

Regulation of ferroptosis

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57 institutes and departments



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Institute of Metabolism and Cell Death
Director: Dr. Marcus Conrad



- Redox biology
- Cell death, Ferroptosis





Cell death

Accidental Cell Death

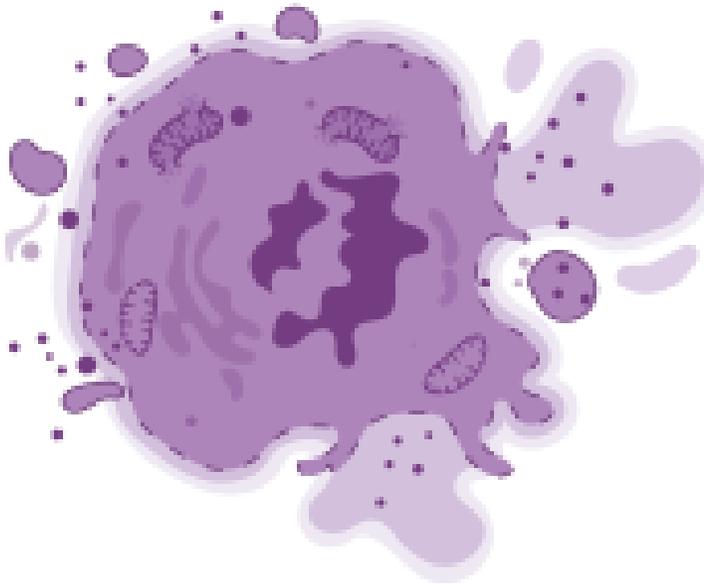
Regulated Cell Death

Apoptosis

Necroptosis

Ferroptosis

etc. ...

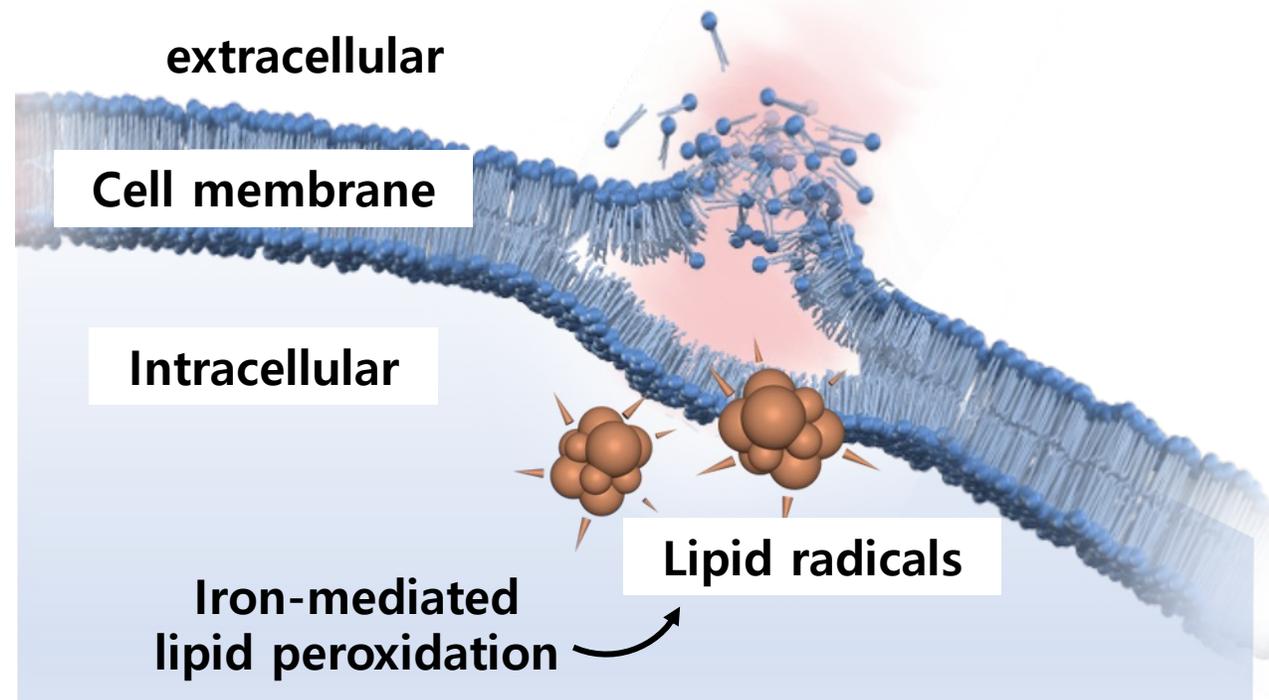




Ferroptosis

An iron-dependent, non-apoptotic regulated cell death characterized by extensive lipid peroxidation. *Dixon et al, Cell. 2012*

Lipid radicals is considered to lead to cellular membrane rupture

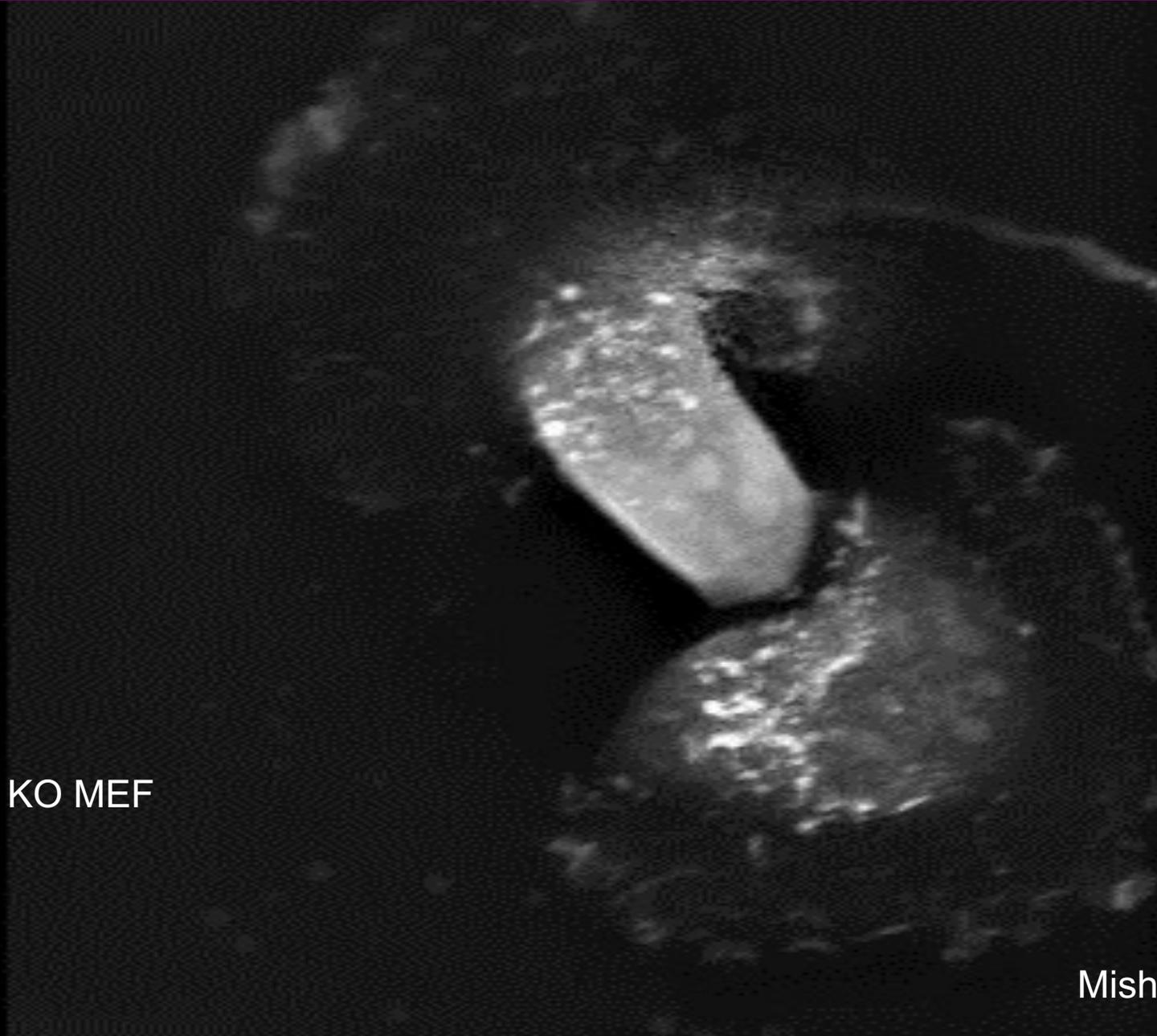




Ferroptosis

TAM-inducible Gpx4 KO MEF

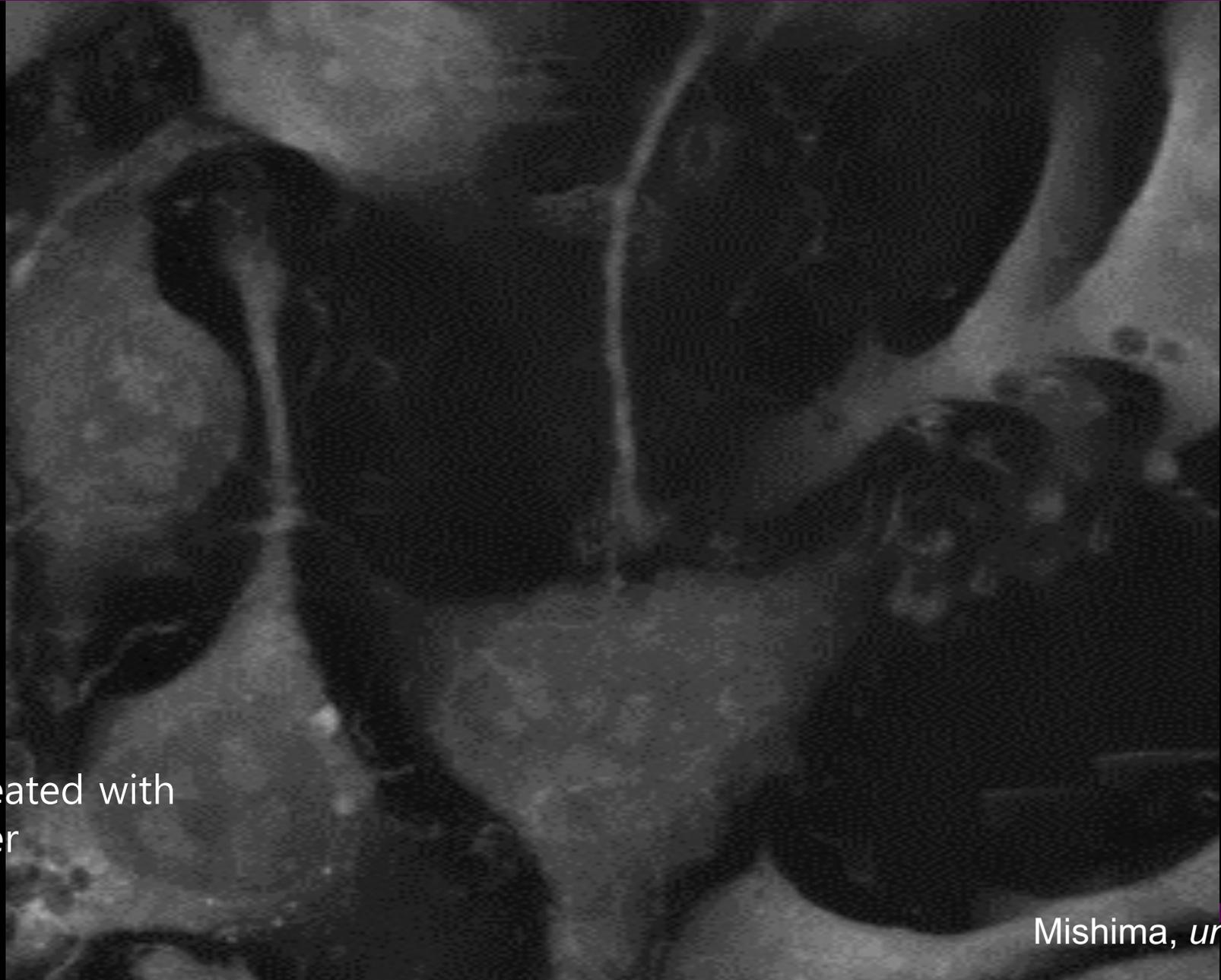
Nanolive imaging



Mishima, *unpublished data*



Apoptosis



HT-1080 cells treated with
apoptosis inducer
(Staurosporine)

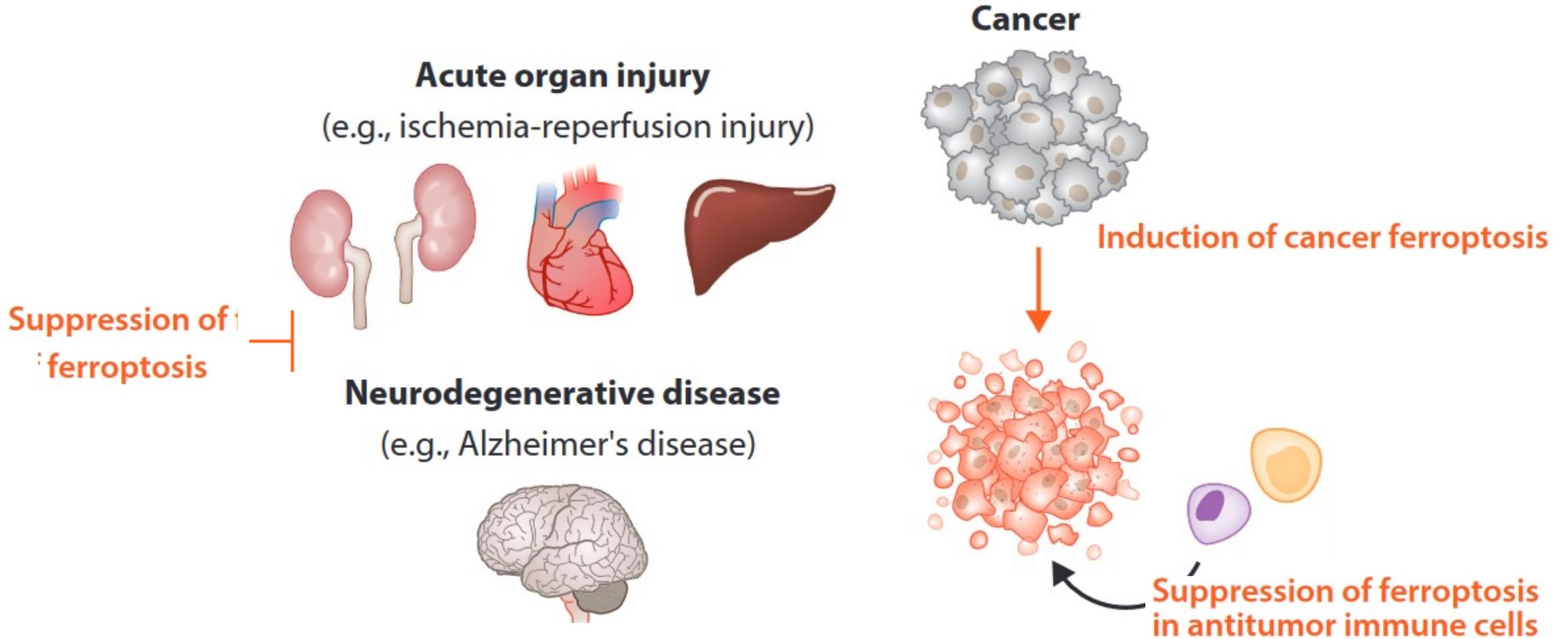
Mishima, *unpublished data*

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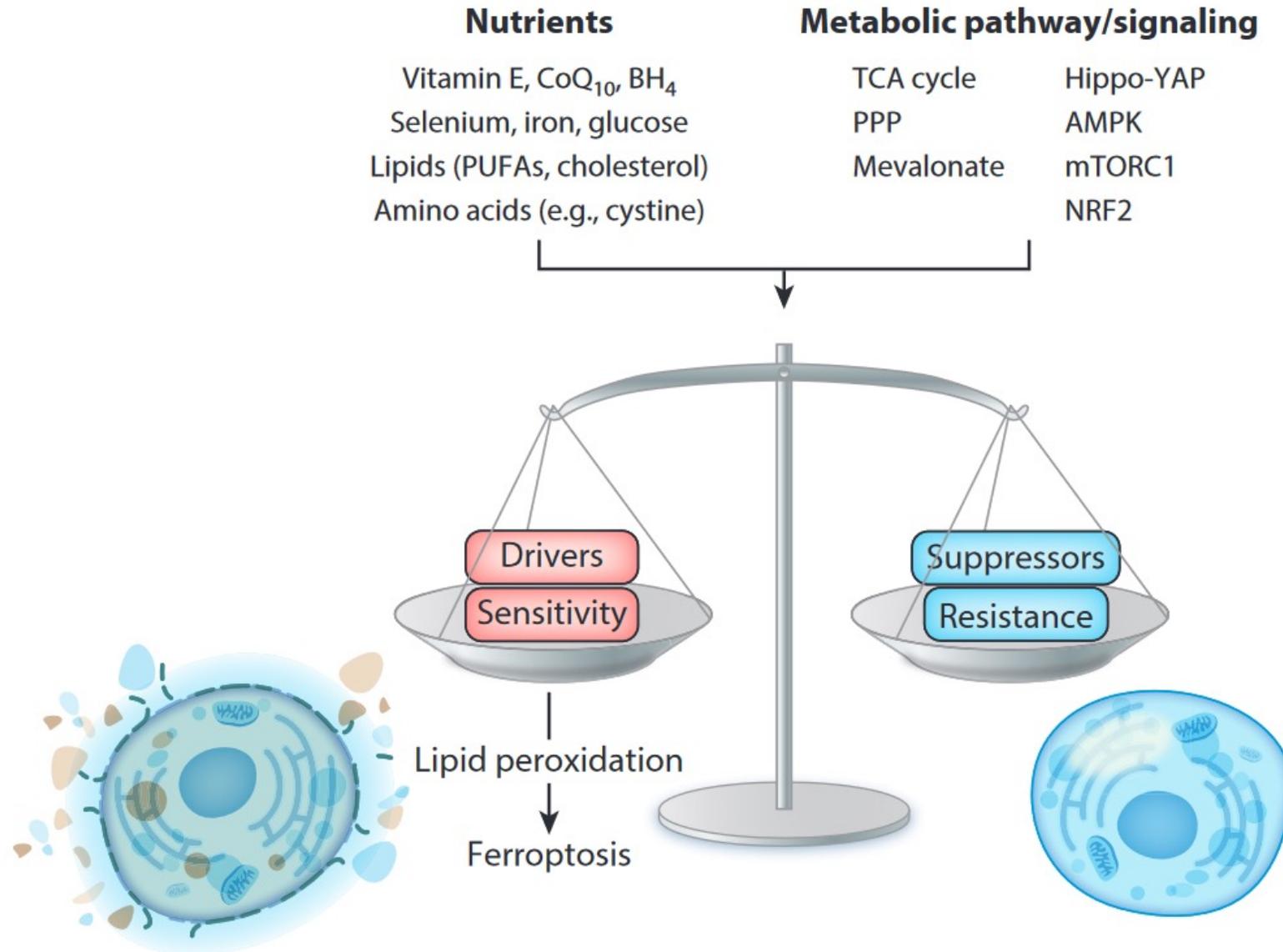


Ferroptosis and diseases

Promising therapeutic target



➔ Ferroptosis Regulation by metabolic pathway/signaling





Today's talk

- 1. History of ferroptosis and its regulation**
- 2. Vitamin K and ferroptosis**

| 2012



Ferroptosis: An Iron-Dependent Form of Nonapoptotic Cell Death

Scott J. Dixon,¹ Kathryn M. Lemberg,¹ Michael R. Lamprecht,³ Rachid Skouta,¹ Eleina M. Zaitsev,¹ Caroline E. Gleason,¹ Darpan N. Patel,¹ Andras J. Bauer,¹ Alexandra M. Cantley,¹ Wan Seok Yang,¹ Barclay Morrison III,³ and Brent R. Stockwell^{1,2,4,*}

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DOI 10.1016/j.cell.2012.03.042

➔ Cystine: an essential amino acid

1950s



Dr. Harry Eagle

- Cystine is essential for cultured cell growth
- Cystine deprivation induces cell death
- Eagle's minimal essential medium (MEM)



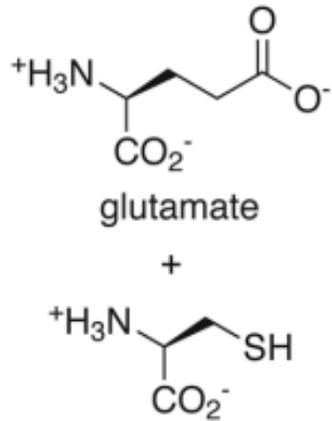
DMEM

Eagle, Science 1955 122:501

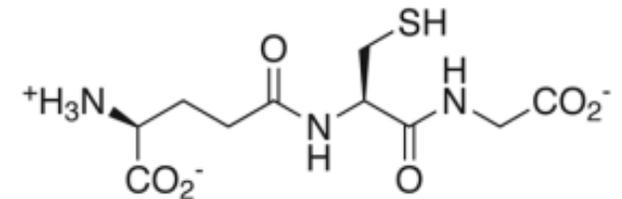
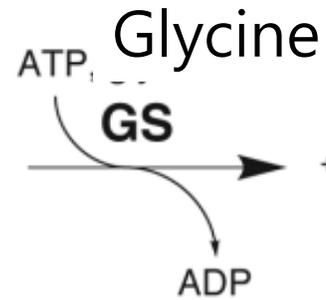
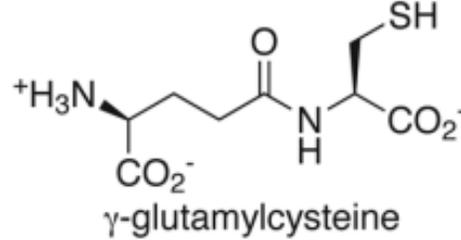
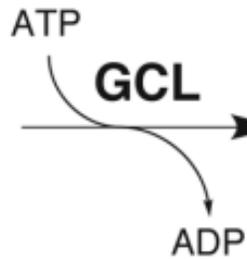
Eagle, JBC 1961 236:1425

➔ Cyst(e)ine is a building block of GSH

Glutamate



Cysteine



Glutathione (GSH)

I 1970s



Dr. Shiro Bannai (Tsukuba Univ.)

(Photo with Marcus Conrad)

- Cystine deprivation induces GSH starvation and accumulation of ROS, causing cell death
- This cell death can be rescued by vitamin E

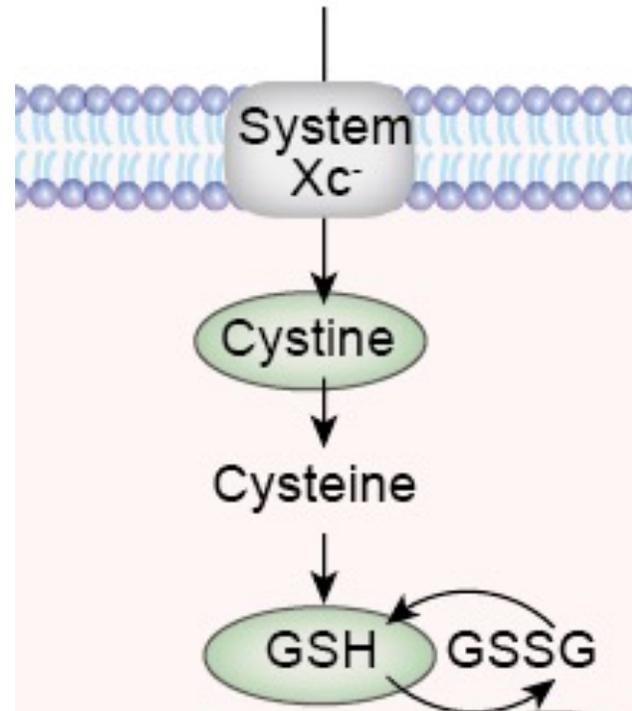
↳ Identification of cystine transporter, xCT

1990s



Dr. Hideyo Sato
(Niigata Univ.)

Cloning of cystine/glutamate antiporter, xCT

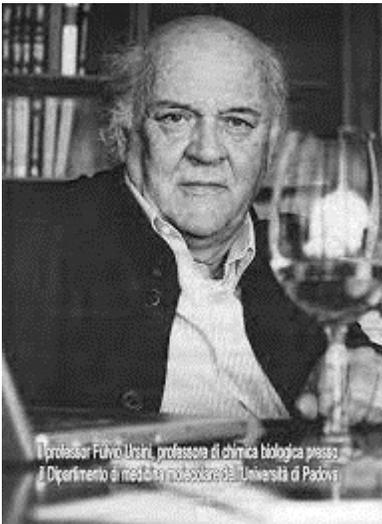


Sato, JBC 1999;274:11455



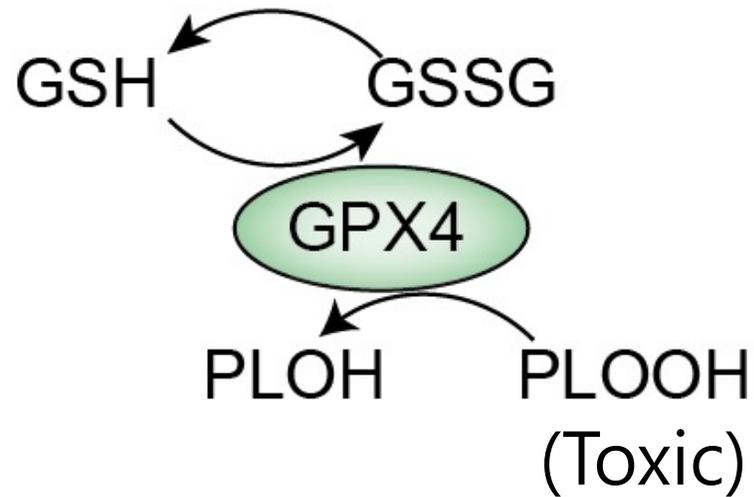
Identification of GPX4

1980s



Dr. Fulvio Ursini
(Univ. of Padova)

- GPX4 (glutathione peroxidase 4) was identified as a lipid peroxidation-detoxifying protein.

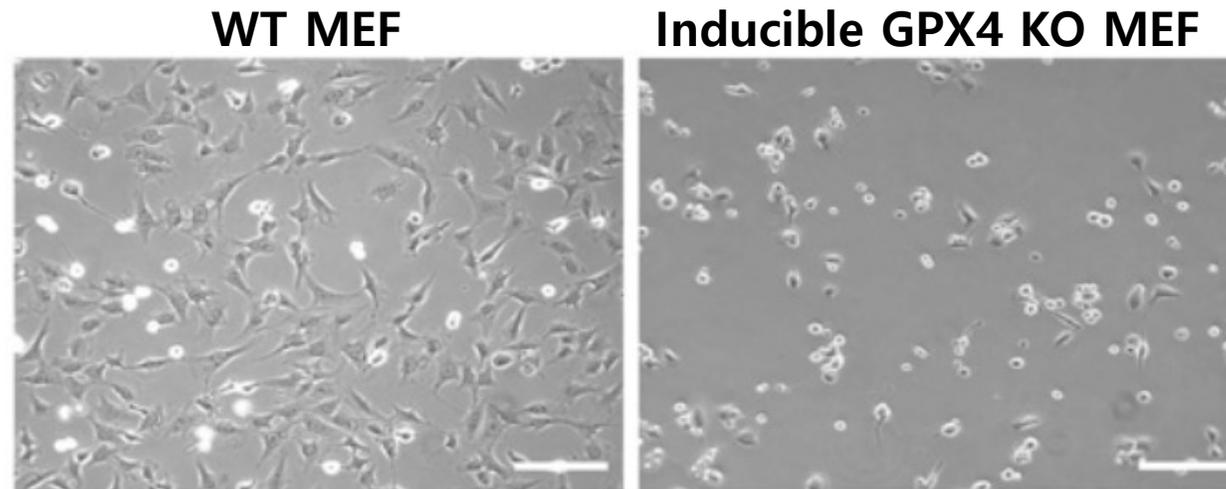


| 2000s



Dr. Marcus Conrad
(Photo when he was young)

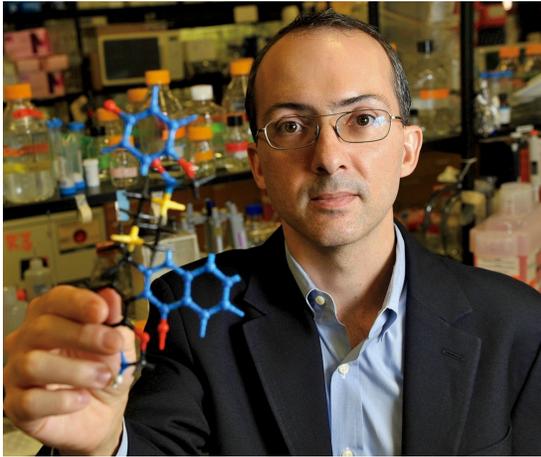
- Loss of GPX4 induces non-apoptotic cell death, characterized by lipid peroxidation





“Ferroptosis” was coined

2012



Dr. Brent Stockwell

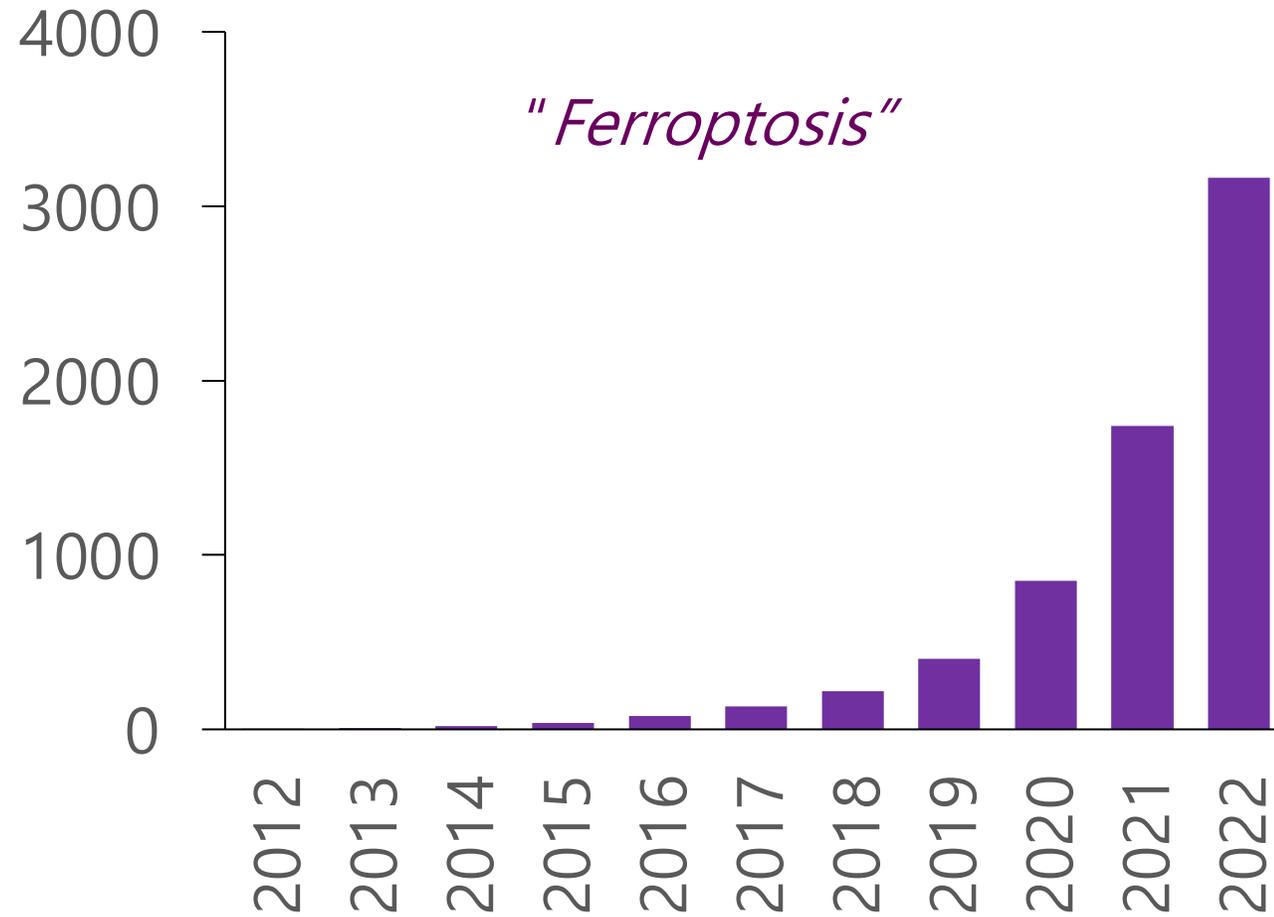
Erastin (xCT inhibitor) and RSL3 (GPX4 inhibitor) induce iron-dependent non-apoptotic cell death

“Ferroptosis”

Dixon, Cell 2012
Yang, Cell 2014



Number of published papers





Ferroptosis contains "oxidative stress"-induced cell death

Cell death forms by

GSH depletion

Cystine deprivation

xCT inhibition

Glutamate-induced oxytosis

"Oxidative stress"-induced cell death

Loss of GPX4

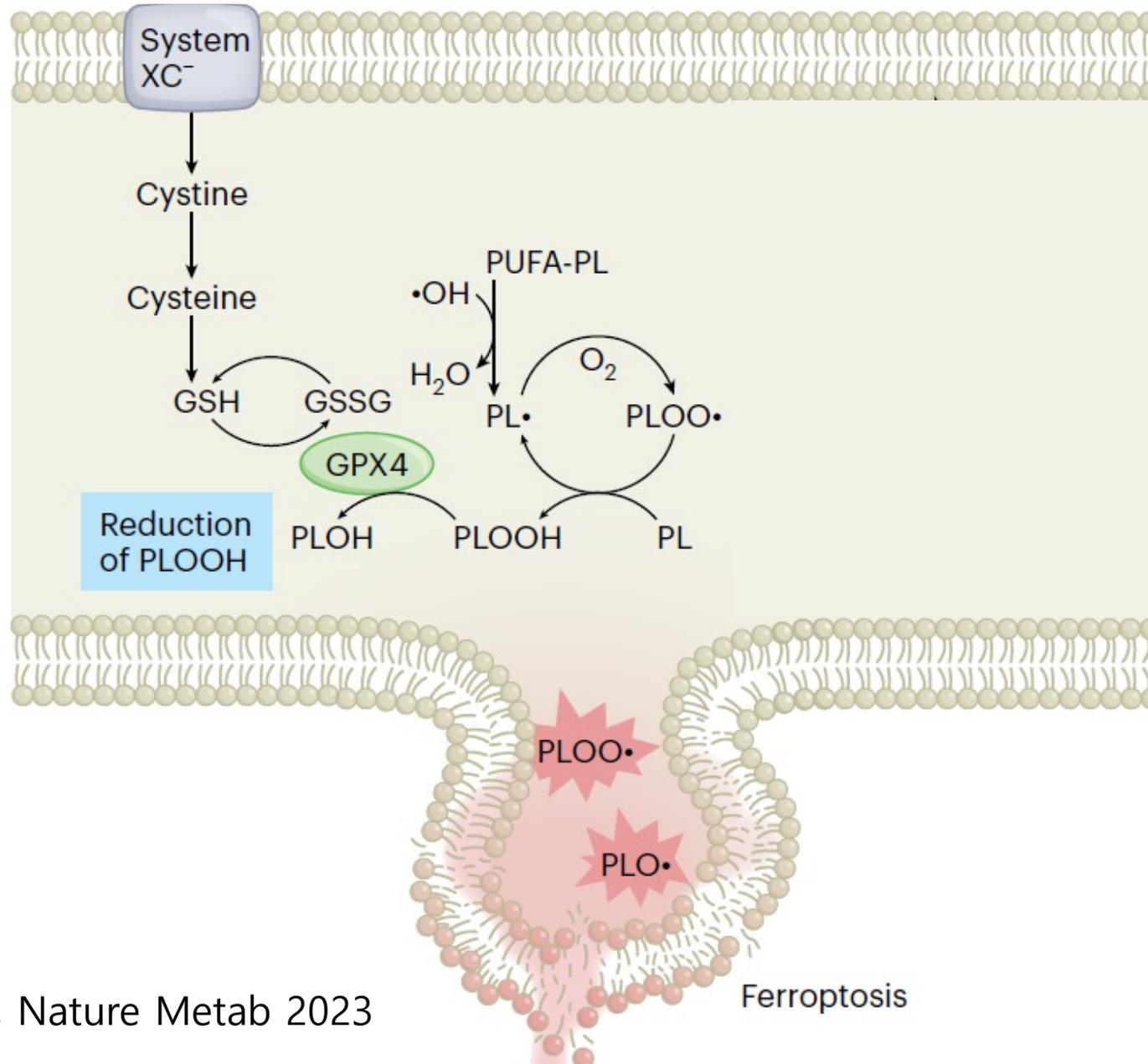
GPX4 inhibition

Genetic loss of GPX4

are all ferroptosis



Cystine-GSH-GPX4 axis: a guardian of ferroptosis





FSP1: GPX4 independent ferroptosis regulation

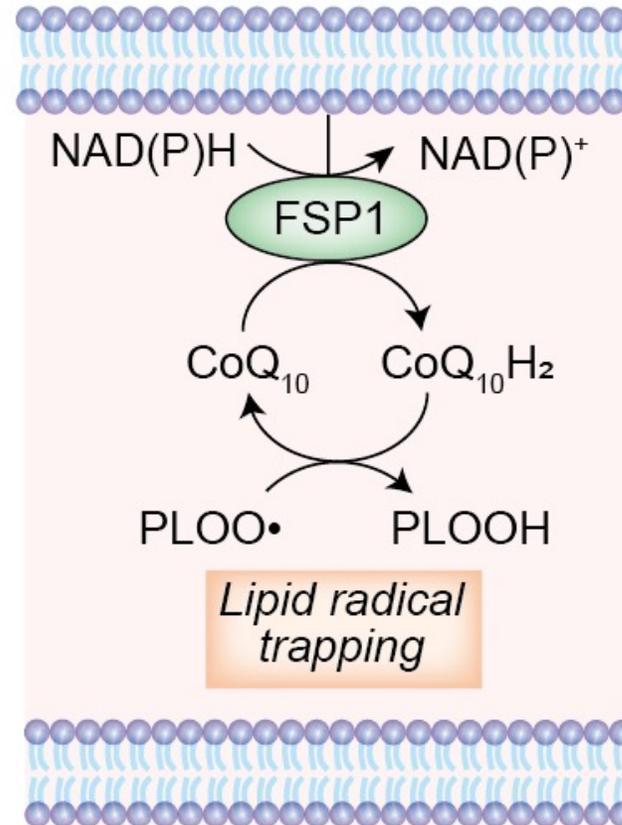
2019



Dr. Marcus
Conrad



Dr. James
Olzmann



-FSP1 (Ferroptosis Suppressor Protein-1)

-Extramitochondrial CoQ10 reductase

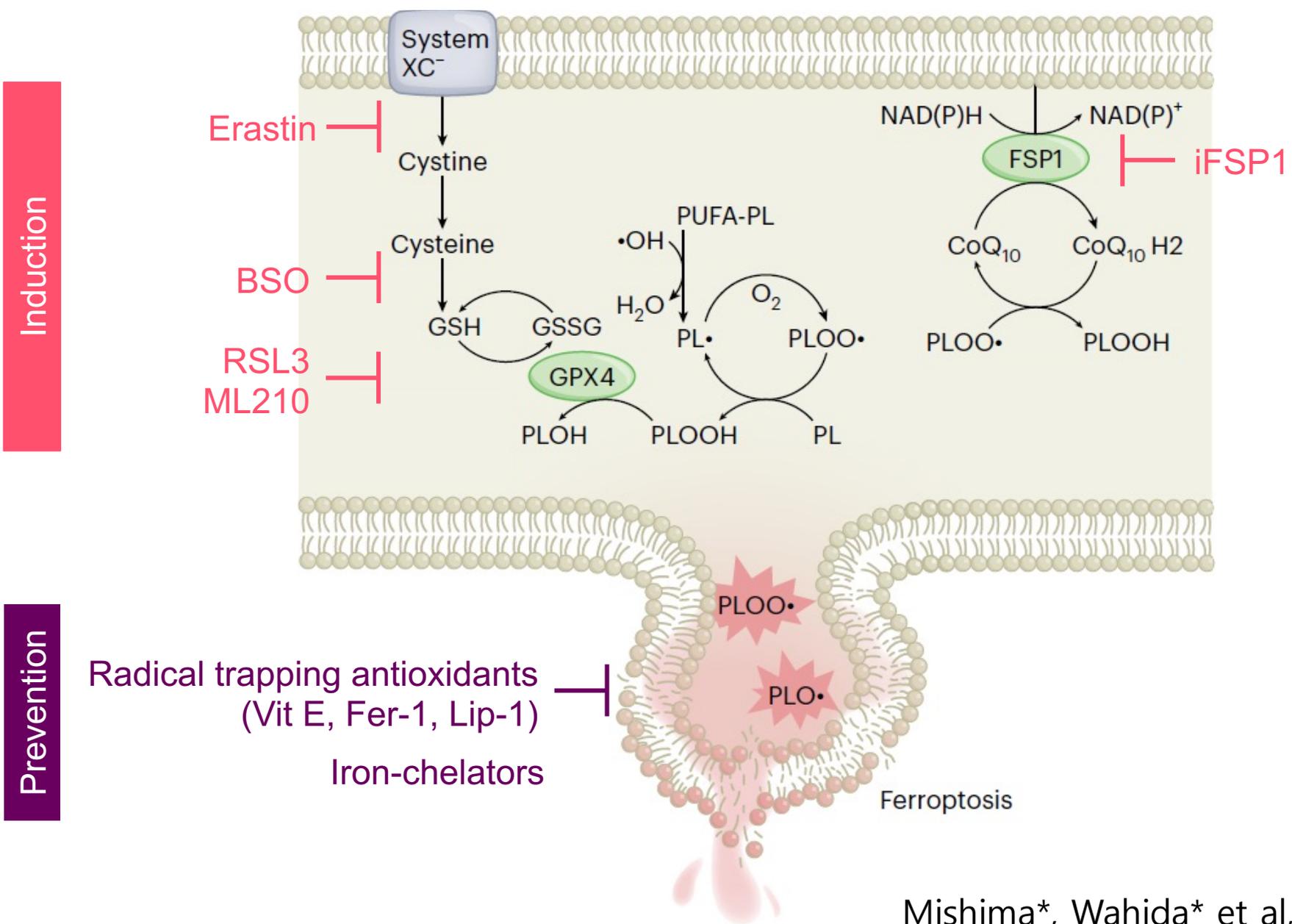
-A reduced form of CoQ10 (CoQH2) scavenges lipid radicals, preventing ferroptosis

Doll, Nature 2019

Bersuker, Nature 2019

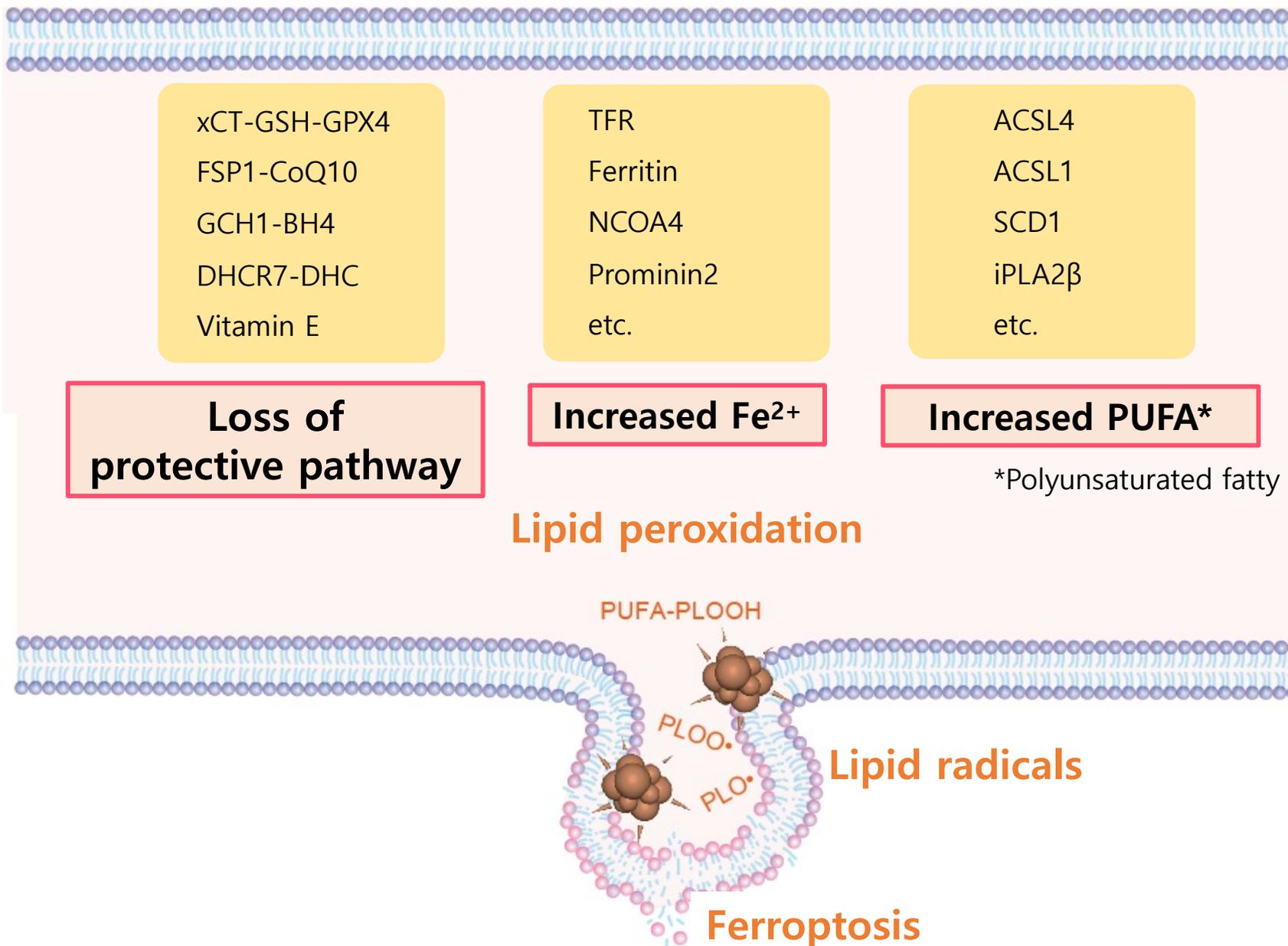


Induction / prevention of ferroptosis





Regulators dictating ferroptosis sensitivity

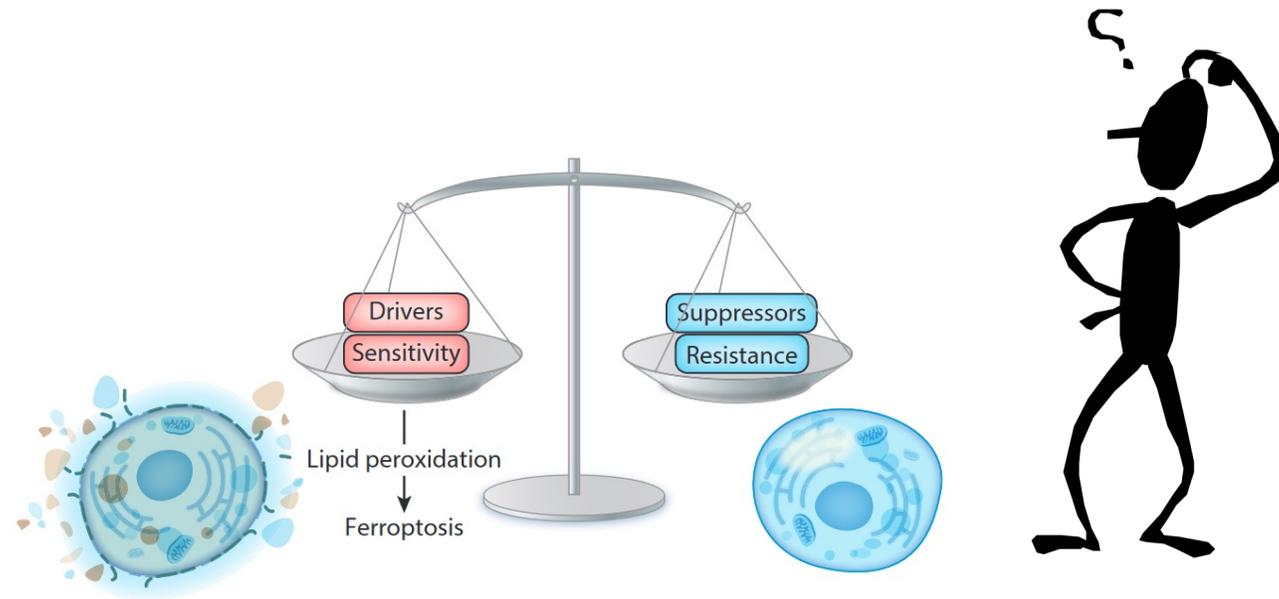


Vitamin K and ferroptosis



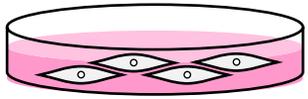
Aim

We first explored yet-unknown metabolites for protecting against ferroptosis.



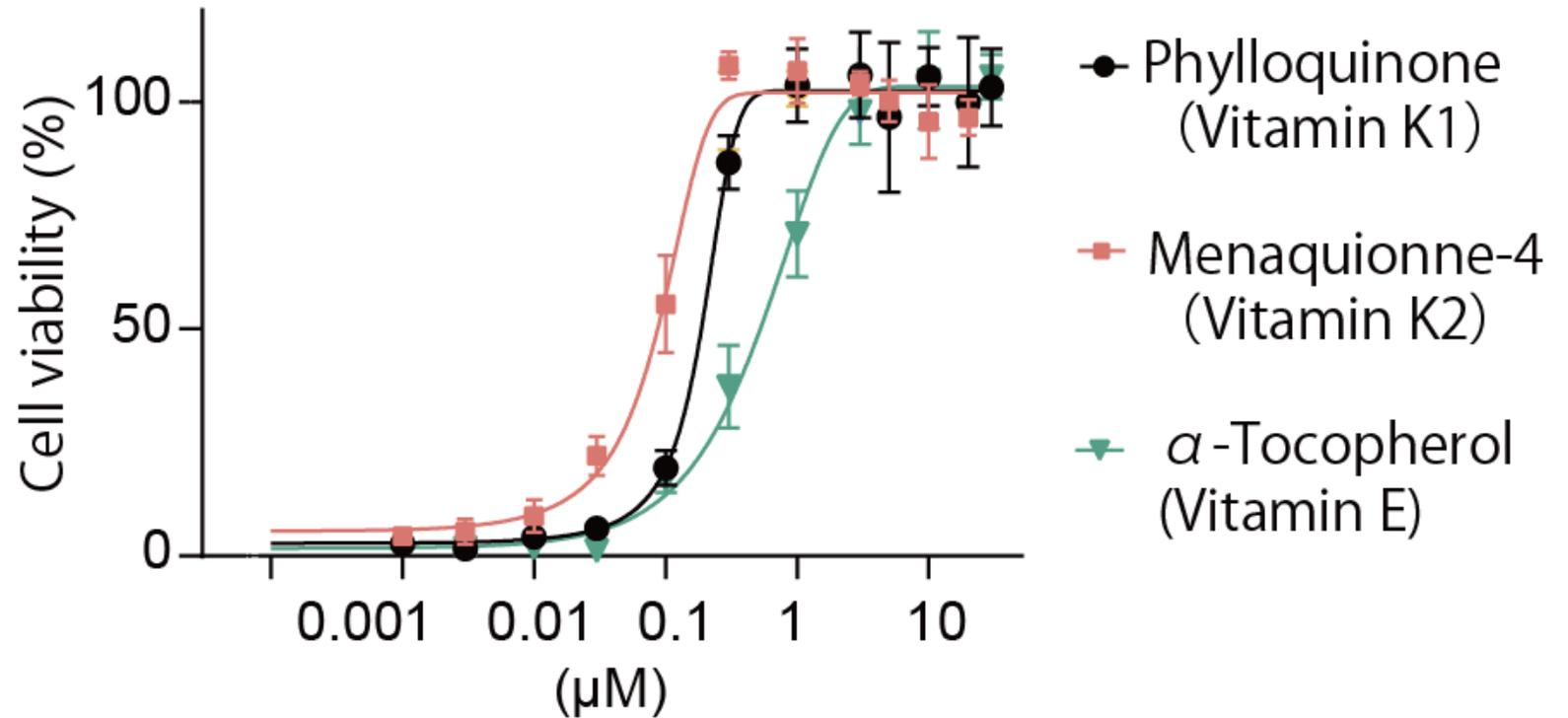
Screening of anti-ferroptotic metabolites

TAM-inducible Gpx4 KO
MEFs



TAM + vitamins
culture for 72 h

Cell viability



Vit K prevents ferroptosis with a lower concentration than Vit E

Mishima... Wahida...et al, Nature 2022

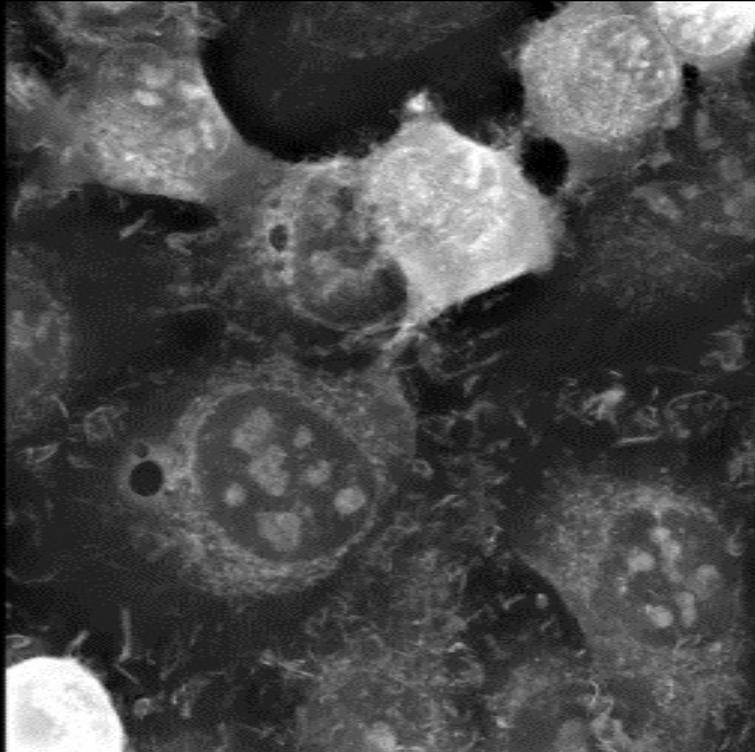
Mishima*, Wahida* et al, Nature Metab 2023



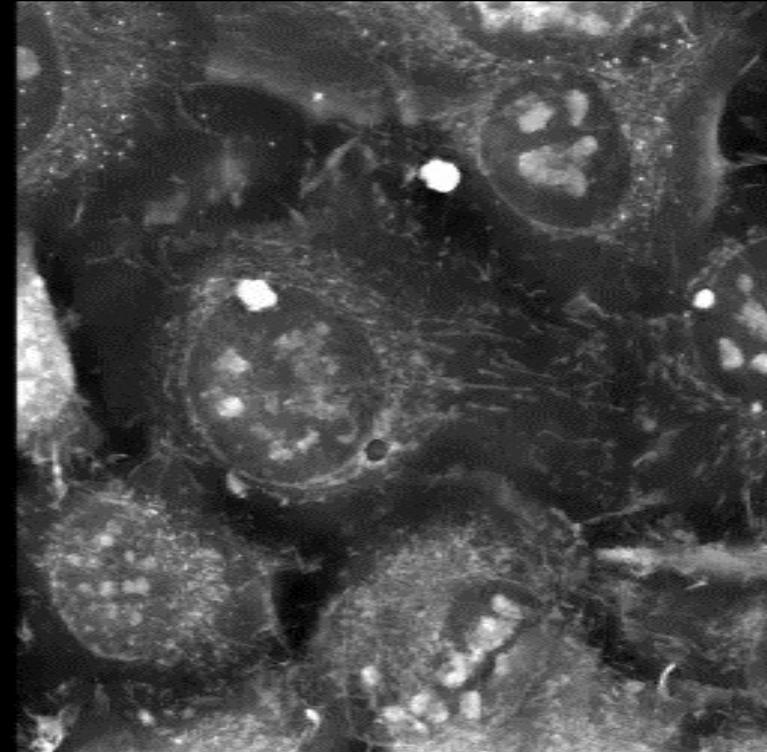
Vitamin K prevents ferroptosis

HT1080 cells

RSL3



RSL3 + MK4



Nanolive movie



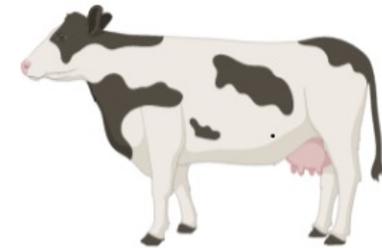
Vitamin K

Function for blood coagulation and bone mineralization

Vitamin K1 Phylloquinone In plant and green vegetables



Vitamin K2 Menaquinone 4
(MK4) In animal products (meat and eggs)
Converted from dietary vitamin Ks



MK7

In Natto (produced by bacteria)

MK8

In Cheese



Mishima... Wahida...et al, Nature 2022

Mishima*, Wahida* et al, Nature Metab 2023



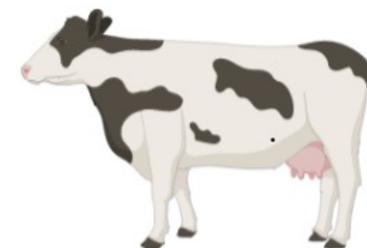
Vitamin K

Function for blood coagulation and bone mineralization

Vitamin K1 Phylloquinone In plant and green vegetables



Vitamin K2 Menaquinone 4
(MK4) In animal products (meat and eggs)
Converted from vitamin K1



Strongest anti-ferroptotic effect

MK7 In Natto (produced by bacteria)

MK8 In Cheese



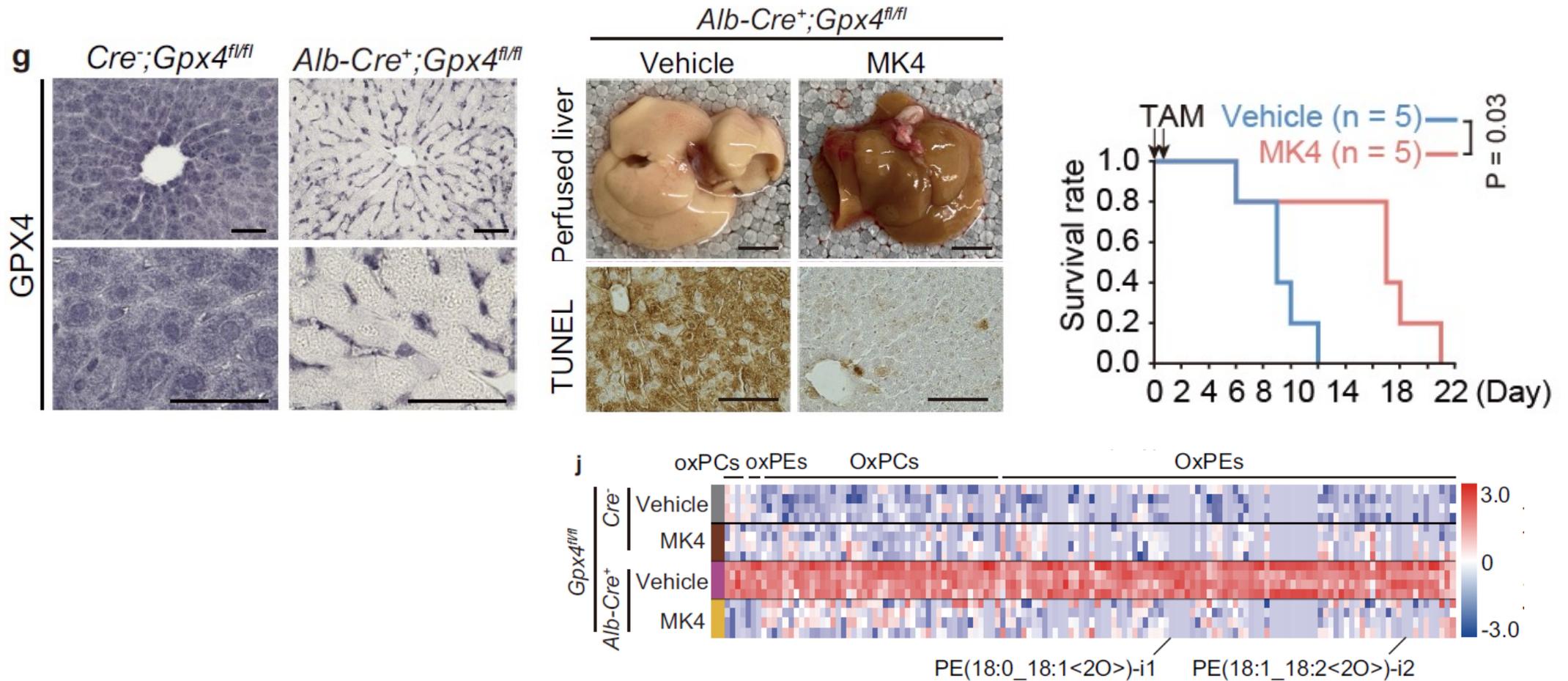
Mishima... Wahida...et al, Nature 2022

Mishima*, Wahida* et al, Nature Metab 2023



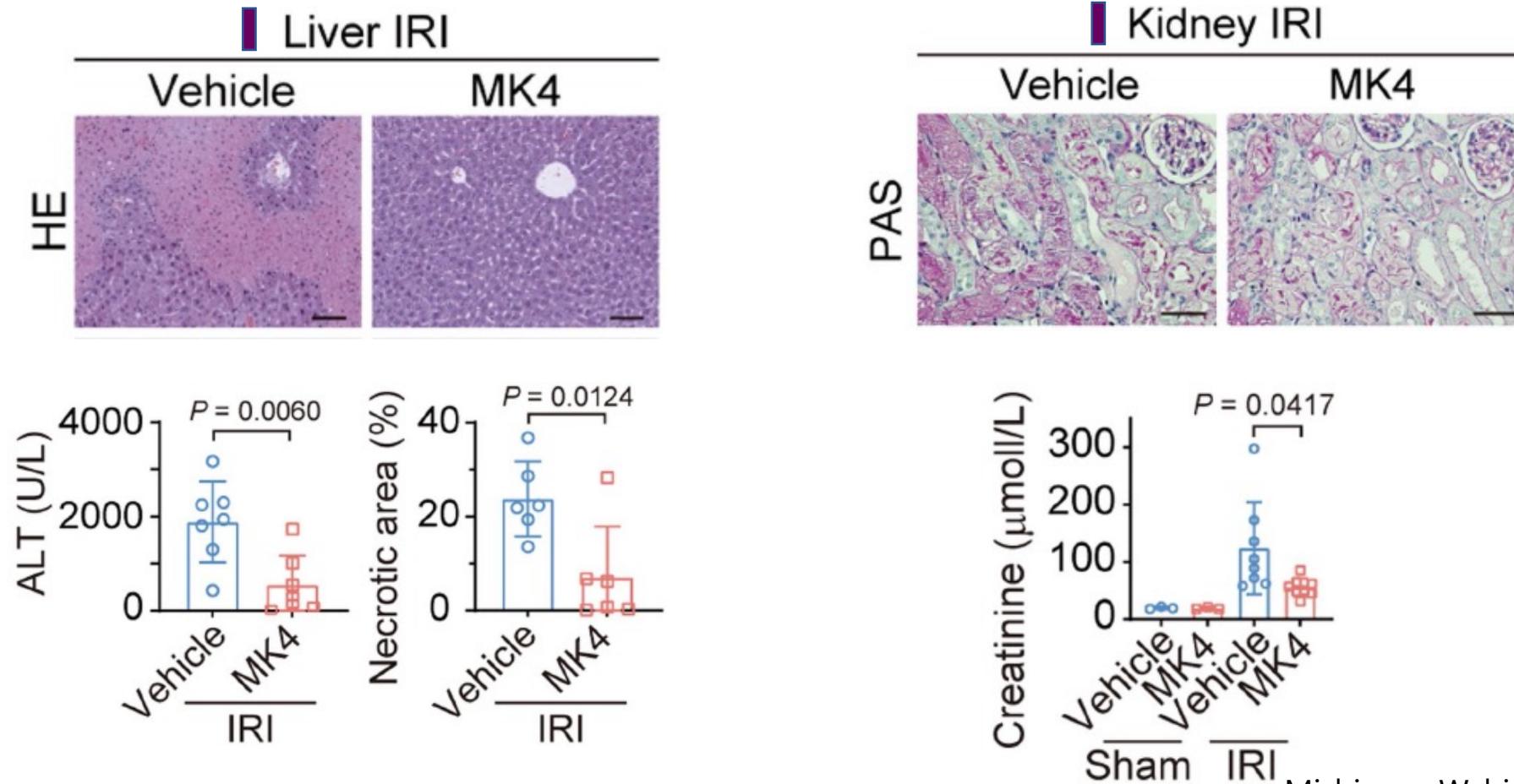
MK4 is tissue-protective against ferroptotic liver damage

Hepatocyte specific Gpx4 inducible KO + MK4 (200 mg/kg/day ip)
(*Alb-cre Gpx4 fl/fl* + low vit E diet)



➔ MK4 is tissue-protective against ischemia-reperfusion injury

C57BL/6 mice + MK4 (200 mg/kg/day ip) pretreatment



Mishima... Wahida...et al, Nature 2022

Mishima*, Wahida* et al, Nature Metab 2023

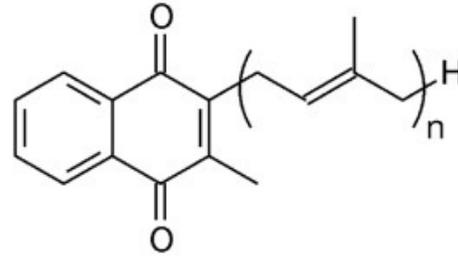


Why VK is cell protective?

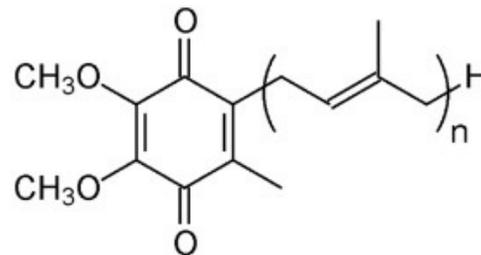


Vitamin K has no radical trapping activity...

Vitamin K



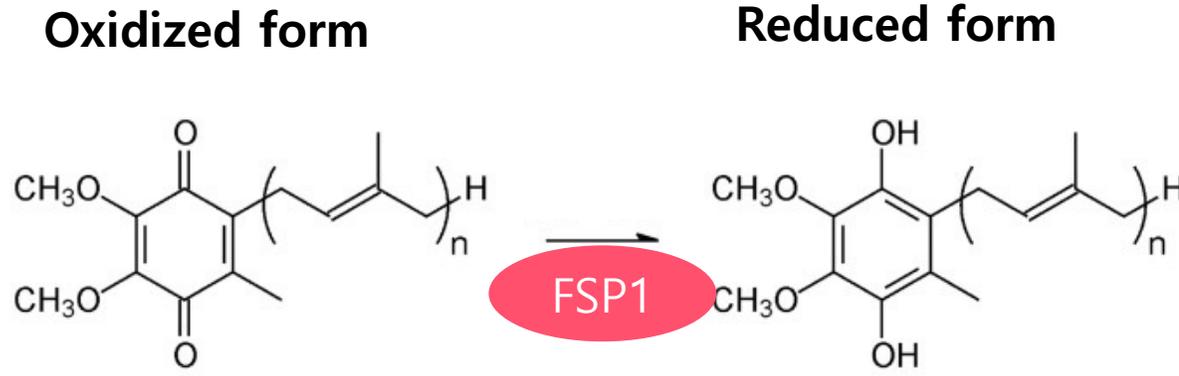
CoQ10



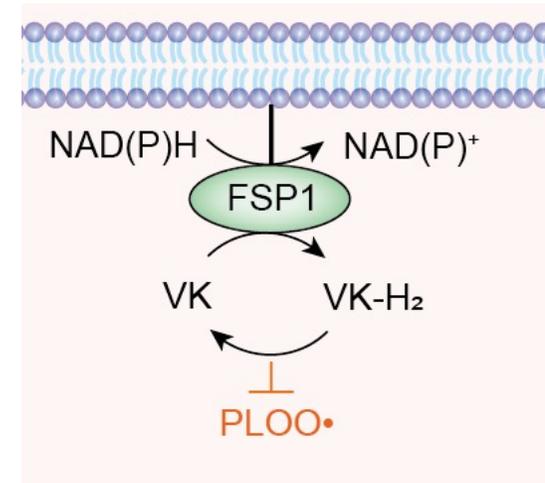
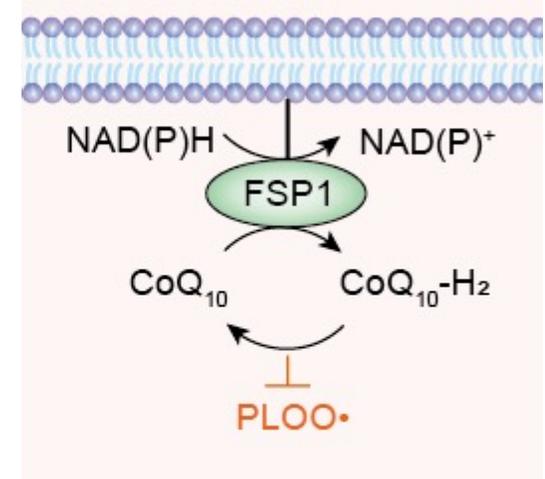
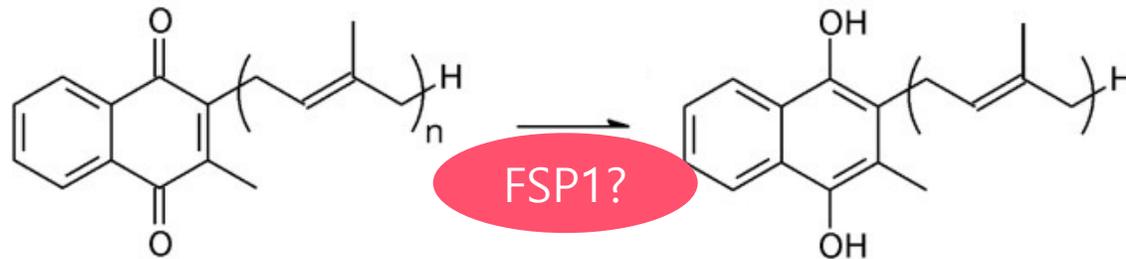


Structural similarity of CoQ10 and VK

CoQ10

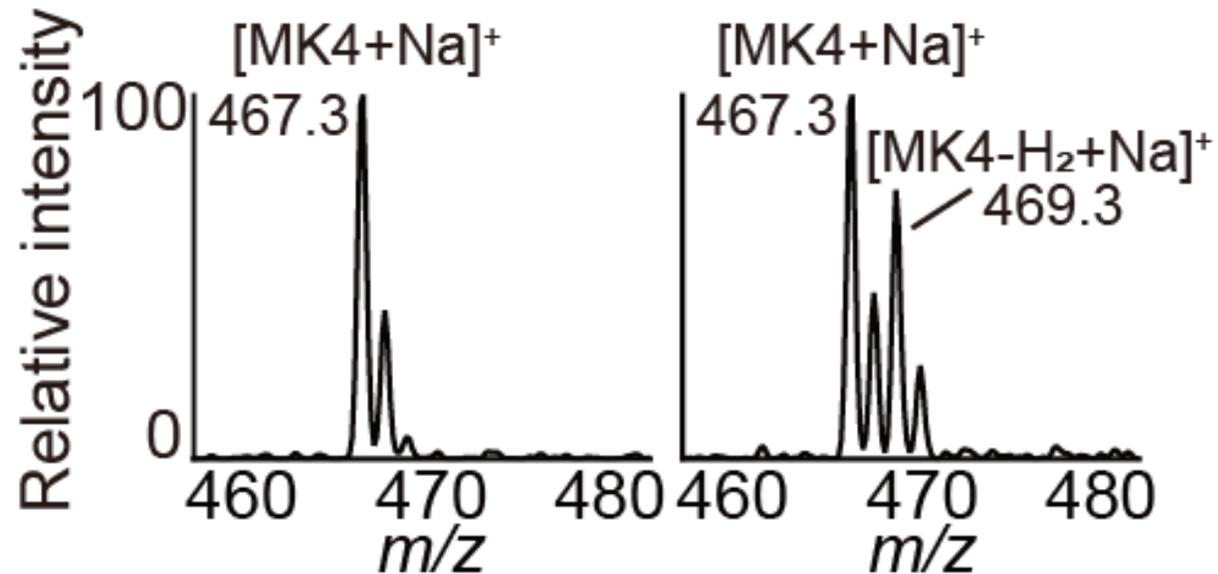


Vitamin K



→ FSP1 is also a vitamin K reductase!

Generation of reduced form of VK !



MK4

MK4 + FSP1 + NADH

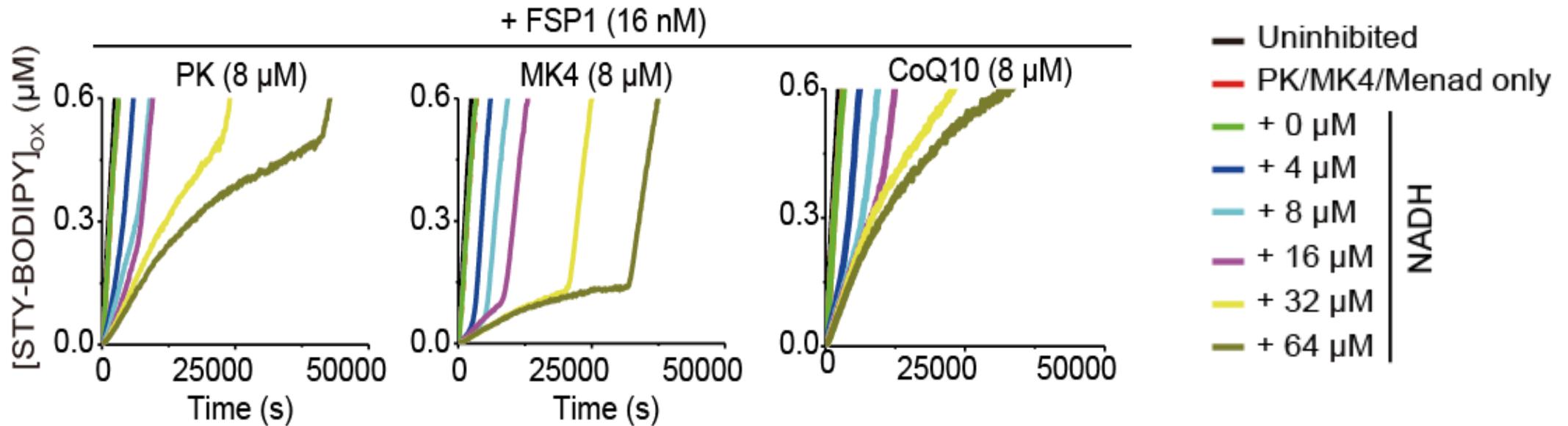
Mass spectra of MK4 ± recombinant FSP1 + NADH

Mishima... Wahida...et al, Nature 2022

Mishima*, Wahida* et al, Nature Metab 2023

→ FSP1-mediated VK-H₂ is potent lipid radical trapping antioxidant

Radical trapping potency: MK4-H₂ > phylloquinone-H₂ ≅ CoQ10-H₂

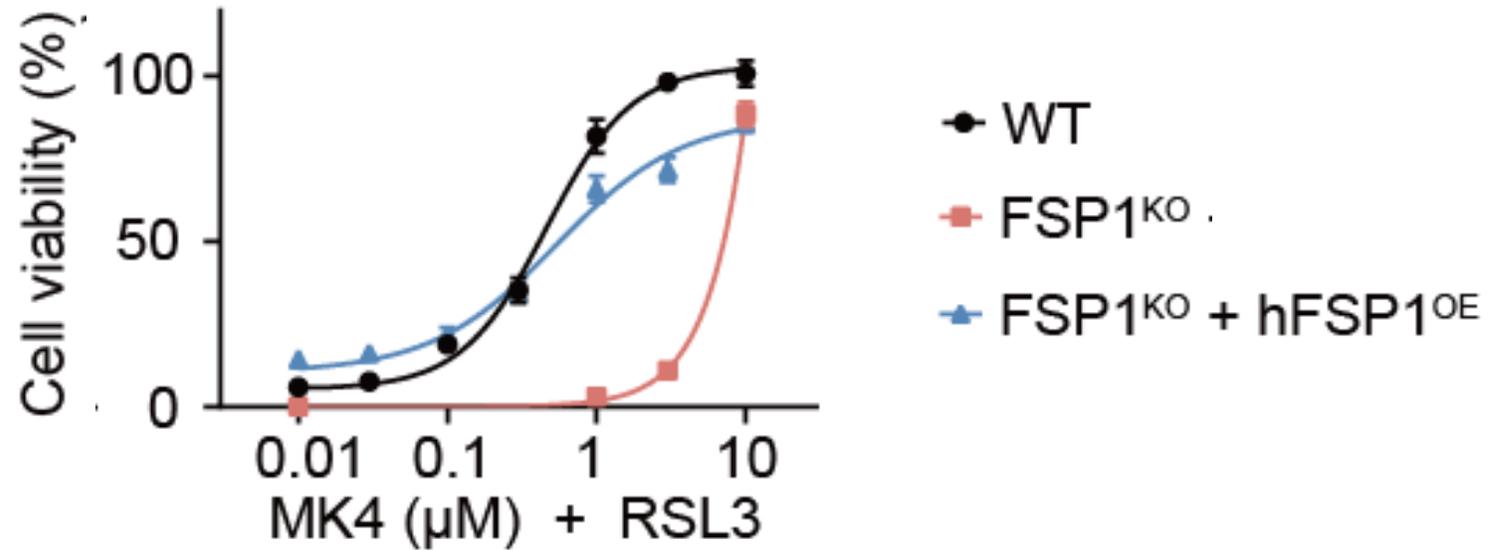
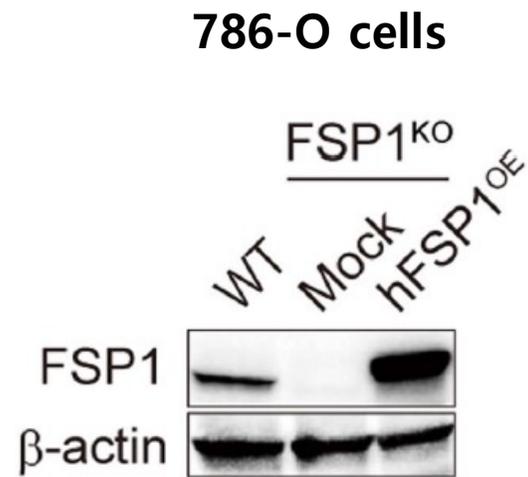


FENIX assay

Mishima... Wahida...et al, Nature 2022

Mishima*, Wahida* et al, Nature Metab 2023

→ FSP1-mediated anti-ferroptotic effect of vitamin K



786-O cells: human kidney cancer cell line

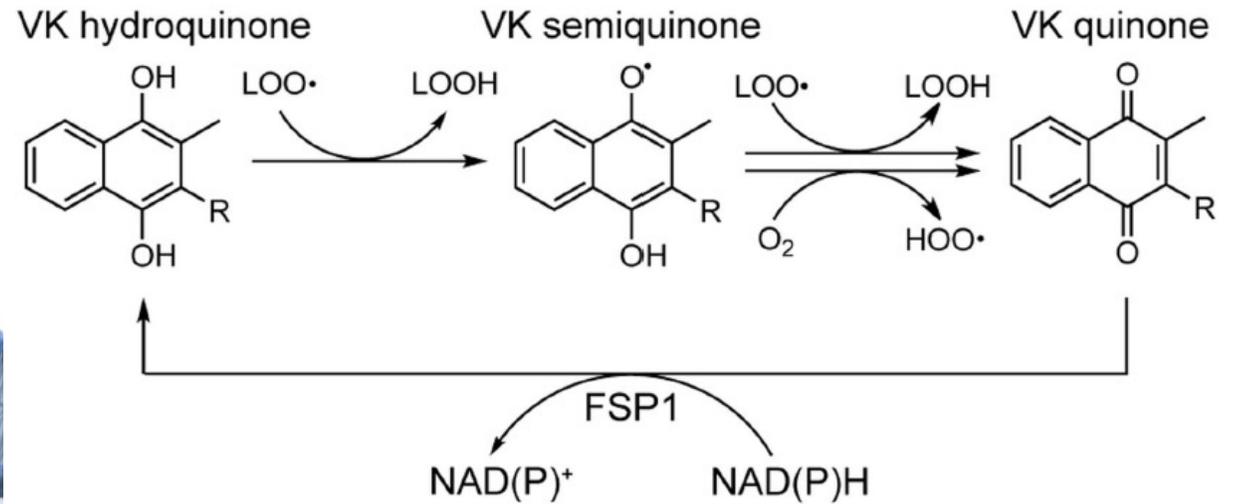
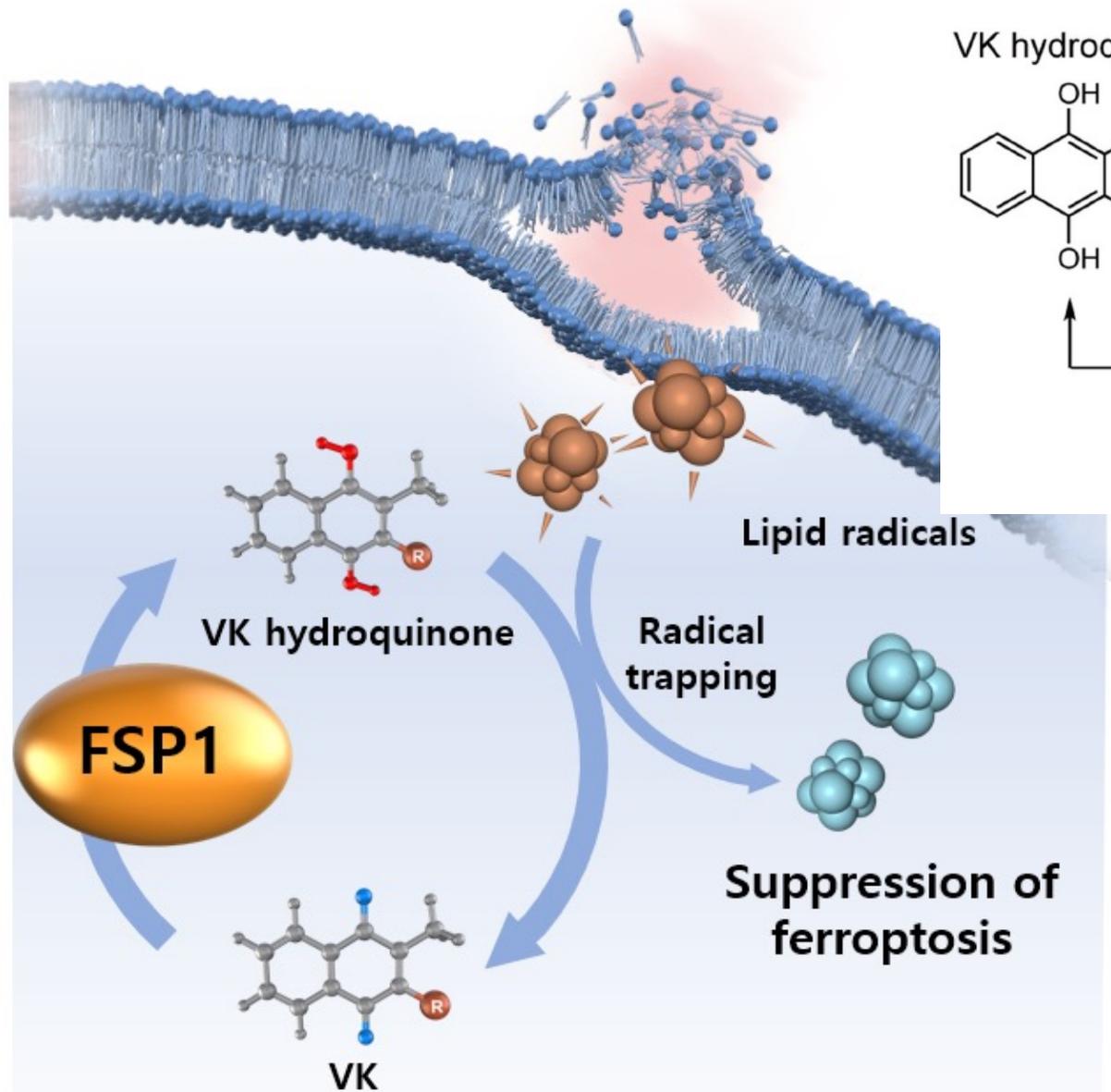
*RSL3: GPX4 inhibitor (ferroptosis inducer)

Mishima... Wahida...et al, Nature 2022

Mishima*, Wahida* et al, Nature Metab 2023



Anti-ferroptotic function of VK via FSP1

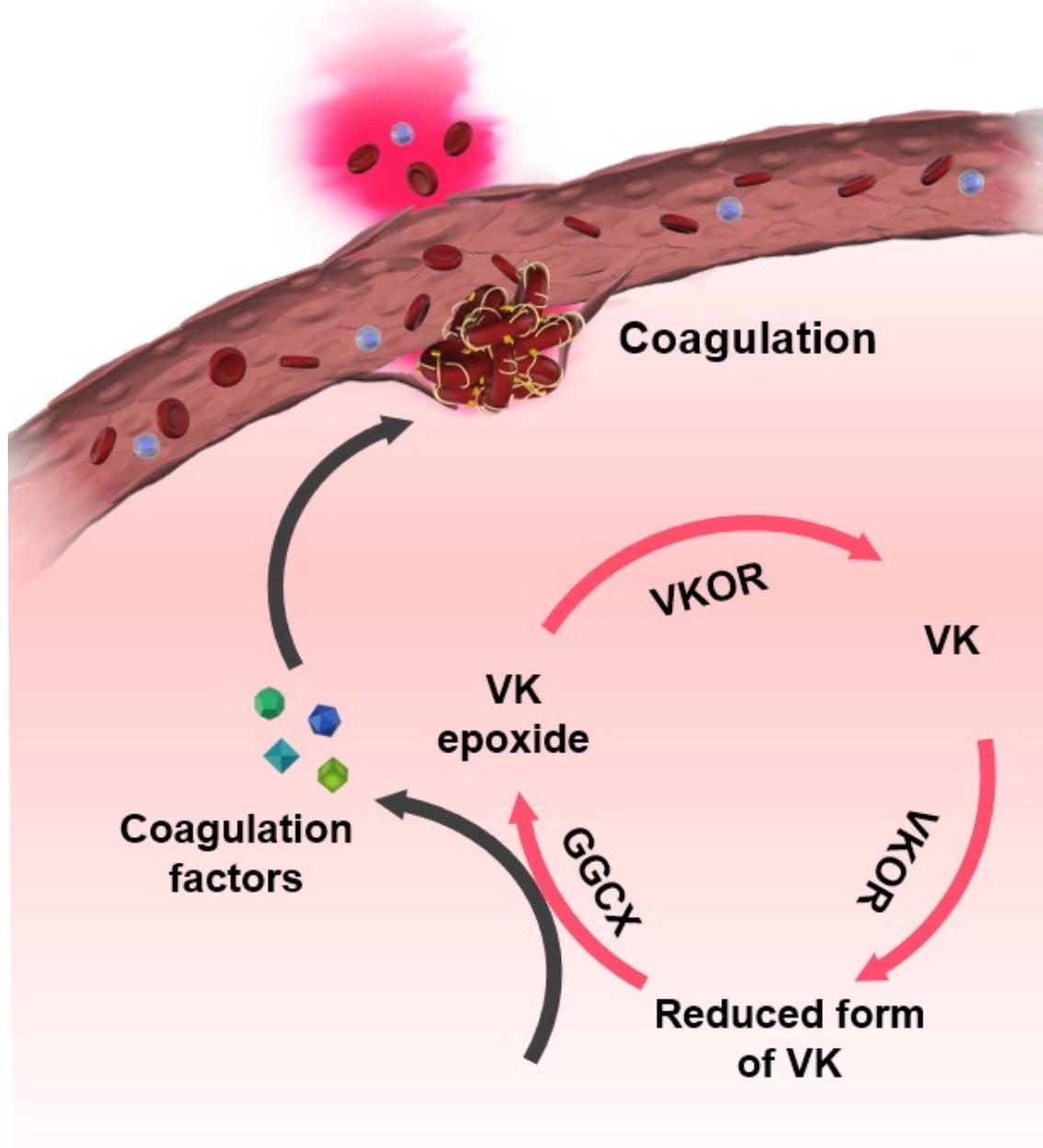


Mishima... Wahida...et al, Nature 2022
Mishima*, Wahida* et al, Nature Metab 2023

FSP1-VK in coagulation

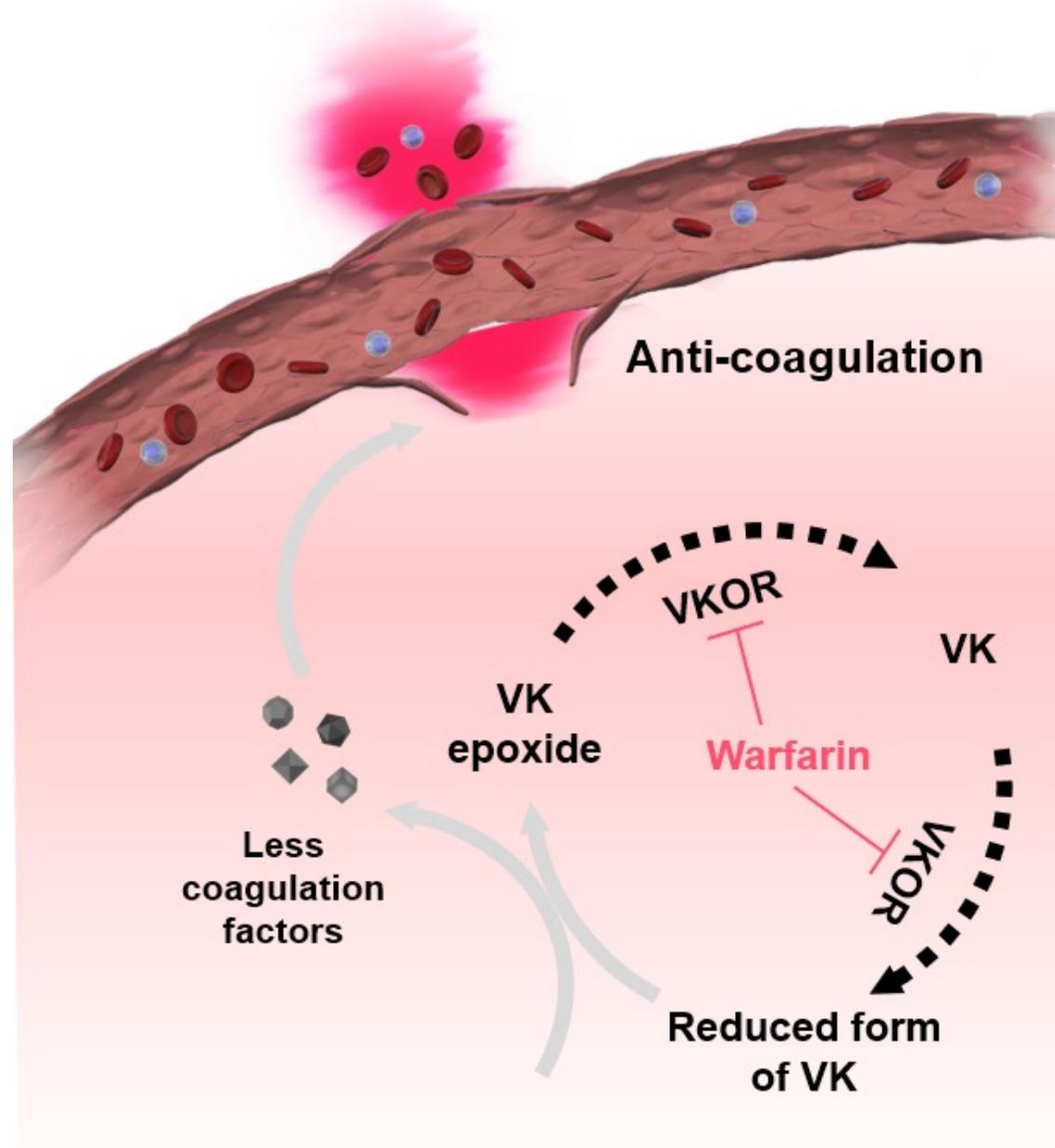


Vitamin K for blood coagulation





Effect of anticoagulant Warfarin



*VKOR: Vitamin K epoxide reductase



Warfarin overdose

Drug-drug interaction

Large variations in the dosage among individuals

Lethal bleeding



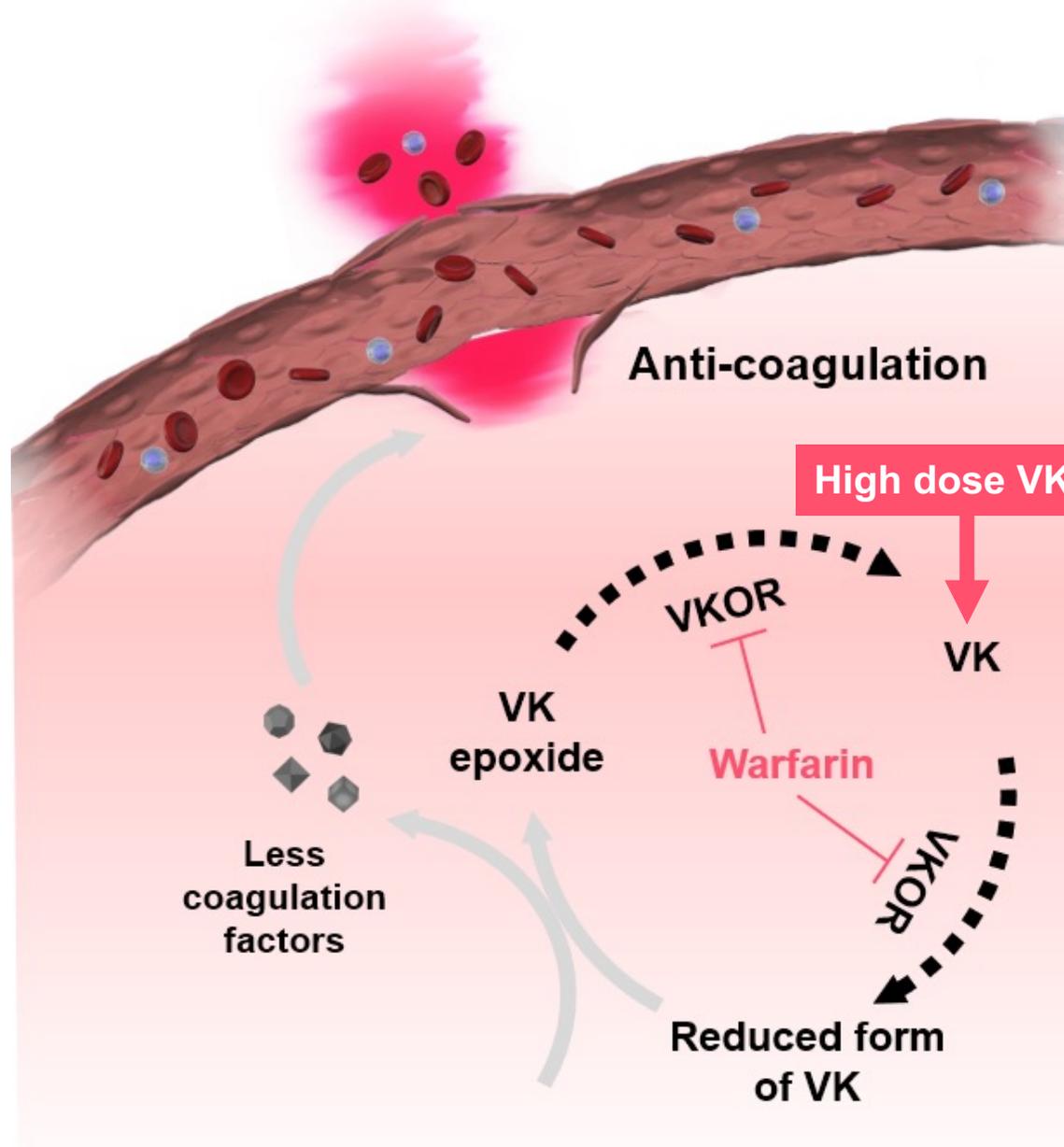
Treatment: reversal by high-dose vitamin K

Mishima... Wahida...et al, Nature 2022

Mishima*, Wahida* et al, Nature Metab 2023

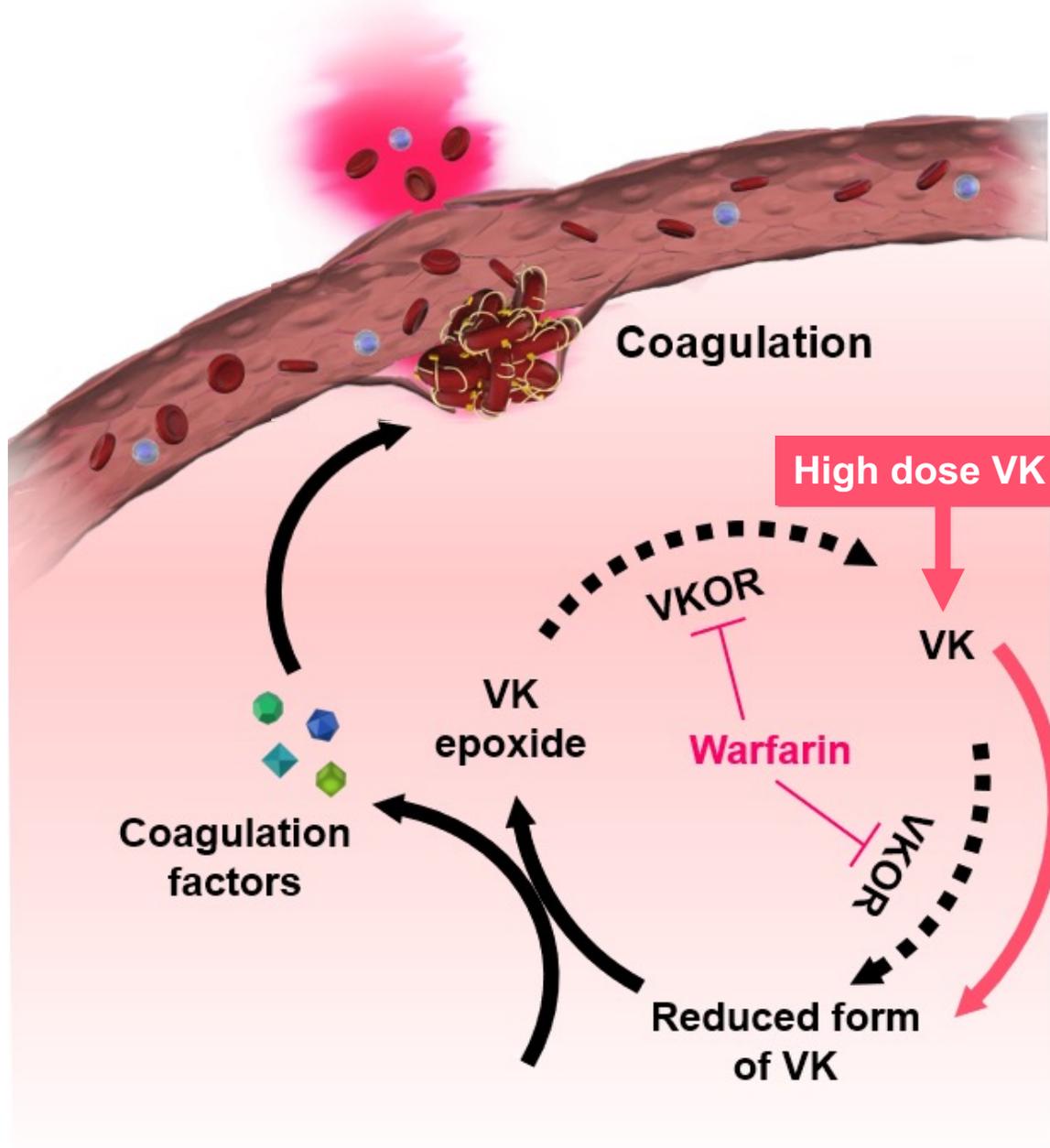


Antidotal effect of VK for warfarin poisoning





Antidotal effect of VK for warfarin poisoning



Unidentified warfarin-resistant VK reductase

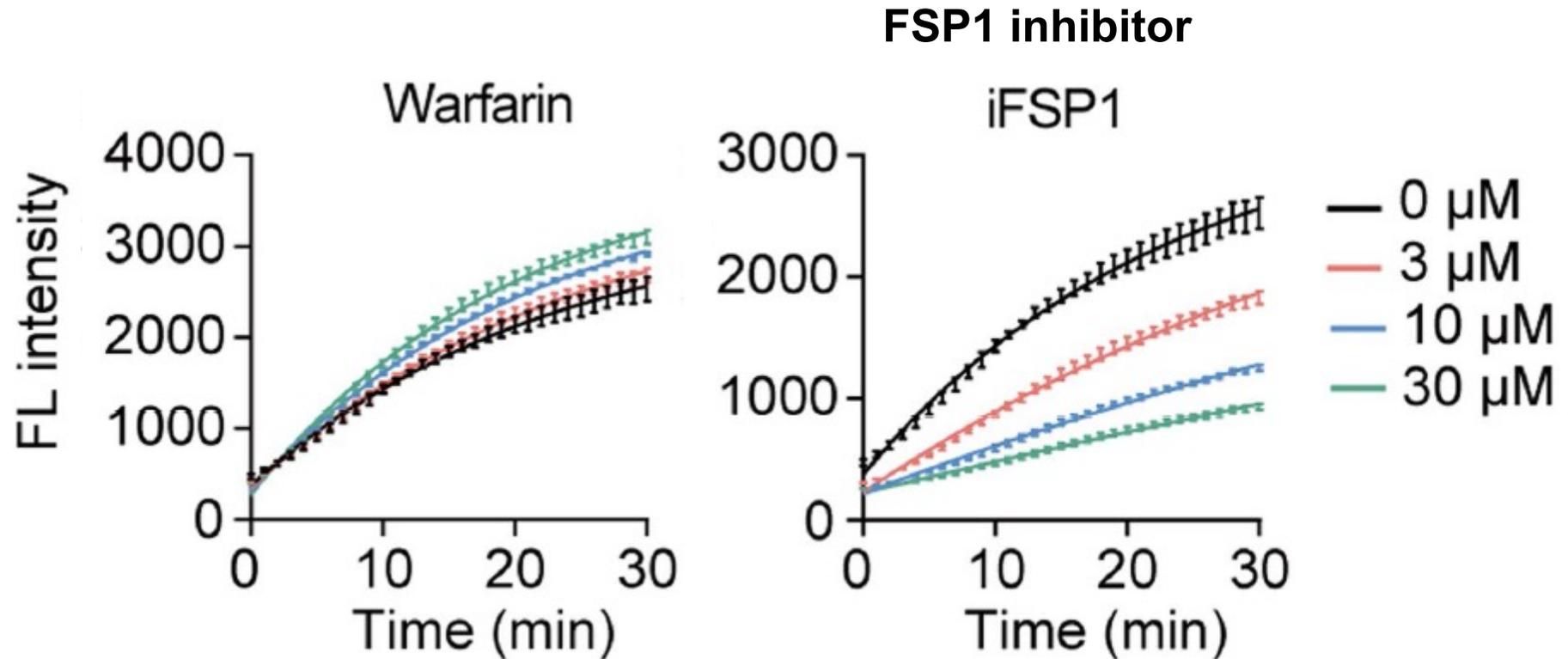
Unidentified over 70 yrs

Is FSP1 the antidotal enzyme for warfarin poisoning?



FSP1 is not inhibited by WF

FSP1 enzymatic assay



hFSP1 + NADH + resazurin + inhibitors



WF poisoning in FSP1 KO mice is not rescued by VK

Warfarin overdose
(Wild type mouse)

+ MK4



Cerebral bleeding

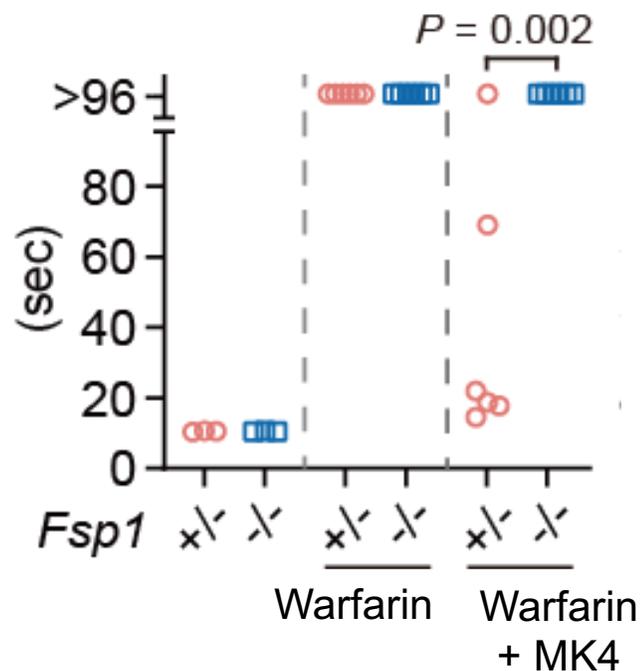
Fsp1^{+/-} mice
Fsp1^{-/-} mice



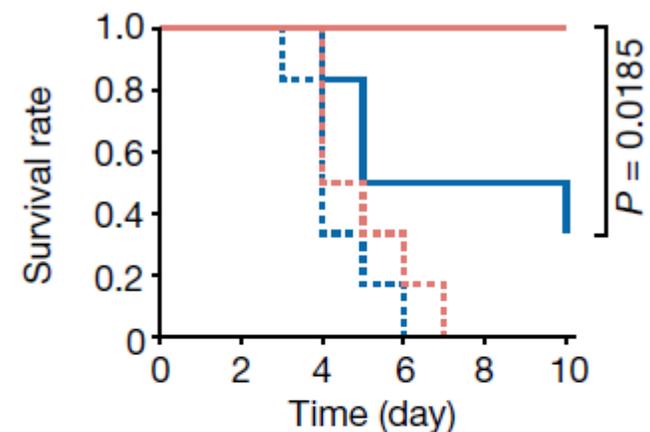
Warfarin
overdose
± MK4



Prothrombin time



Survival rate



- *Fsp1*^{+/-} + warfarin + MK4 (n = 6)
- *Fsp1*^{-/-} + warfarin + MK4 (n = 6)
- ⋯ *Fsp1*^{+/-} + warfarin (n = 6)
- ⋯ *Fsp1*^{-/-} + warfarin (n = 6)

Mishima... Wahida...et al, Nature 2022

Mishima*, Wahida* et al, Nature Metab 2023



WF poisoning in FSP1 KO mice is not rescued by VK

Warfarin overdose
(Wild type mouse)

+ MK4



Cerebral bleeding

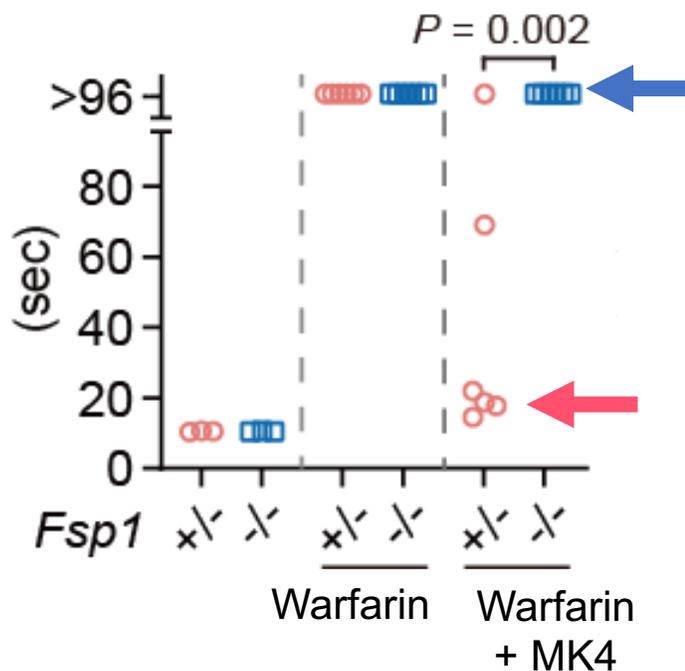
Fsp1^{+/-} mice
Fsp1^{-/-} mice



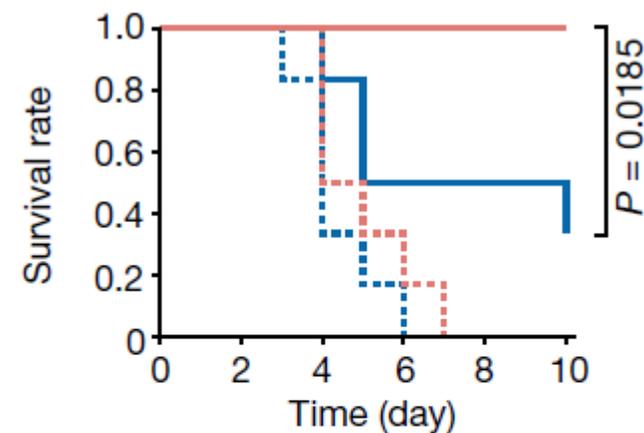
Warfarin
overdose
± MK4



Prothrombin time



Survival rate

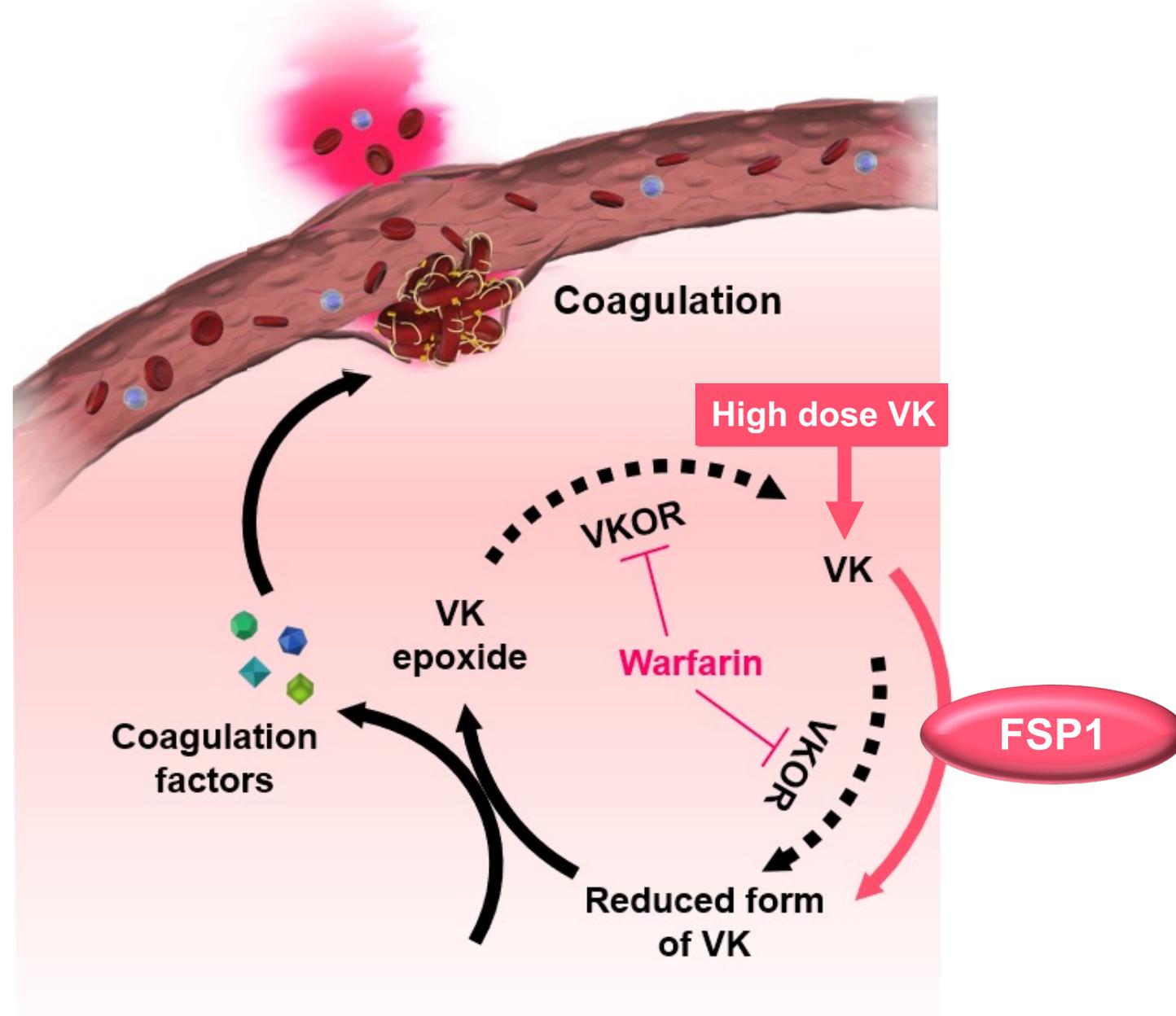


- *Fsp1*^{+/-} + warfarin + MK4 (n = 6)
- *Fsp1*^{-/-} + warfarin + MK4 (n = 6)
- *Fsp1*^{+/-} + warfarin (n = 6)
- *Fsp1*^{-/-} + warfarin (n = 6)

Mishima... Wahida...et al, Nature 2022

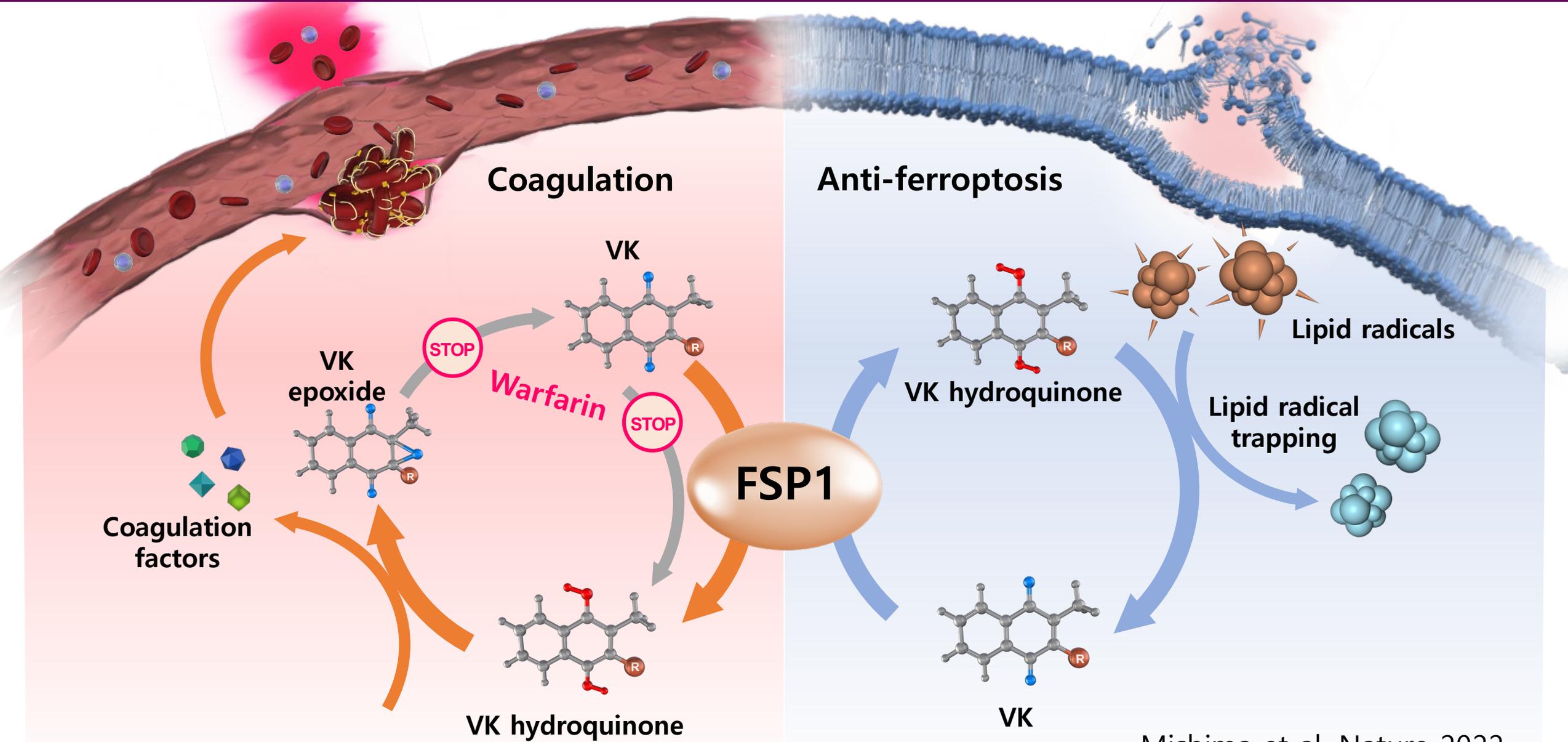
Mishima*, Wahida* et al, Nature Metab 2023

→ FSP1 is the antidotal effect of VK for warfarin poisoning



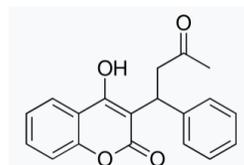
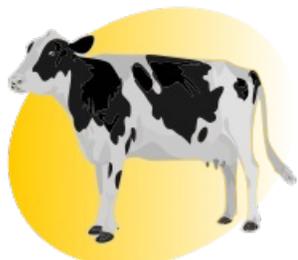


Summary: FSP1-mediated 2 functions of VK





Human history and warfarin



Dicoumarol was isolated from sweet clover as an anticoagulant

1939

Approved as rat poisoning 1948

Approved by FDA 1954

President Eisenhower received warfarin following a heart attack 1955

1943 Synthesis of warfarin

1951 A suicide-attempted patient with warfarin poisoning was recovered by VK treatment.

Then, known as a specific antidote

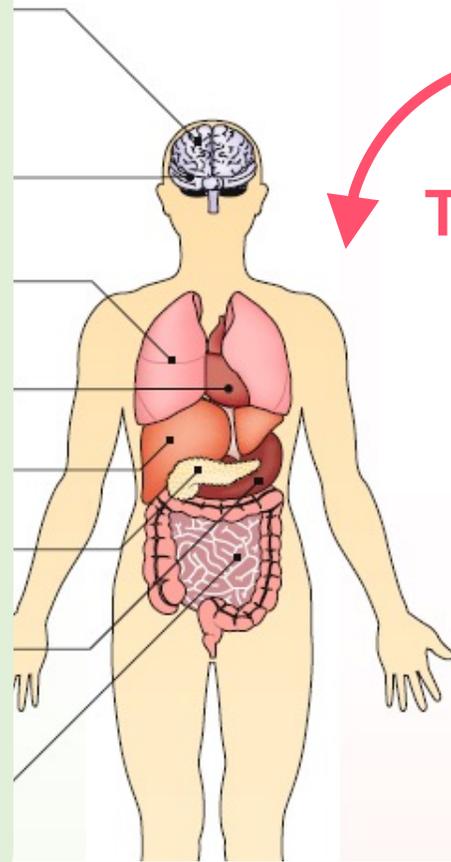
Used worldwide

2022 Identification of antidotal VK reductase, FSP1



VK for ferroptosis-related diseases?

- Alzheimer dis
 - Parkinson dis
 - ALS
- } Neurodegeneration
- Stroke
 - COPD
 - Myocardial infarction
 - Liver injury, NASH
 - Pancreatic injury
 - Kidney injury
 - Intestine injury



High dose VK
Therapeutic effect ?



***No reports of clinical toxicity of high doses of VK**



vitamin K vs CoQ

VK

0.05 – 0.5 nmol/g tissue

<<

CoQ

~ 100 nmol/g tissue

- Pharmacological dose of VK is anti-ferroptotic
- Effect by endogenous VK would be minor in our bodies

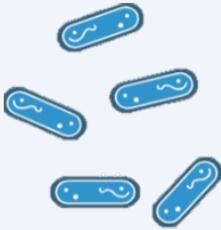
➔ Is anti-ferroptosis the evolutionally original function of VK?

Ancient

Phylloquinone



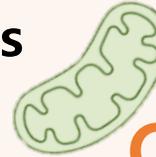
Menaquinone



Function of VK

Antioxidant / anti-ferroptosis ?
(Heat, UV and Fe^{2+})

Electron carriers
(mito.)



CoQ₁₀

Anti-ferroptosis
(extra-mito.)

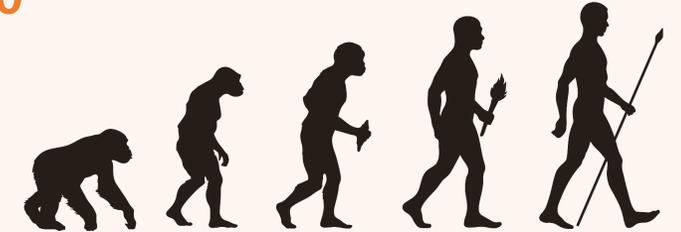
VK

-74 mV Redox potential (Eo') +100 mV

Anaerobic
environment

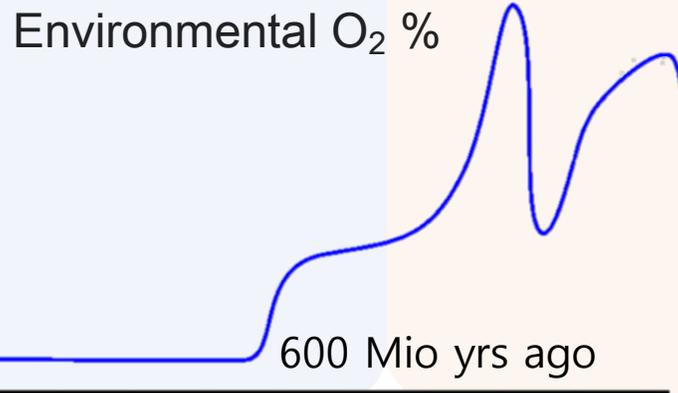
Aerobic
environment

Present



Function of VK

Blood clotting



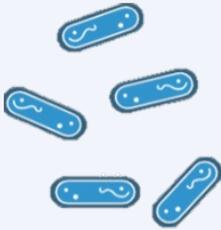
➔ Is anti-ferroptosis the evolutionally original function of VK?

Ancient

Phylloquinone



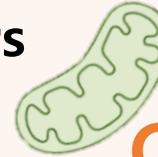
Menaquinone



VK

Electron carriers

(mito.)



Anti-ferroptosis
(extra-mito.)

CoQ₁₀

Present

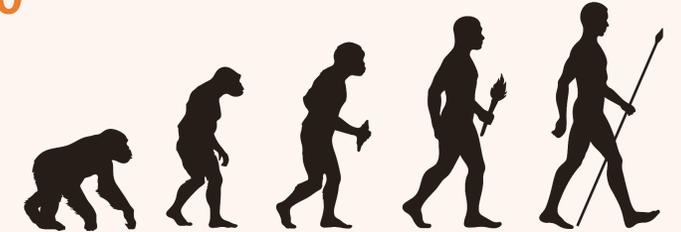
-74 mV

Redox potential (Eo')

+100 mV

Anaerobic
environment

Aerobic
environment



Function of VK

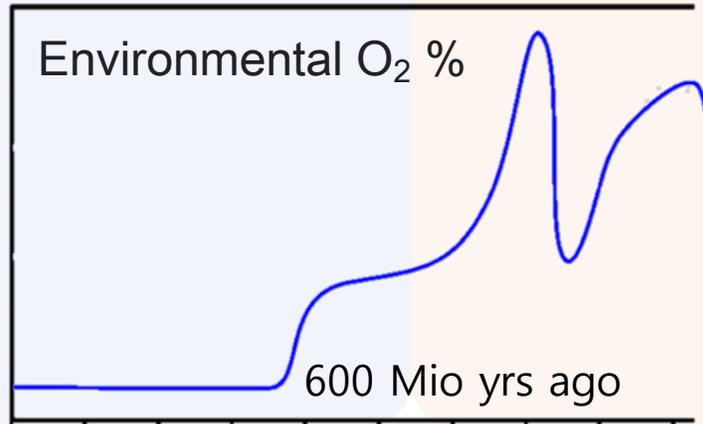
Antioxidant / anti-ferroptosis ?
(Heat, UV and Fe²⁺)

Function of VK

Blood clotting

Environmental O₂ %

600 Mio yrs ago

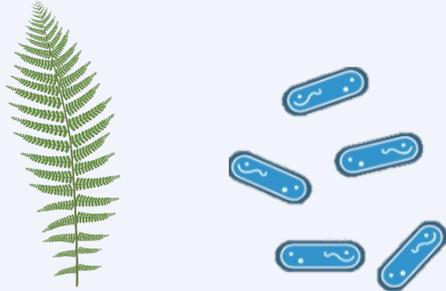


➔ Is anti-ferroptosis the evolutionally original function of VK?

Ferroptosis



Ancient



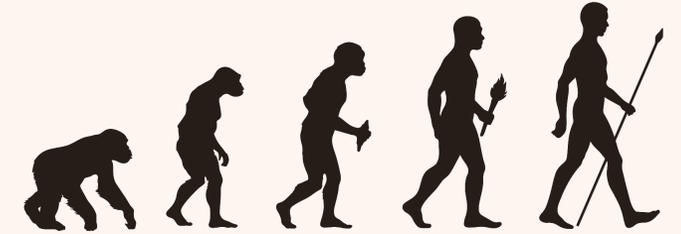
VK

Electron carriers



CoQ₁₀

Present



-74 mV Redox potential (Eo') +100 mV

Anaerobic environment

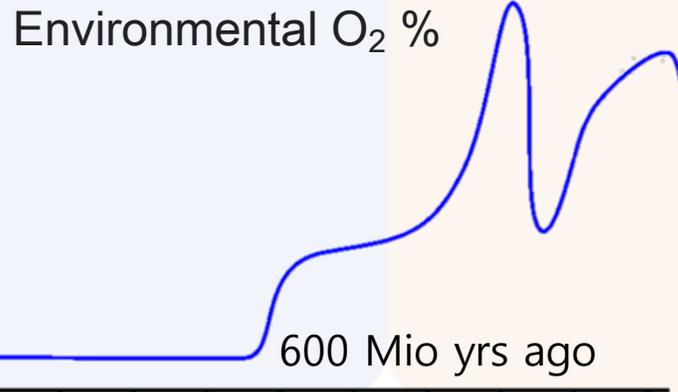
Aerobic environment

Function of VK

Antioxidant / anti-ferroptosis ?
(Heat, UV and Fe²⁺)

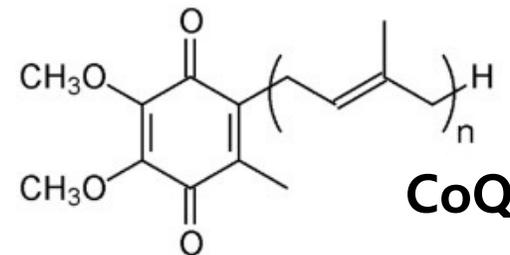
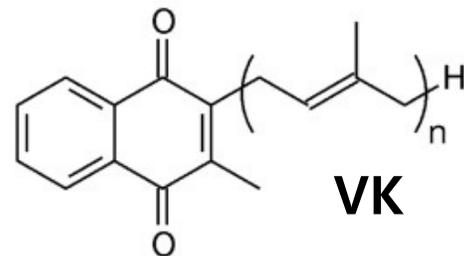
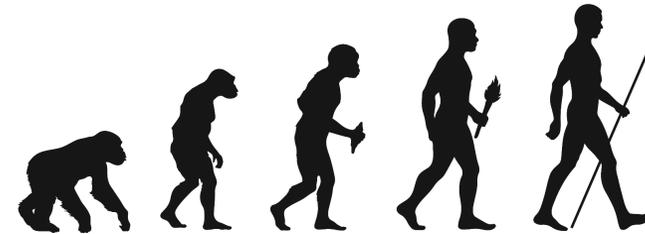
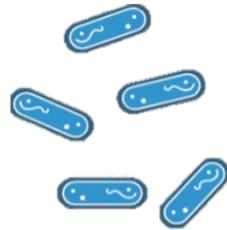
Function of VK

Blood clotting





Exploring the physiological significance of the anti-ferroptotic function of VK in mammals, plants and bacteria.



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