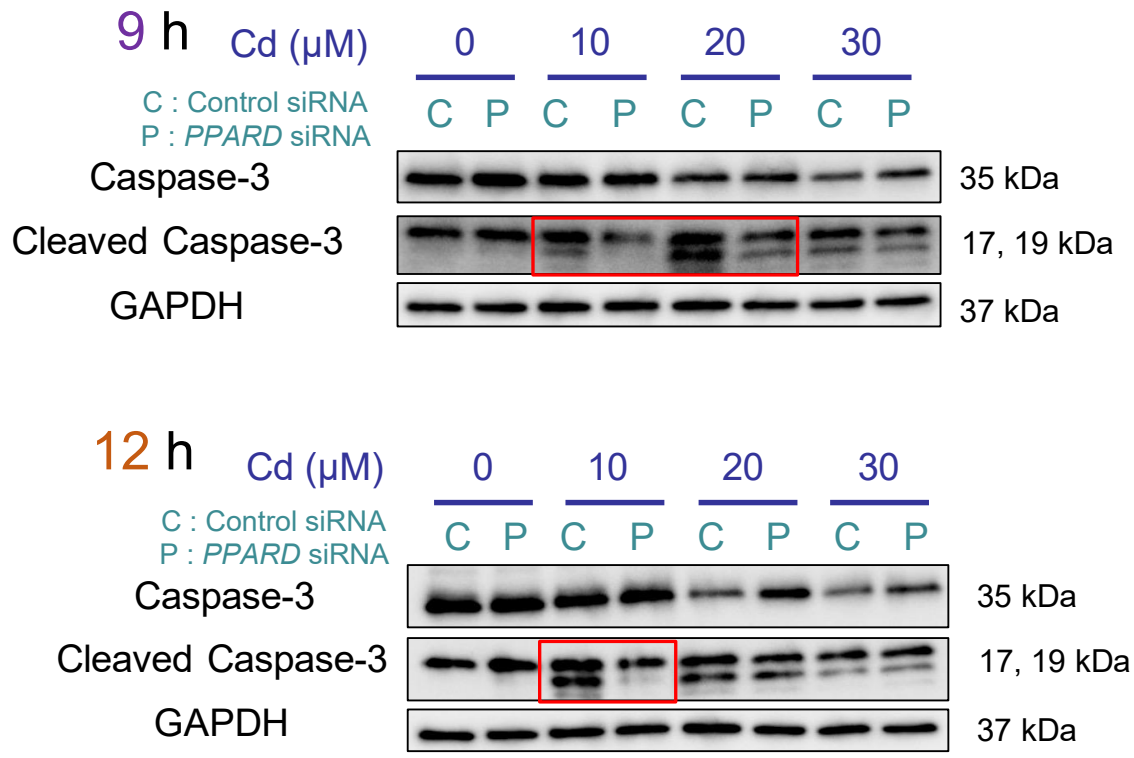
Values are means ± S.D. (n = 3).
*Significantly different from control group , $P < 0.05$.
#, $P < 0.05$.

Cd-induced apoptosis was inhibited by *PPARD* knockdown.

Mori *et al.*, *Int. J. Mol. Sci.*, 2022

Effect of PPARD knockdown on caspase-3 activity by Cd

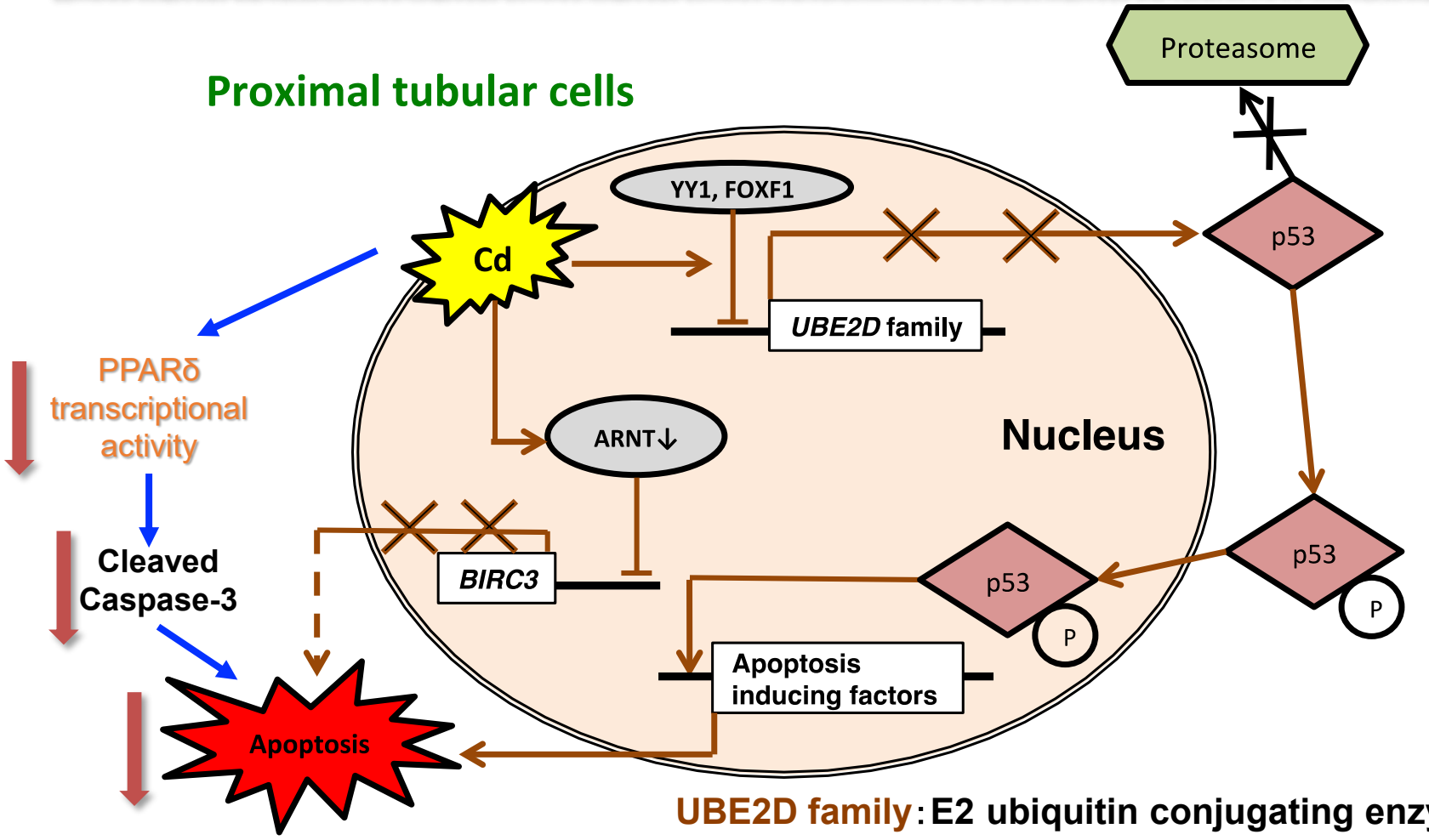




Increased cleaved caspase-3 level by Cd was inhibited by *PPARD* knockdown.

Mori *et al.*, *Int. J. Mol. Sci.*, 2022

The pathway of Cd toxicity through the inhibition of transcription factors in proximal tubular cells



UBE2D family: E2 ubiquitin conjugating enzymes

p53 : Tumor suppressor, apoptosis inducer, a target of Ube2d family

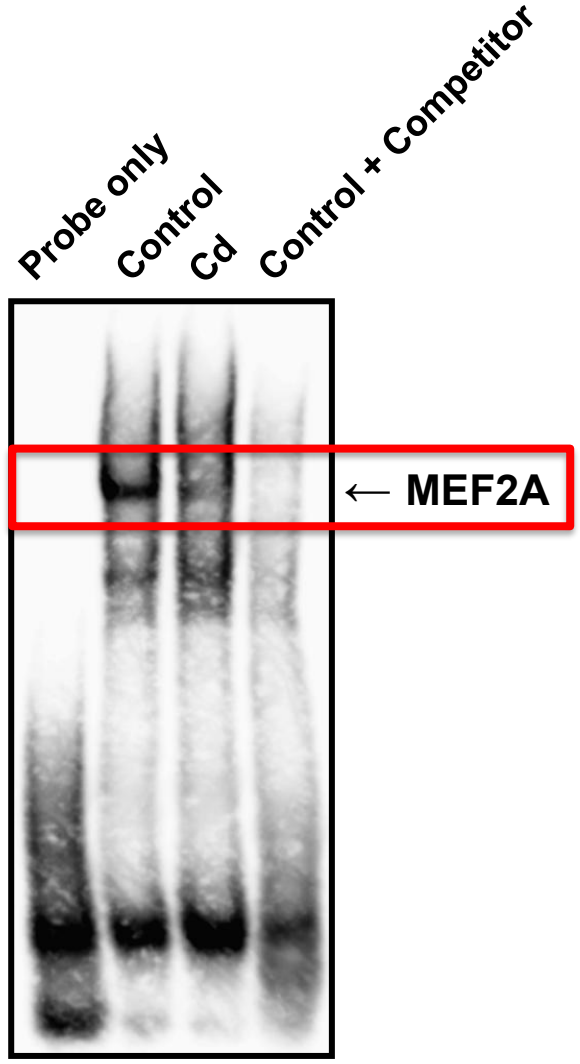
BIRC3 : Apoptosis inhibitor

PPAR : Peroxisome Proliferator-Activated Receptor

DNA binding activity of MEF2A in HK-2 cells treated with Cd

MEF2A: myocyte-specific enhancer factor 2A

HK-2 cells
↓Cd 40 μM for 3 h
Nuclear extracts
↓Incubation with DNA probe
Separated on a polyacrylamide gel
↓
Transfer to a nylon membrane
↓
Detection

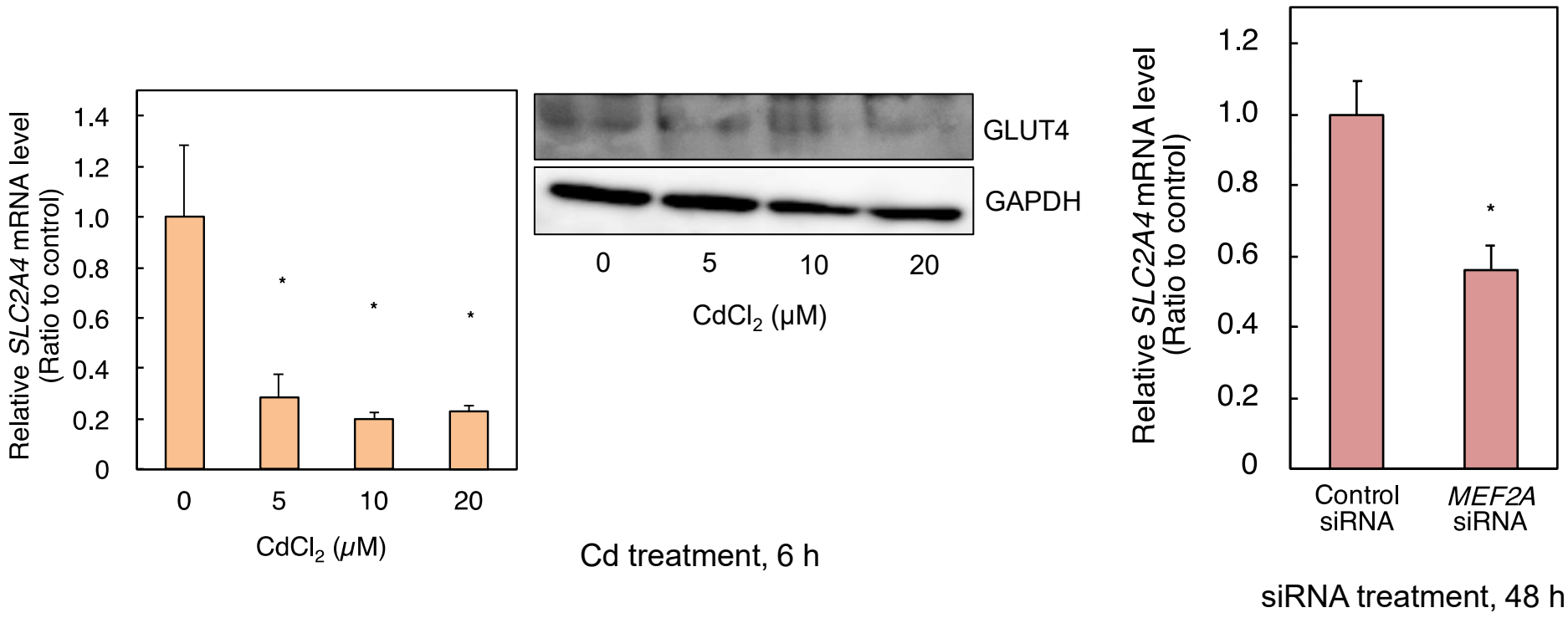


Cd decreased transcription activity of MEF2A in HK-2 cells

Lee *et al.*, FASEB J., 2021

GLUT4 (*SLC2A4*) expression changes by Cd and *MEF2A* siRNA treatment

***SLC2A4*: coding gene for GLUT4 (glucose transporter 4) protein**



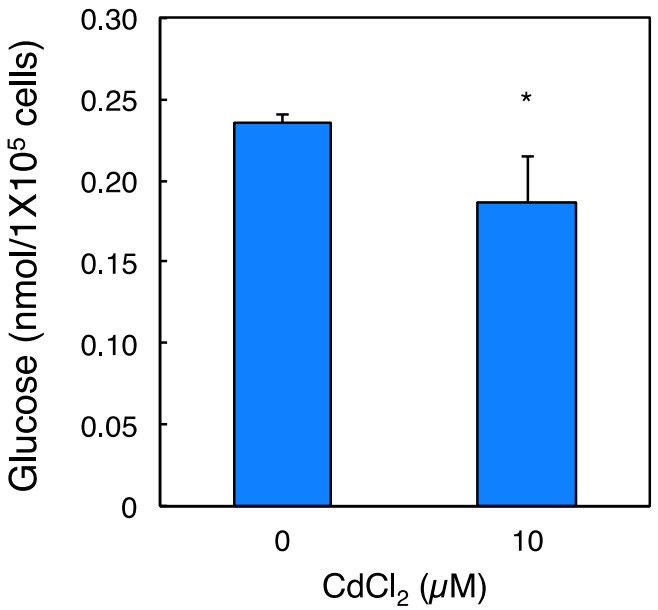
mRNA levels were examined using real-time RT-PCR. mRNA levels were normalized to *GAPDH*.
Values are means ± S.D. (n=3). *Significantly different from control, $p < 0.05$.

Not only Cd treatment but also *MEF2A* siRNA decreased the GLUT4 (*SLC2A4*) expression

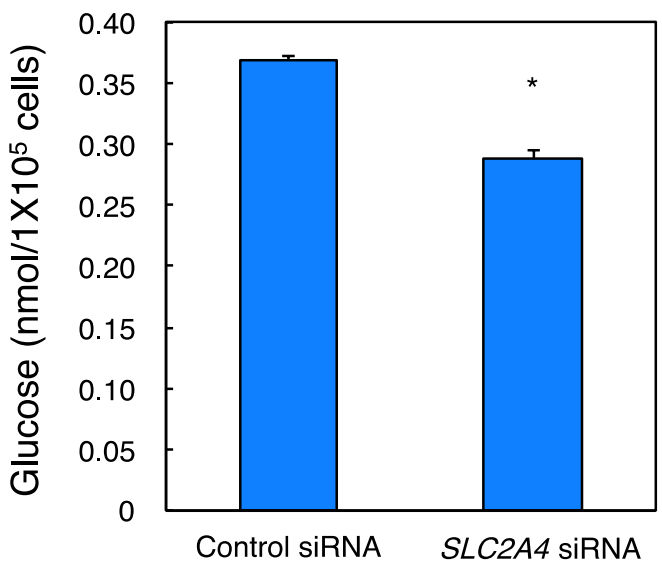
Lee *et al.*, FASEB J., 2021

Effects of Cd and *SLC2A4* siRNA on the cellular glucose level in HK-2 cells

Cd treatment, 6 h



siRNA treatment, 48 h



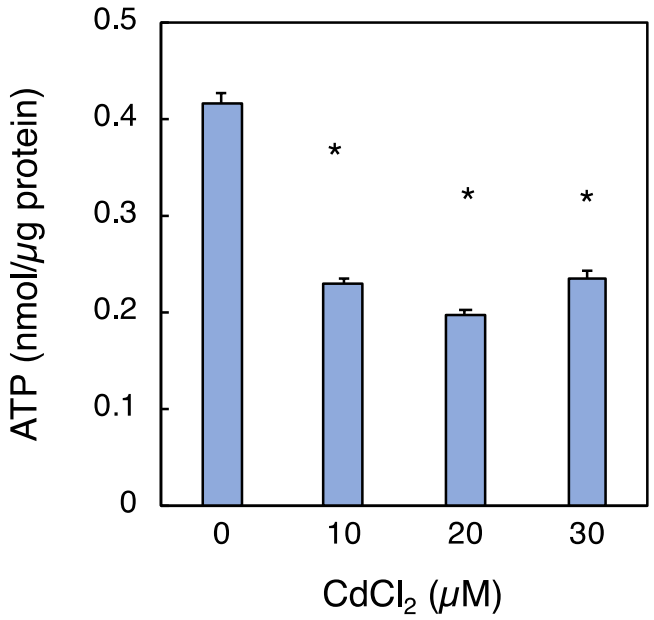
The glucose level was measured according to the Glucose Assay Kit. The glucose level was normalized to cell number. Values are means ± S.D. (n=3). *Significantly different from the control group. P<0.05.

Not only Cd treatment but also *SLC2A4* knockdown decreases the cellular glucose level in HK-2 cells

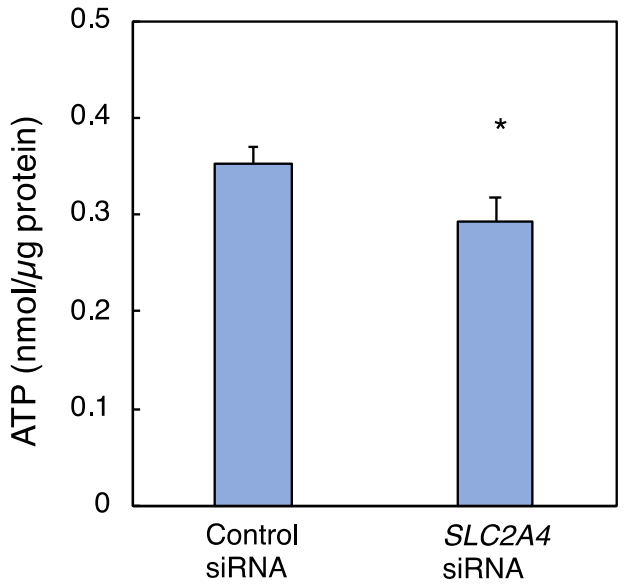
Lee *et al.*, FASEB J., 2021

Effect of Cd or *SLC2A4* siRNA on the ATP level in HK-2 cells

Cd treatment, 6 h



siRNA treatment, 48 h

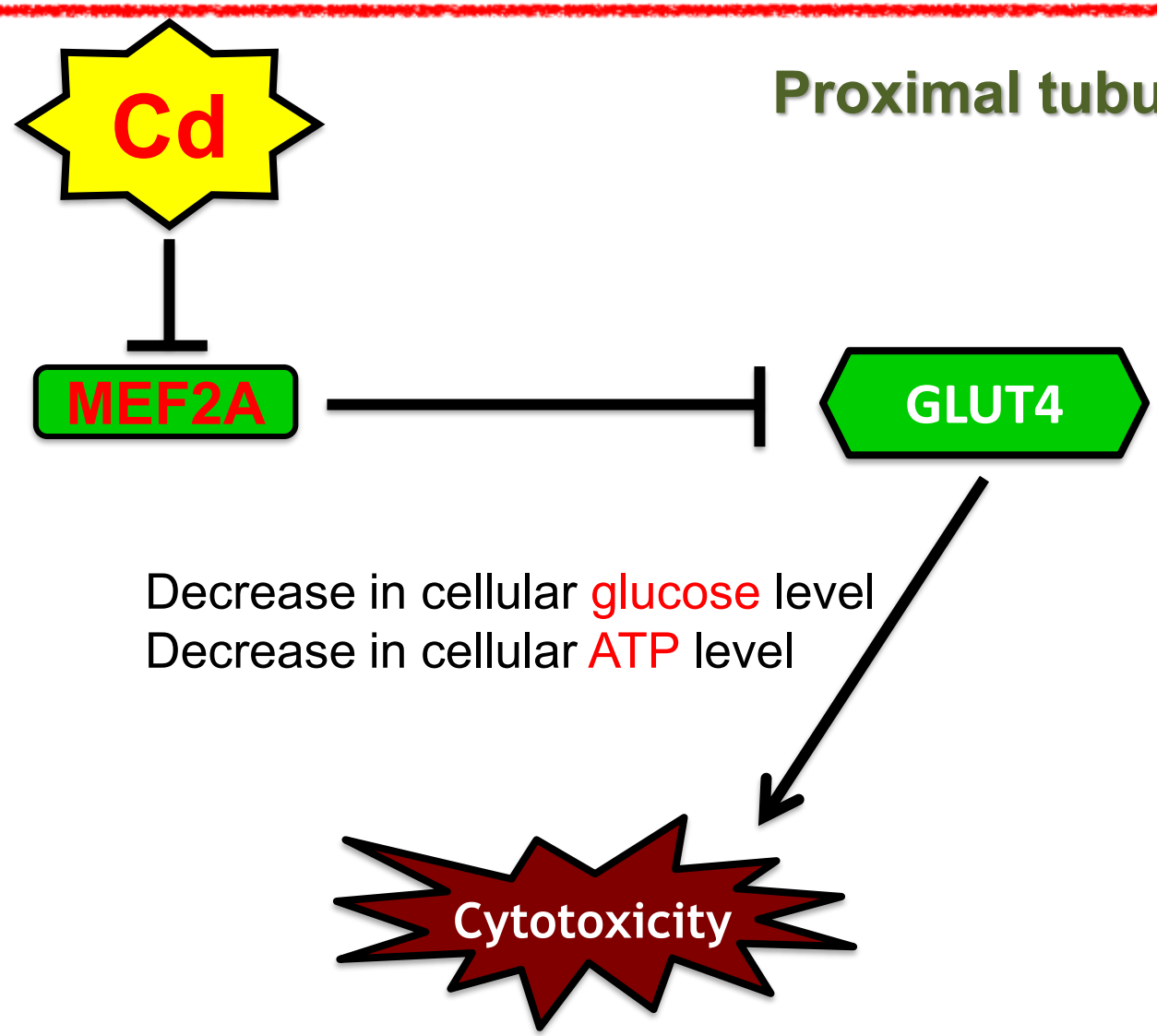


Cellular ATP levels were measured with CellTiter-Glo® 2.0 Reagent Kit. Values are the means ± S.D. (n = 4). *Significantly different from the control group. $P<0.05$.

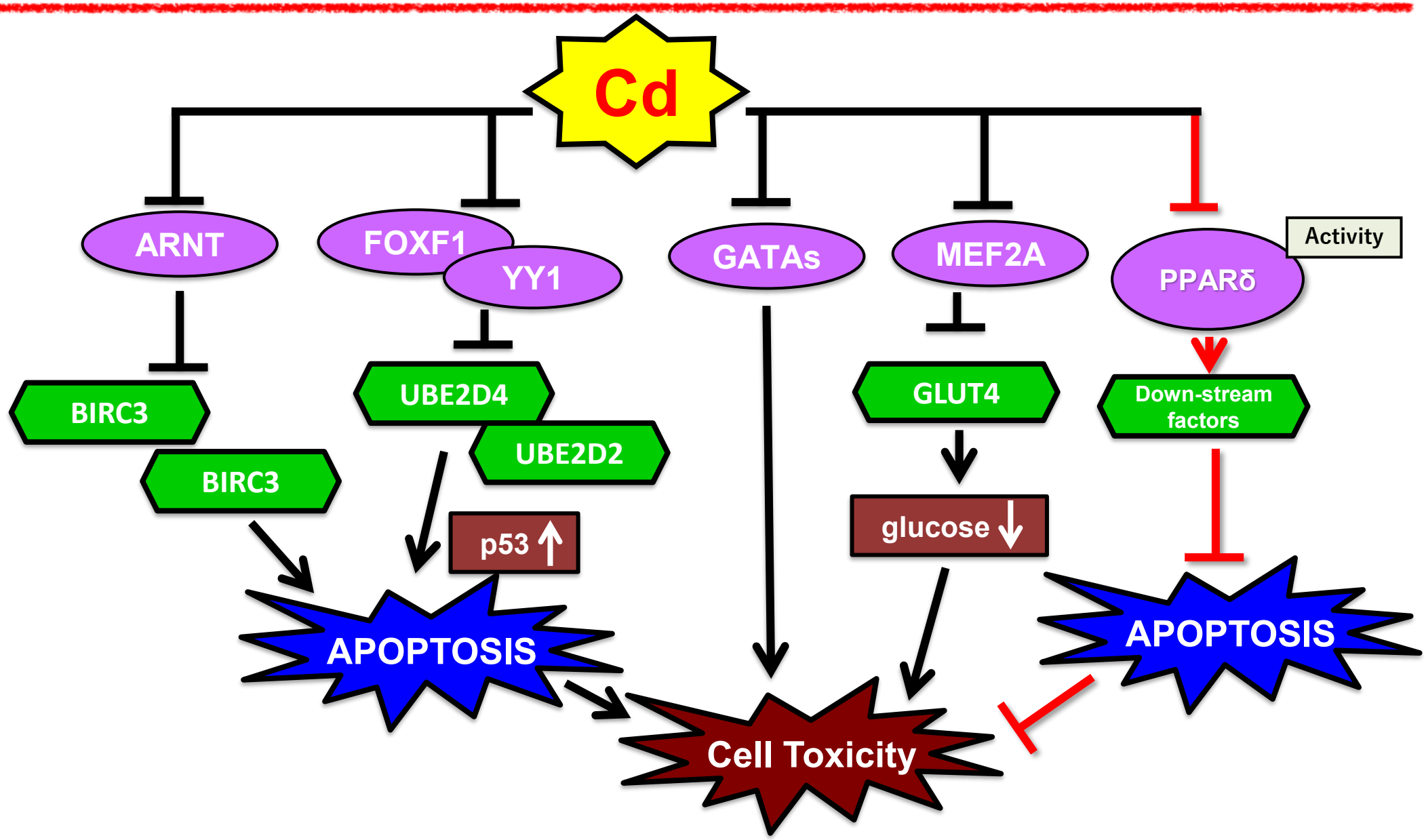
Not only Cd treatment but also *SLC2A4* knockdown decreases the cellular ATP level in HK-2 cells

Lee *et al.*, FASEB J., 2021

MEF2A pathway in Cd renal toxicity



カドミウムの毒性発現における細胞内転写経路の関与



謝 辞

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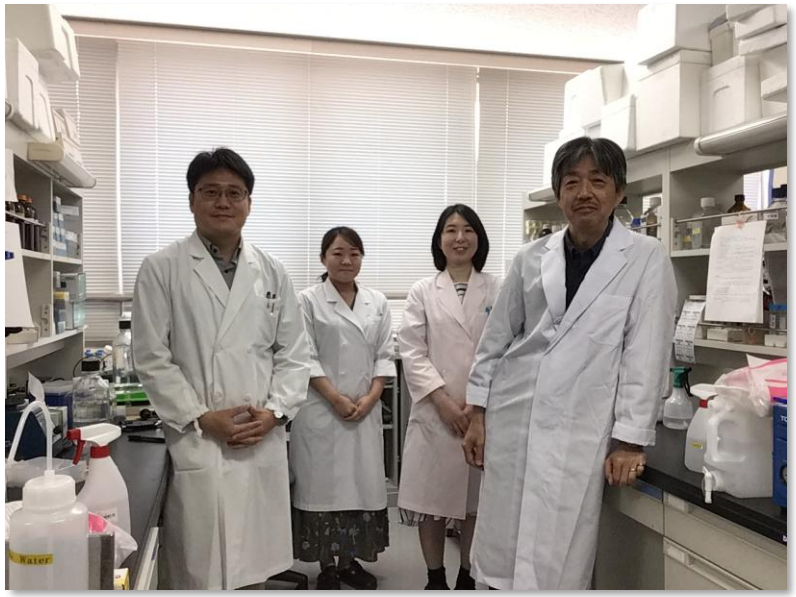

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藤原 泰之　教授

ご清聴ありがとうございました

AICHI GAKUIN
UNIVERSITY