3rd International Conference on

Chronic Obstructive Pulmonary Disease

July 11-12, 2016 Brisbane, Australia

Pharmacological and genetic approaches determine protease and oxidative stress as exacerbating factors in a mouse model of obstructive lung diseases

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Kumamoto University

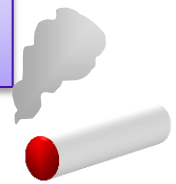
復興の意気や溢るる
Full of Kumamoto University Spirit
熊本大学
（五高寮歌より）

Department of Molecular Medicine, Graduate School of Pharmaceutical Sciences, Kumamoto University

Kumamoto Castle after the Kumamoto earthquake (M6.5+M7.3 Apr.16. 2016)

Obstructive lung diseases – ex. COPD, cystic fibrosis (CF)

Respiratory disease associated with major symptoms of airway narrowing and lung overexpansion



Heavy air pollution, Smoking

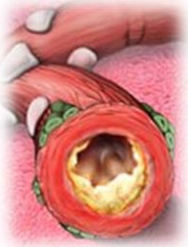
Individual factors

Inflammation

Oxidative Stress

Proteases

Pathology

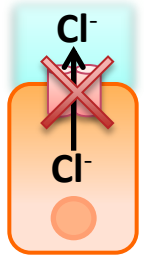


Emphysema

Mucus obstruction

Symptomatic treatments

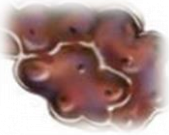
- **Chronic obstructive pulmonary diseases (COPD)**
  - Fourth leading cause of death worldwide
  - Patients are increasing

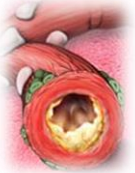


- **Cystic fibrosis (CF)**
  - Autosomal recessive genetic disorder
  - Pulmonary disease, being the primary cause of death

Researches on obstructive lung diseases in our lab

Obstructive pulmonary disease model in mice





Mice models	Inflammation /Emphysema	Mucus
Papain intratracheal treatment	○	×
Elastase intratracheal treatment	○	×
LPS intratracheal treatment	○	×
Bleomycin intratracheal treatment	○	×
Genetic modified mice		
IFN-γ Tg	○	×
IL-13 Tg	○	×
SAM Tg	○	×
CFTR KO, CFTR mutant knock in	○	×
Tabaco smoke exposure	○	×

（Takayama et al., 2006 ）

No models were available that show mucus obstruction

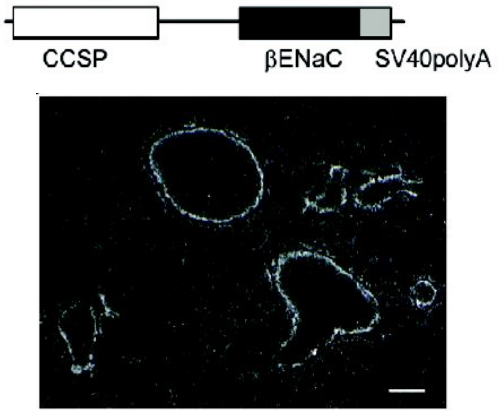
Airway specific βENaC (Scnn1b) -transgenic mice

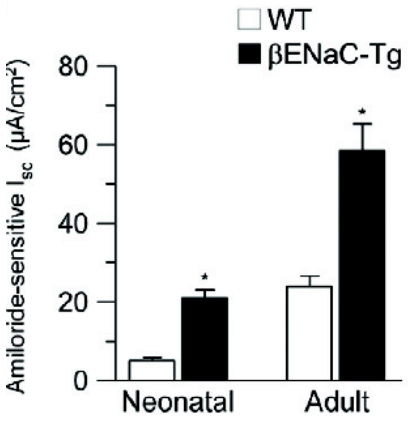
Finally, mice with CF lung disease

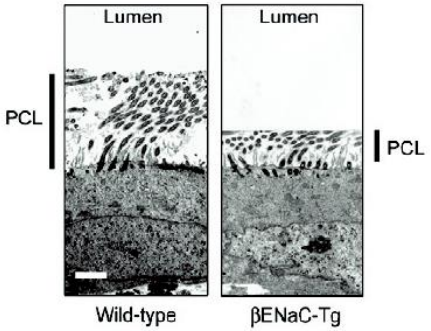
Raymond A Frizzell & Joseph M Pilewski

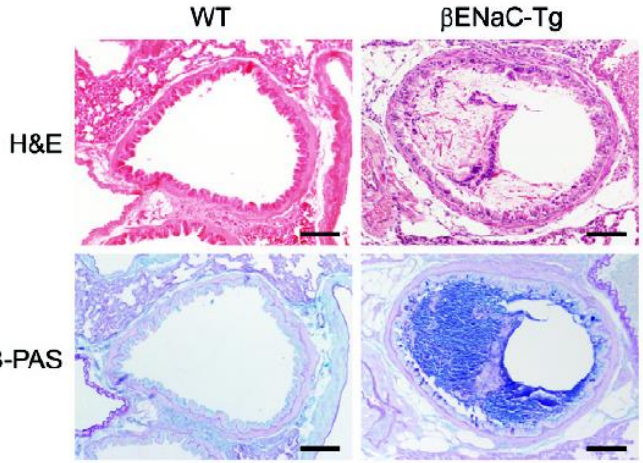
Increasing sodium absorption by overexpressing the epithelial sodium channel in mouse airways results in mucus accumulation and inflammation, changes that occur in the lungs of individuals with cystic fibrosis. The development of lung disease in these mice should provide insights into a disease that has long been lacking an animal model (pages 487–493).

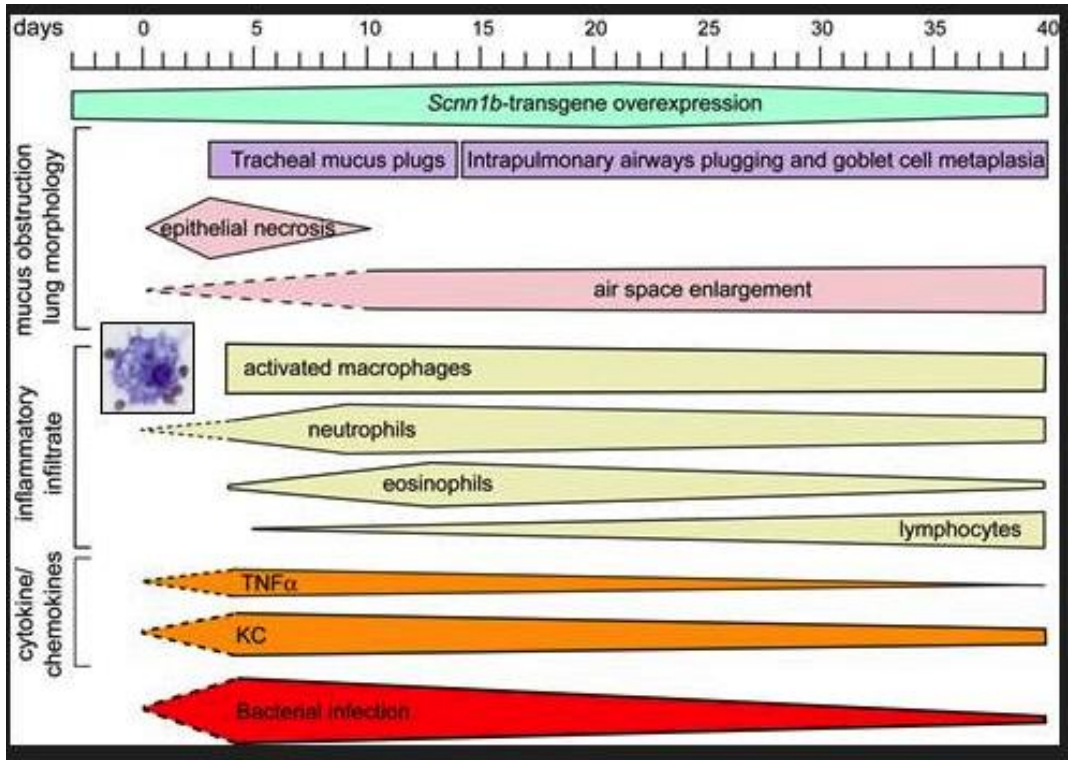
VOLUME 10 | NUMBER 5 | MAY 2004 NATURE MEDICINE





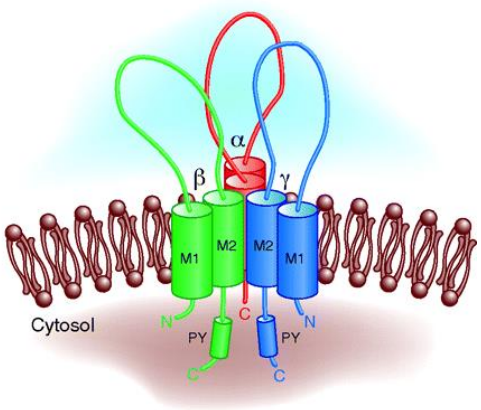






Mall *et al., Nat Med.* 2004

Epithelial sodium channel (ENaC)



- Transporter of Na^+ across tight epithelia

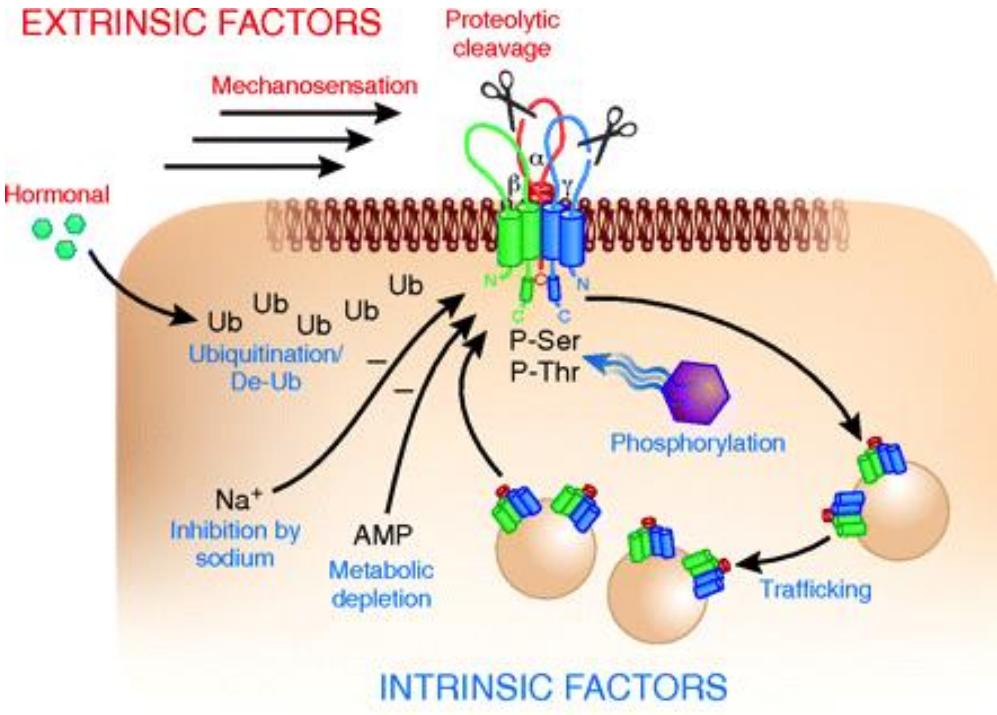
(distal nephron, urinary bladder, lung airway, distal colon, and ducts of salivary and sweat glands)

- Three structurally related subunits

(α-, β-, and γ-ENaC, as an α, β, γ heterotrimer at the plasma membrane)

- The various mechanisms of ENaC regulation

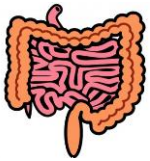
e.g.) hormone activation
mechanical stretch
proteolytic cleavage
intracellular trafficking
ubiquitination
various kinases
Sodium
metabolic substrates



Bhalla V. and Hallows KR. *JASN*. 2008

How important is ENaC in human body ?

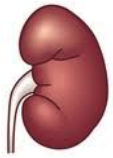
Sodium regulated by ENaC helps maintain the body water distribution, which affect blood pressure and airway surface liquid - water goes where the sodium goes……

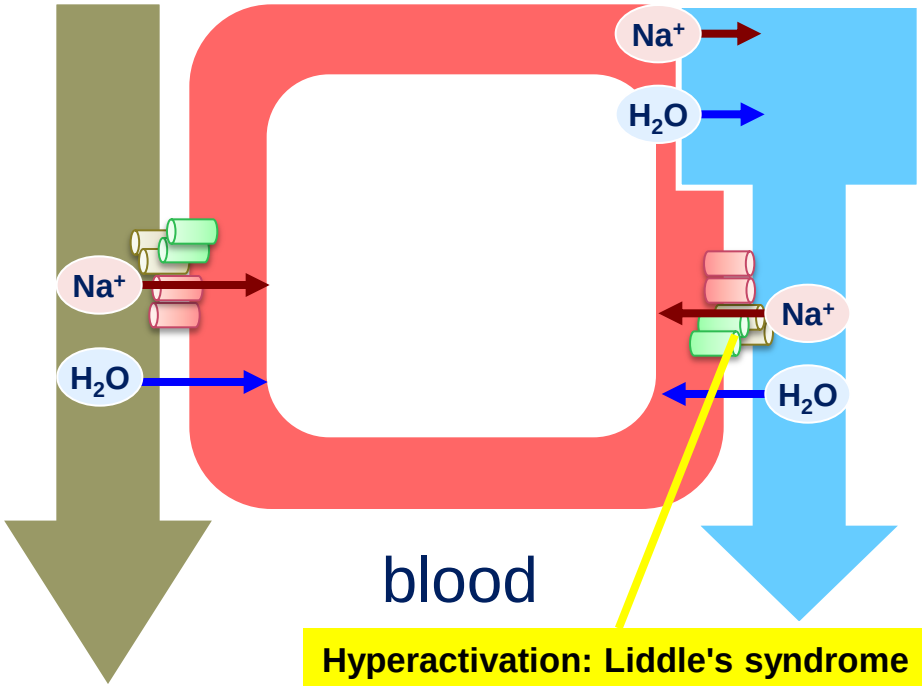


colon

Body fluid

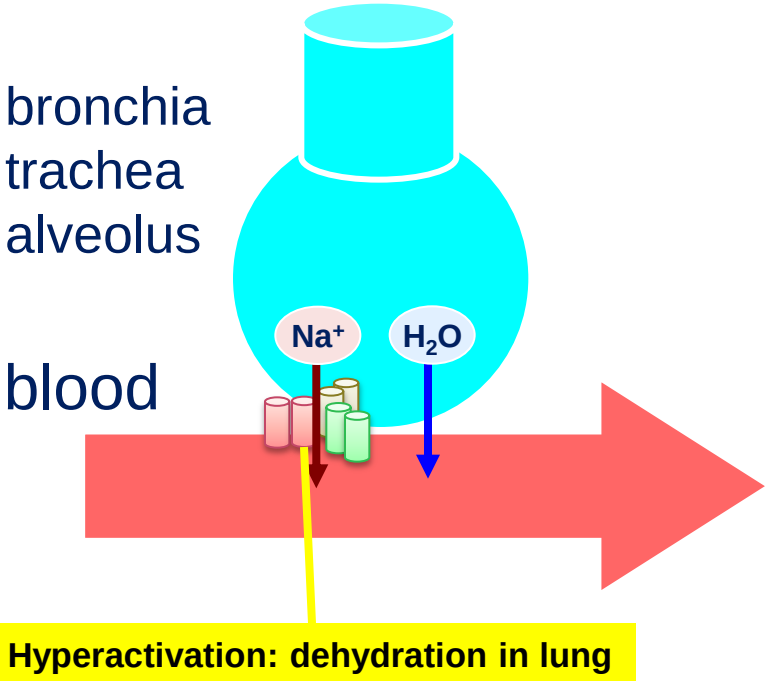
kidney





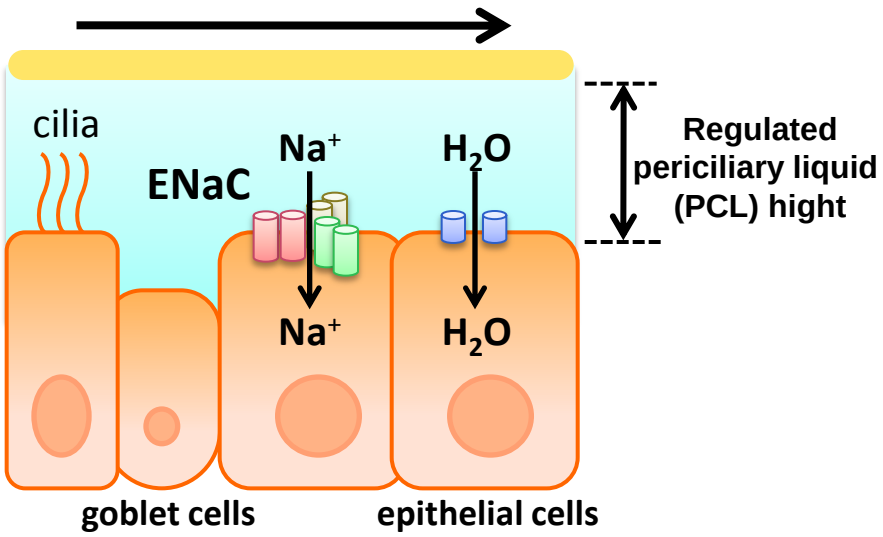
Airway surface

lung

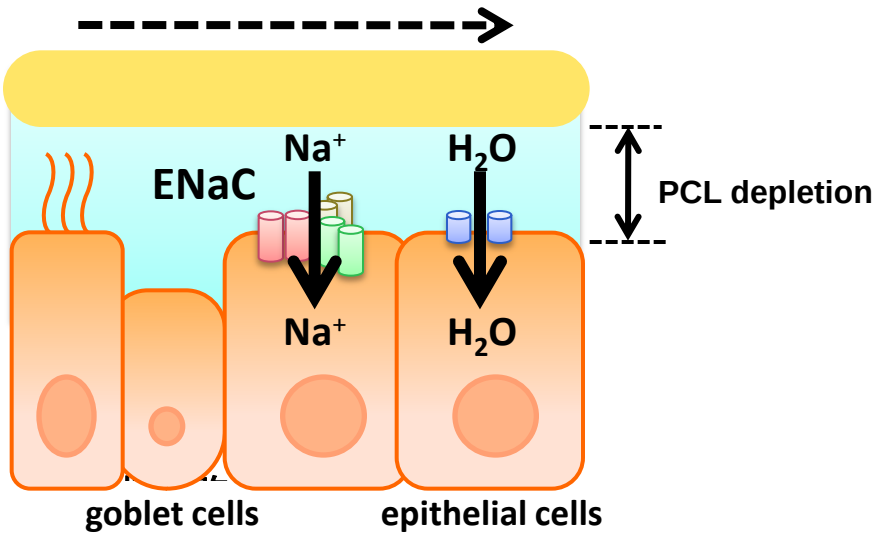


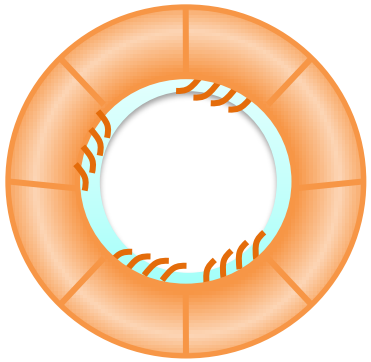
ENaC regulates airway surface liquid

< normal airway >

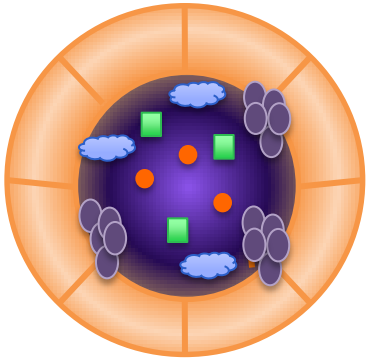


< ENaC overexpressing mouse >
(Cystic fibrosis airway)





Proper mucus clearance



- Mucus stasis
- Inflammation
- Goblet cell hyperplasia

chemokines elastase neutrophils Mucus cell granule

Mall *et al., Nat Med.* **2004**

βENaC protein expression versus GOLD category in 37 COPD patients.

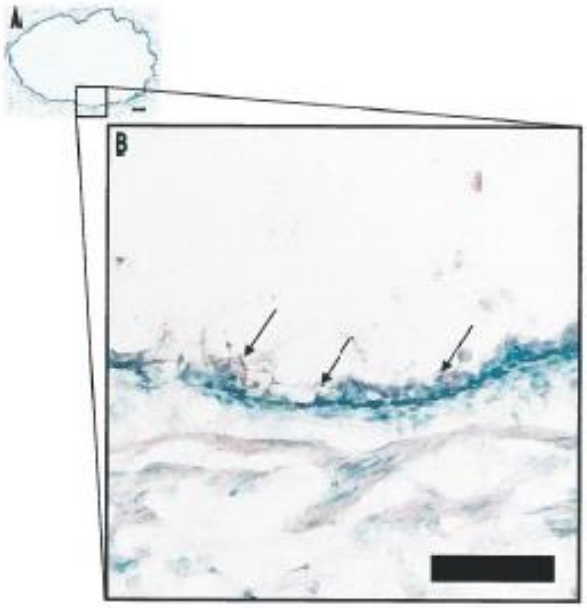


Figure 6.13: Light micrographs showing beta-ENaC protein expression in the small airway epithelium.
Rabbit polyclonal beta-ENaC antibody was used as a primary antibody to detect human 3-ENaC protein in the small airway epithelium. A) A whole small airway from a GOLD 2 patient showing beta-ENaC protein staining in purple with the epithelium staining green. B) The closer view of the boxed region on A with the airway epithelium staining for f3-ENaC protein (arrows). (bar = 100 um)

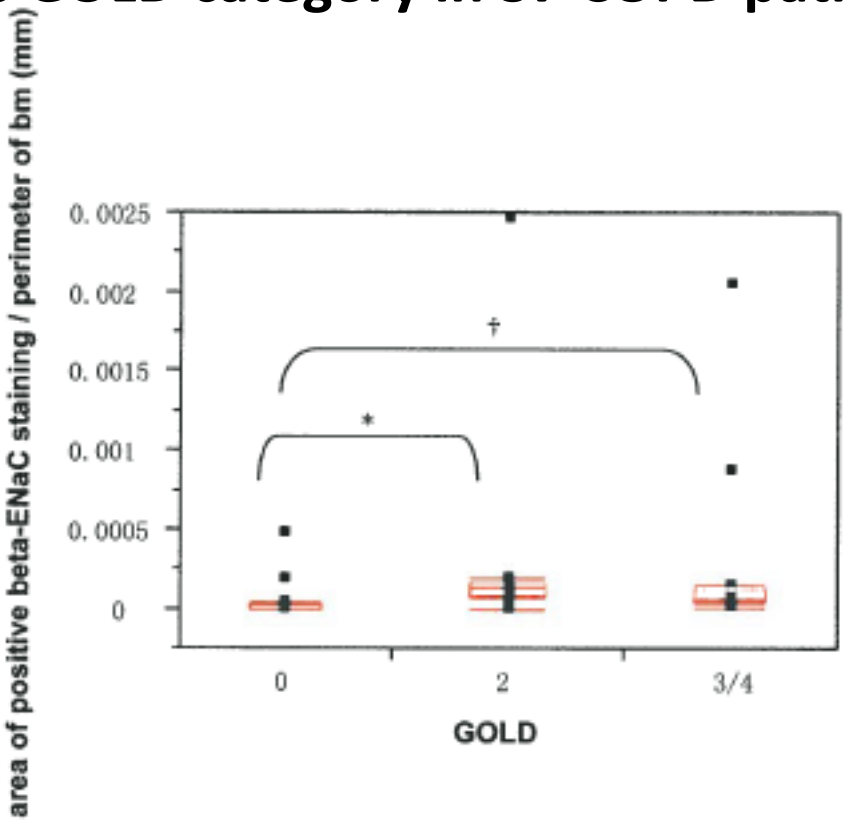


Figure 6.14: β-ENaC protein expression versus GOLD category in 37 COPD patients. β-ENaC protein expression was calculated as the ratio of area of positive staining in the airway epithelium to the perimeter of basement (bm) membrane of the airway. The data are expressed as box plots. The red line inside the box represents median β-ENaC protein expression in each GOLD stage and the boxes denote the lower and higher quantiles while the tails represent the range of the data with the exception of data outside the tails that are possible outliers. The Kruskal-Wallis test was carried out to analyze the difference in β-ENaC protein expression among three GOLD categories.

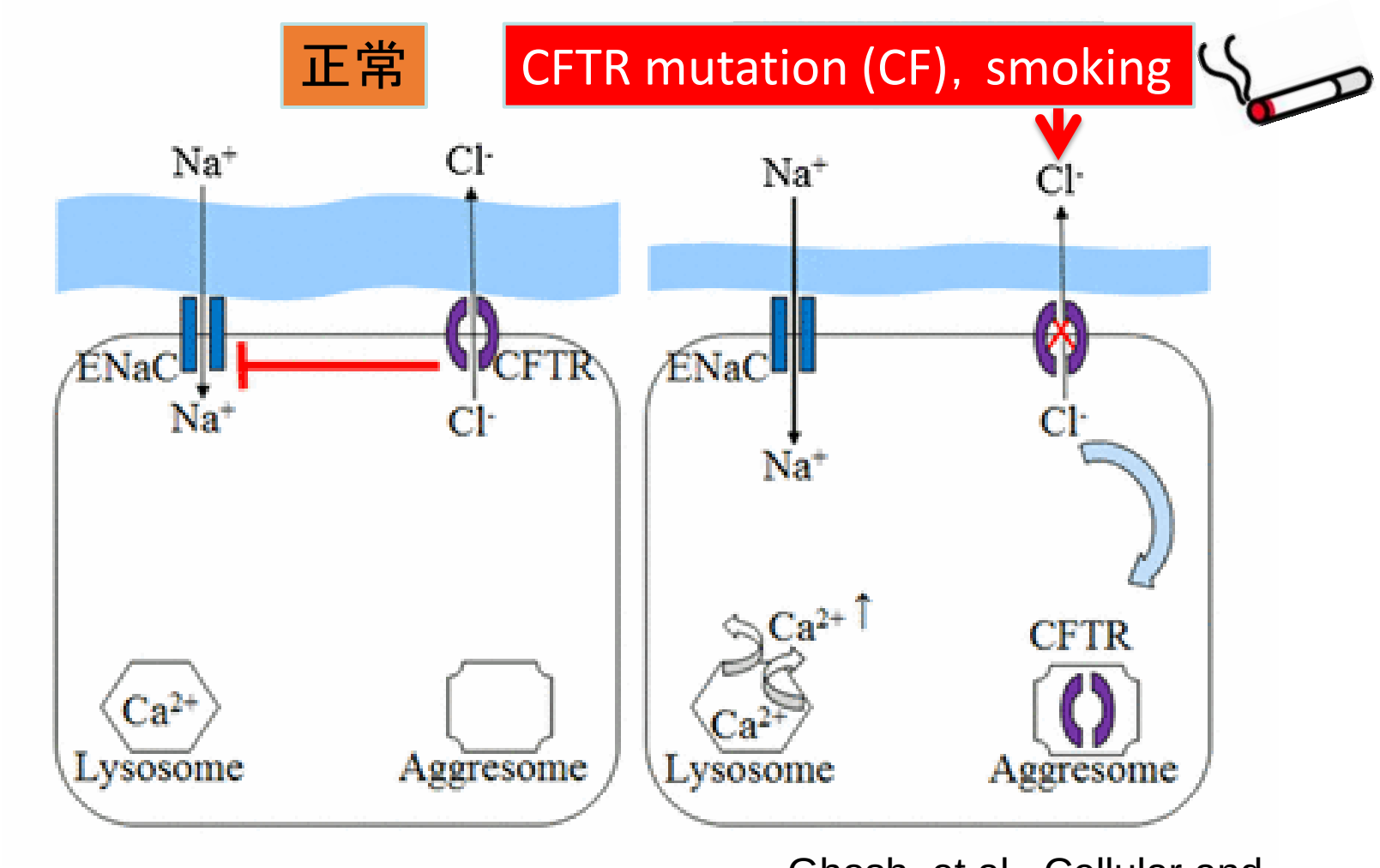
* significant increase of β-ENaC protein expression between GOLD 0 (n=12) and 2 (n=13)(p = 0.01).

† significant increase of β-ENaC protein expression between GOLD 0 (n=12) and 3/4 (n=12) (p = 0.01).

(Master thesis of BKM Chan, The University of British Columbia 2008

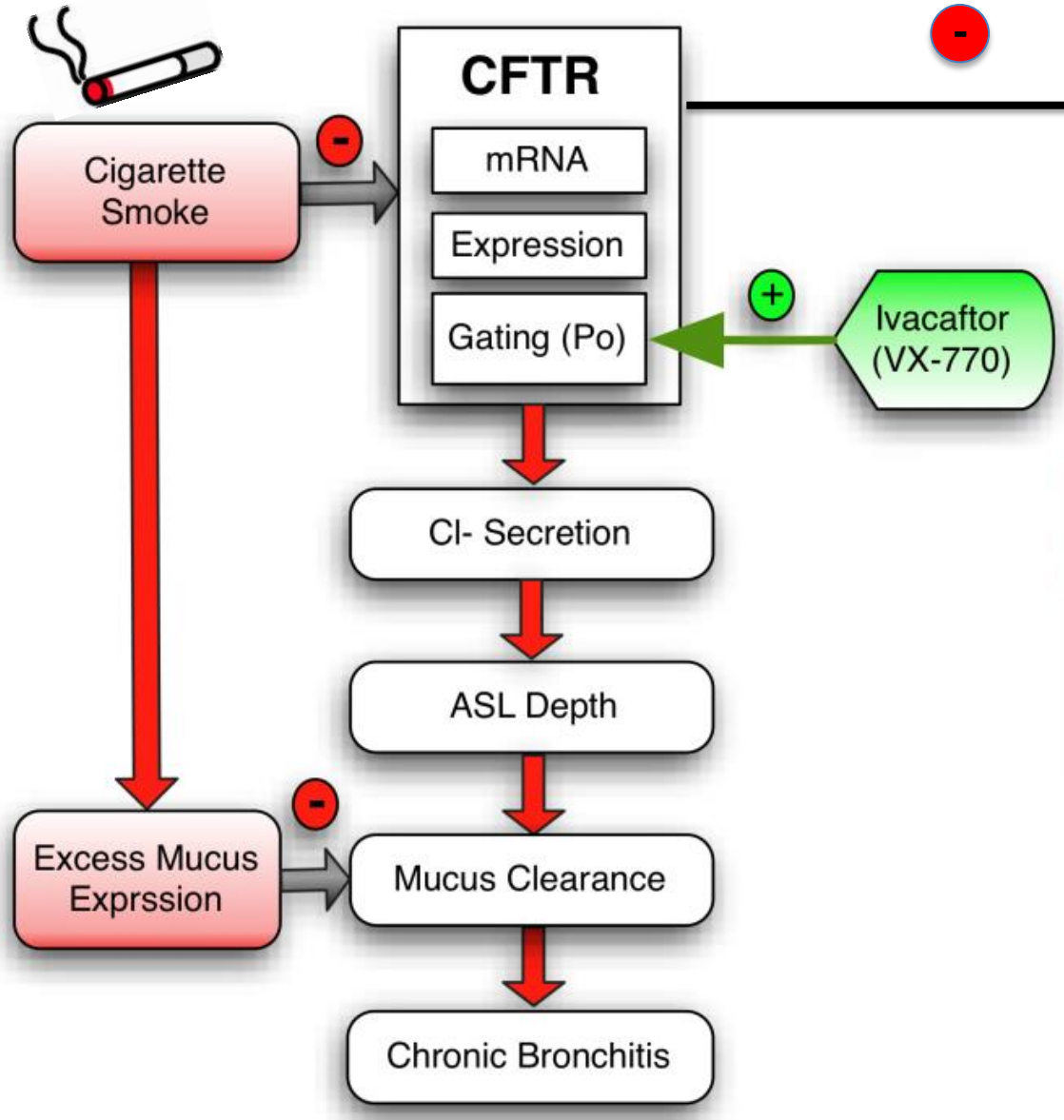
Tabaco smoke and CFTR dysfunction activate ENaC

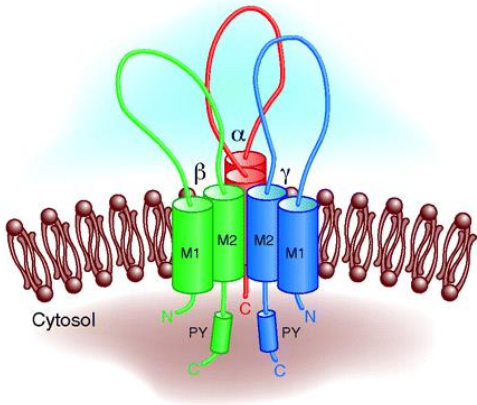
~Implication of ENaC-Tg as a model of COPD~



Ghosh, et al., Cellular and Molecular Life Sciences, 2015

CFTR activation as a target of COPD





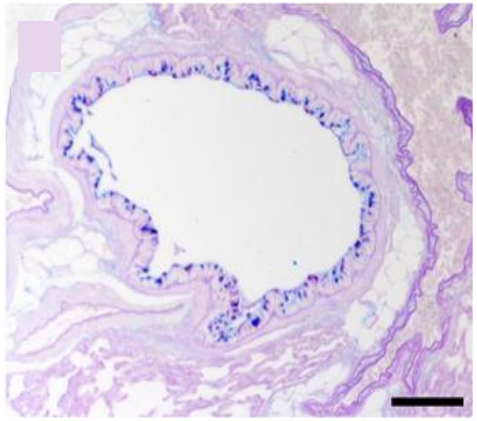
CF therapeutic agent
(CFTR potentiator)

Lin, et al., A3871, ATS 2015

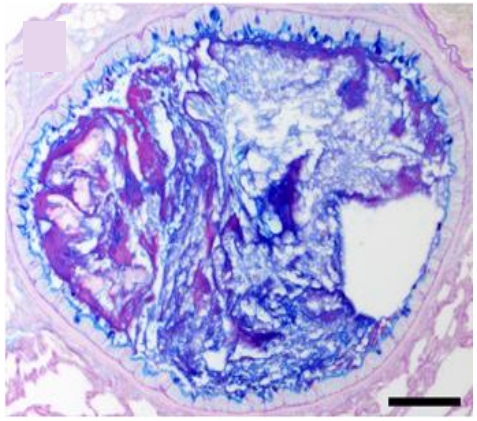
Could airway-specific ENaC-Tg mice be the model of obstructive lung diseases such as CF?

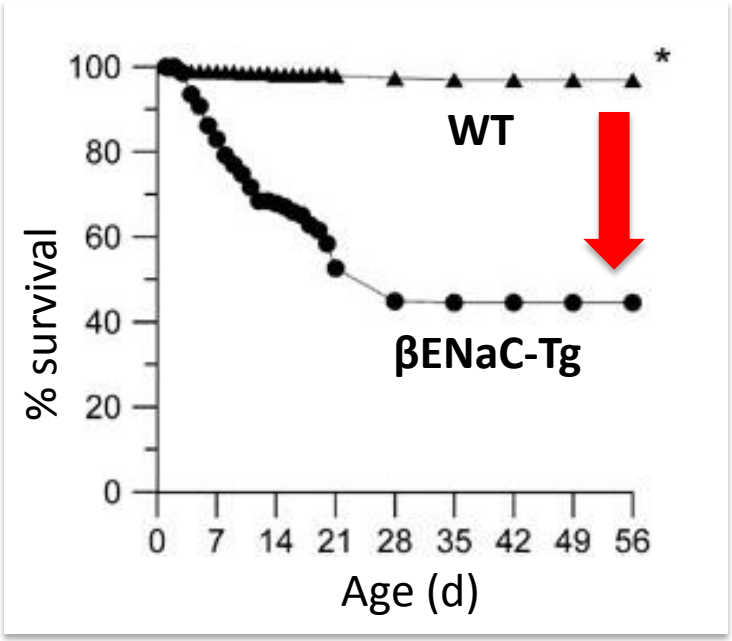
Mice models	Inflammation /Emphysema	Mucus
Airway-specific ENaC-Tg mice	○	○

WT



βENaC-Tg





(Mall *et al., Nat Med* 2004)

Yes! But 50% pulmonary mortality needs to be improved!!

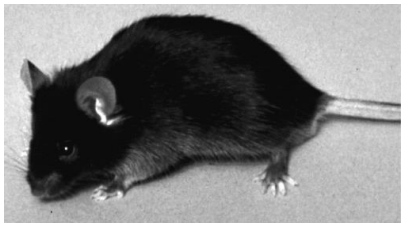
Development of low-mortality βENaC-Tg mice

C3B6 line (JAX)

High-mortality mice

(Mall *et al., Nat Med* 2004)

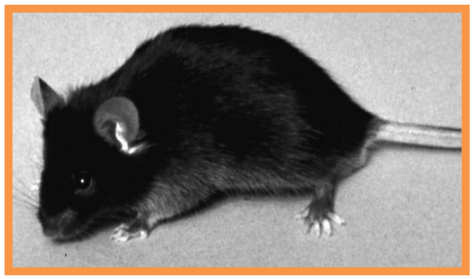
B6 line



Resistance for airway imflammation mice

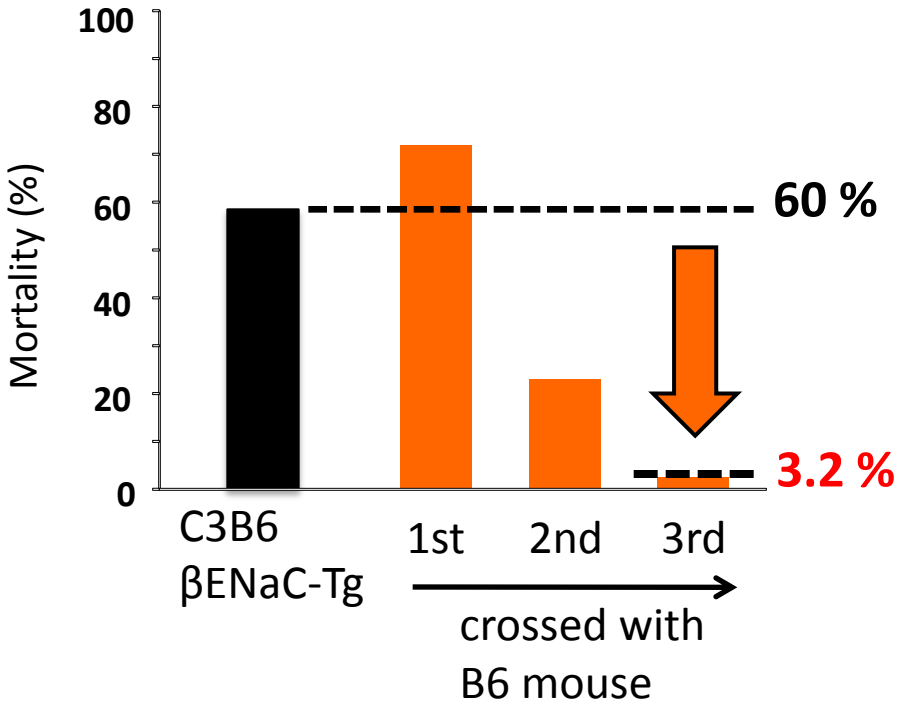
(Churg and Wright, *Karger* 2007)

crossed



low-mortality βENaC-Tg mice

(Shuto T, *Sci Rep.*, in revision)



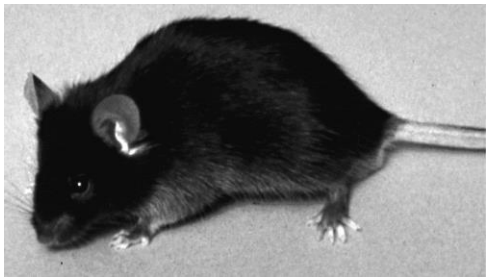
Test

Mucus, inflammation

Emphysema, lung function

Gene expression pattern (Microarray)

Pulmonary phenotypes of low-mortality βENaC-Tg mice



low-mortality βENaC-Tg mice

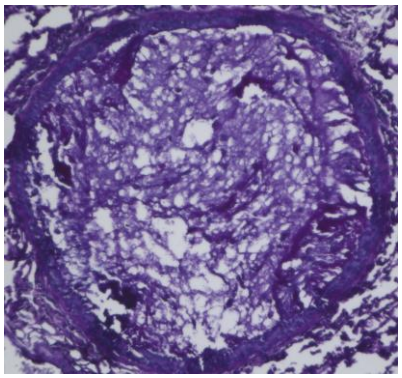
(Shuto T, *Sci Rep.*, in revision)

Inflammation

Lung function

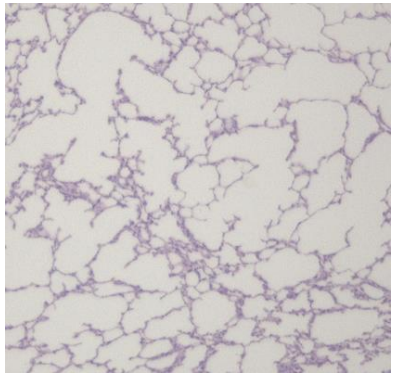
flexiVent system (SCIREQ Inc.)

Mucus plug



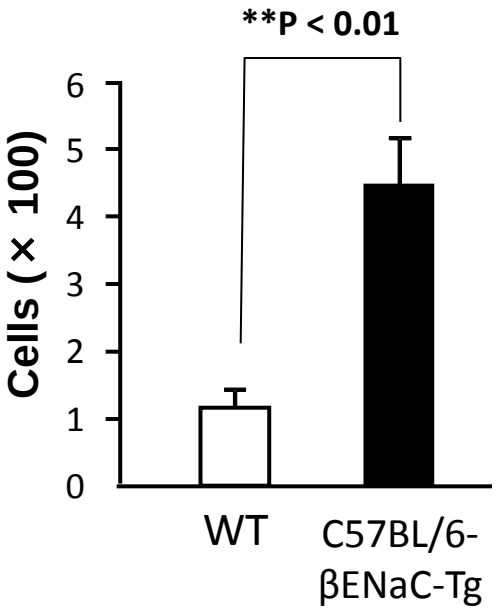
Bronchia

Emphysema



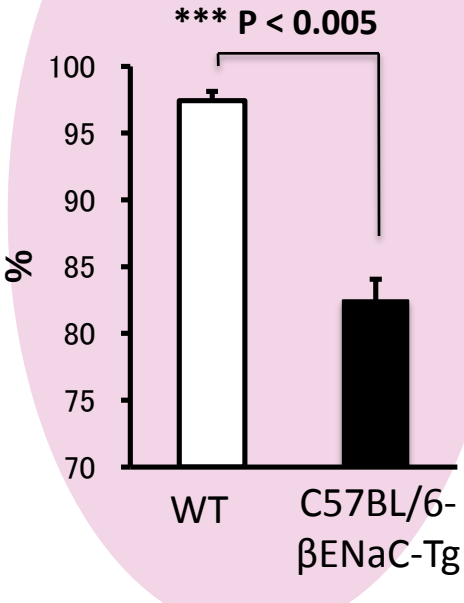
Pulmonary alveolus

Neutrophils



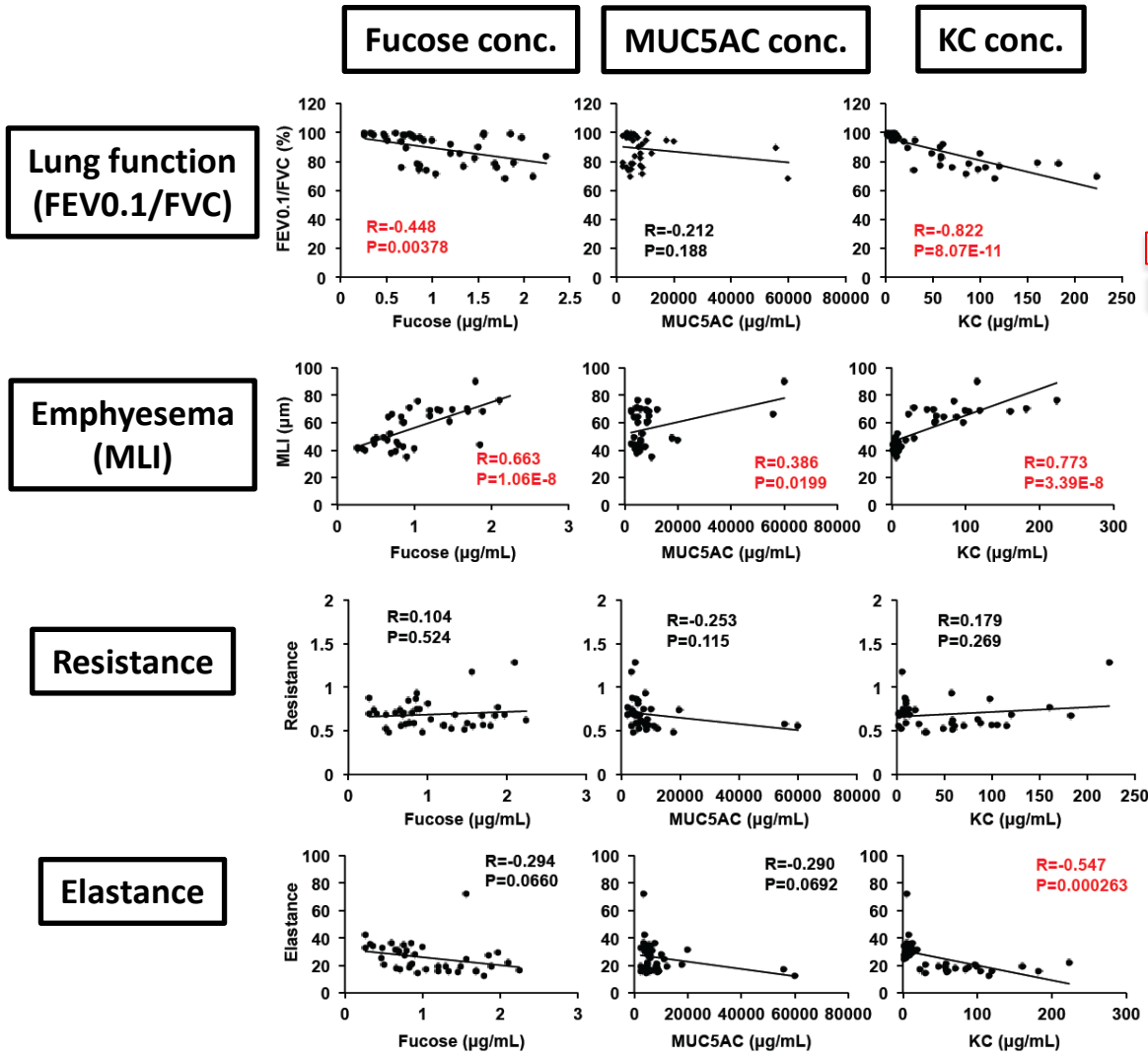
FEV0.1%

Forced expiratory volume % in 0.1 second

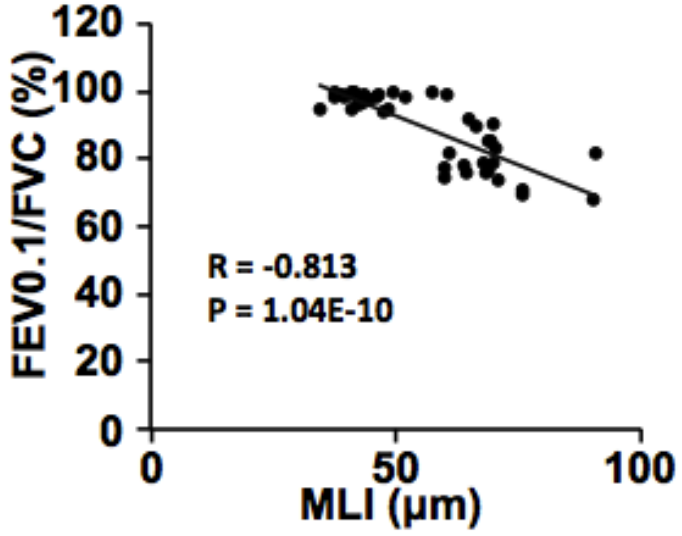


Mucus hypersecretory, airway inflammatory and emphysema-like phenotypes were observed in our low-mortality βENaC-Tg mice line

Pulmponary emphysema and dysfunction in βENaC-Tg mice



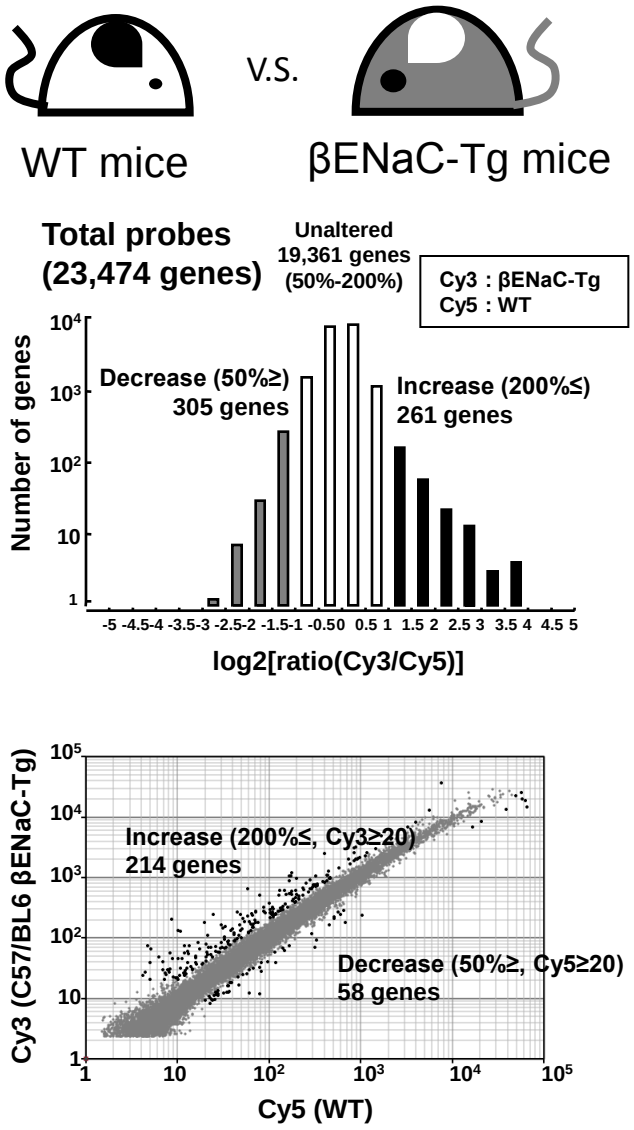
	Correlation coefficients		
	Fucose (μg/ml)	MUC5AC (mg/ml)	KC (μg/ml)
FEV0.1/FVC	-0.448*	-0.212	-0.822*
MLI	0.663*	0.386*	0.773*
Resistance	0.104	-0.253	0.179
Elastance	-0.294	-0.290	-0.547

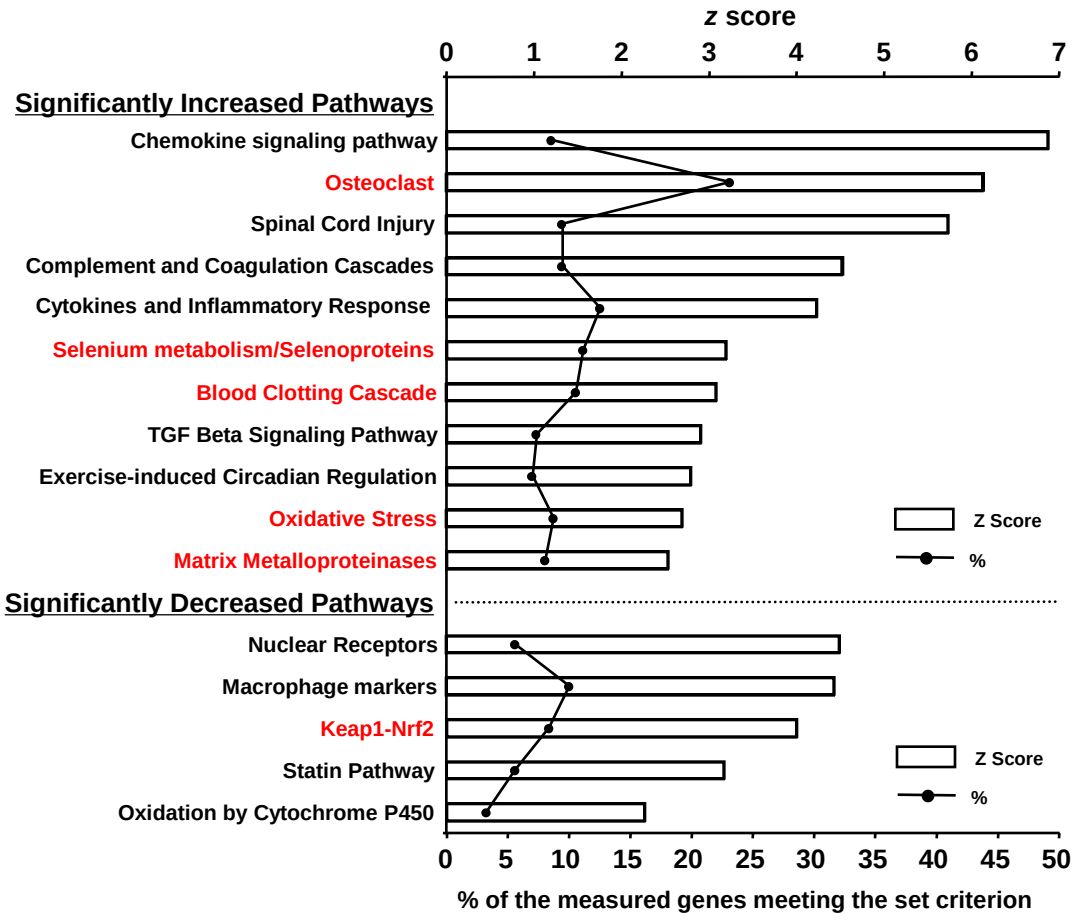


(Shuto T, *Sci Rep.*, in revision)

Pulmponary emphysema and dysfunction are the key hallmarks of βENaC-Tg mice

Gene expression analysis (3D-Gene , TORAY)





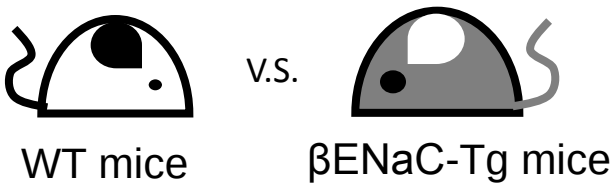
- Proteases-antiproteases imbalance
- Oxidative stress

(Shuto T, *Sci Rep.*, in revision)

How airway-specific β-ENaC-Tg mice therapeutically targeted?

Fact 1

Microarray analysis of lung mRNA

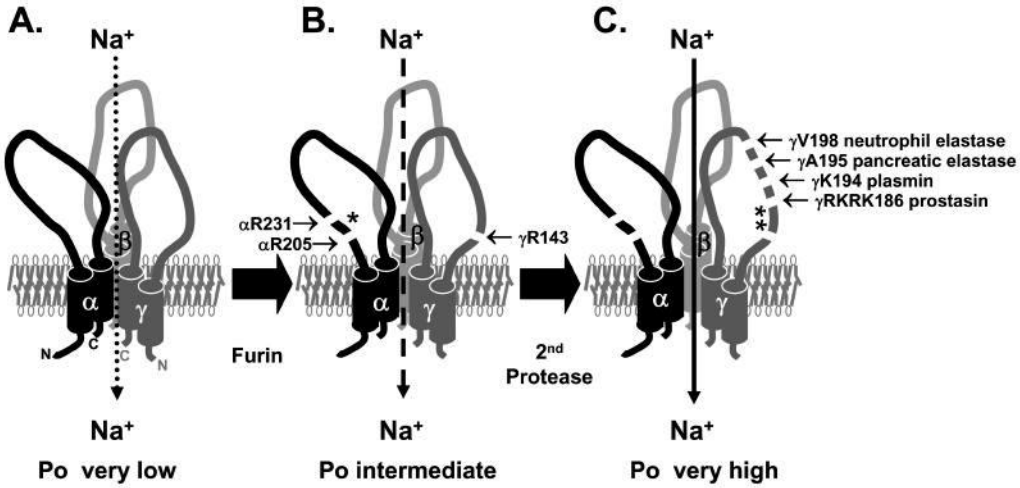


Typical symptom of COPD

- **Proteases**
- Oxidative stresses
- Inflammations

Activated in βENaC-Tg mice

Fact 2



Passero CJ, Hughey RP, Kleyman TR. *Curr Opin Nephrol Hypertens.* 2010

ENaC is activated by proteolytic cleavage (serine proteases) and release of inhibitory peptides

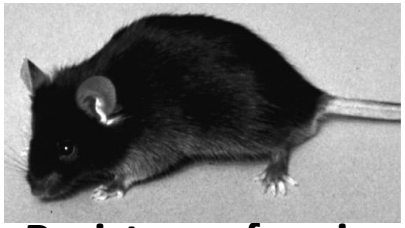
Serine proteases inhibitors may be effective for the pulmonary symptom of airway-specific βENaC-Tg mice

Pulmonary phenotypes of low-mortality βENaC-Tg mice

C3B6 line

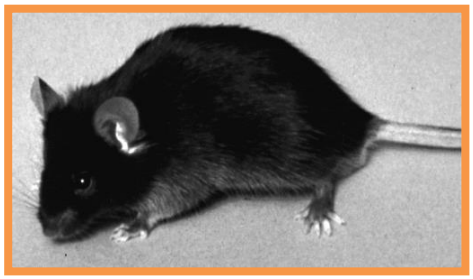
High-mortality mice
(Mall *et al.*, *Nat Med* 2004)

C57BL/6 line



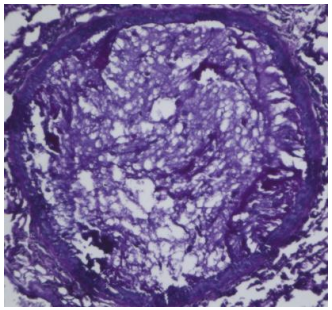
Resistance for airway imflammation mice
(Churg and Wright, *Karger* 2007)

Crossing



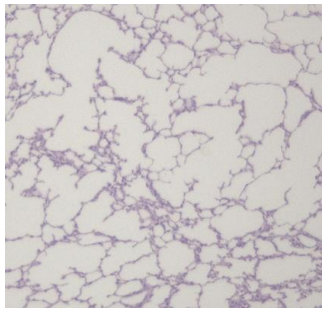
low-mortality βENaC-Tg mice
(C57BL6-βENaC-Tg mouse)

Mucus plug



Bronchia

Emphysema



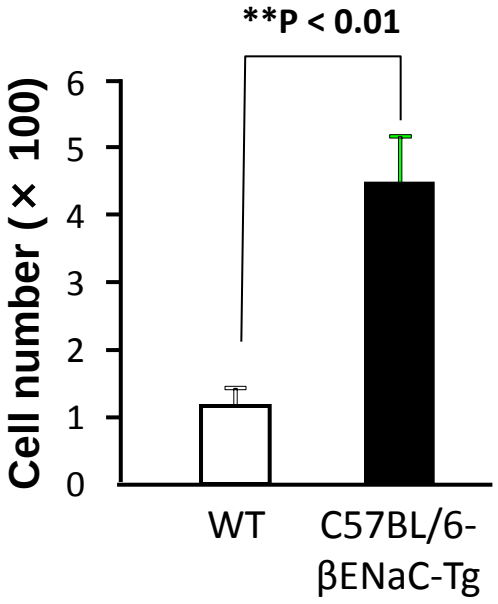
Pulmonary alveolus

Microarray

- **Proteases -antiproteases imbalances**
- Oxidative Stress

Inflammation

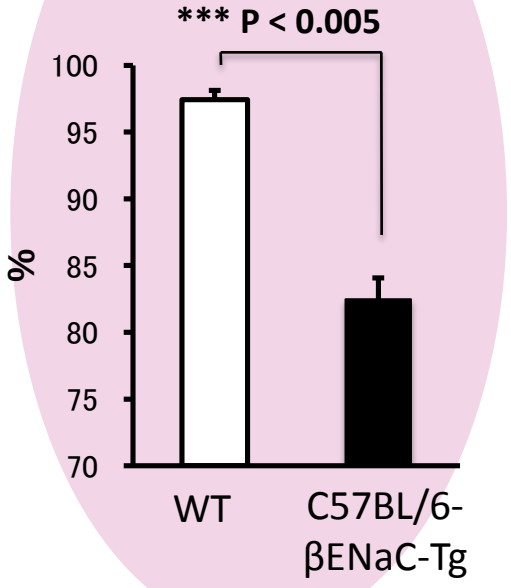
Neutrophils



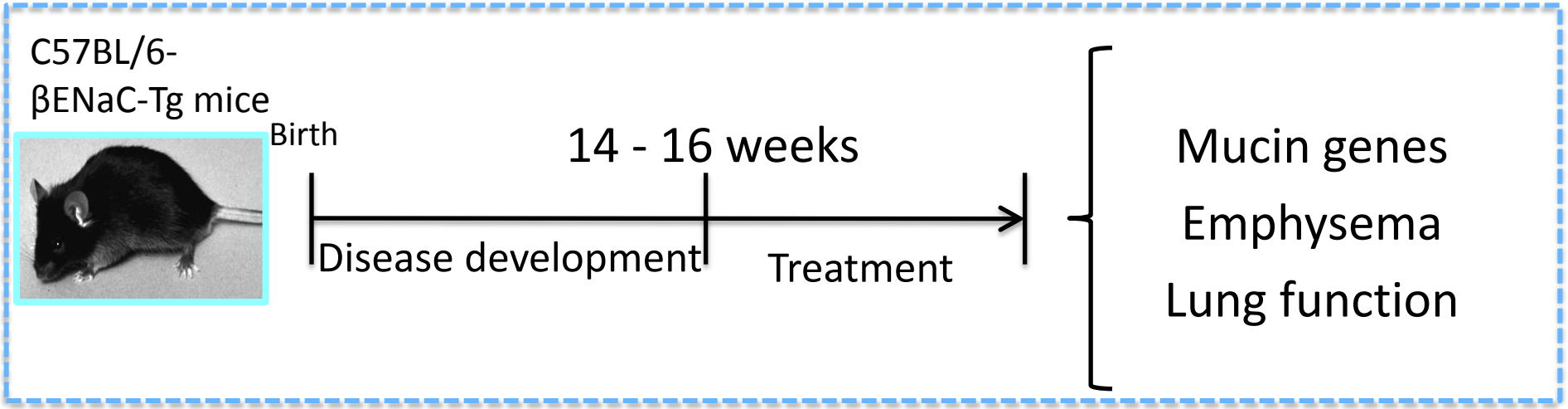
Lung function

FEV0.1%

（Forced expiratory volume % in 0.1 second）

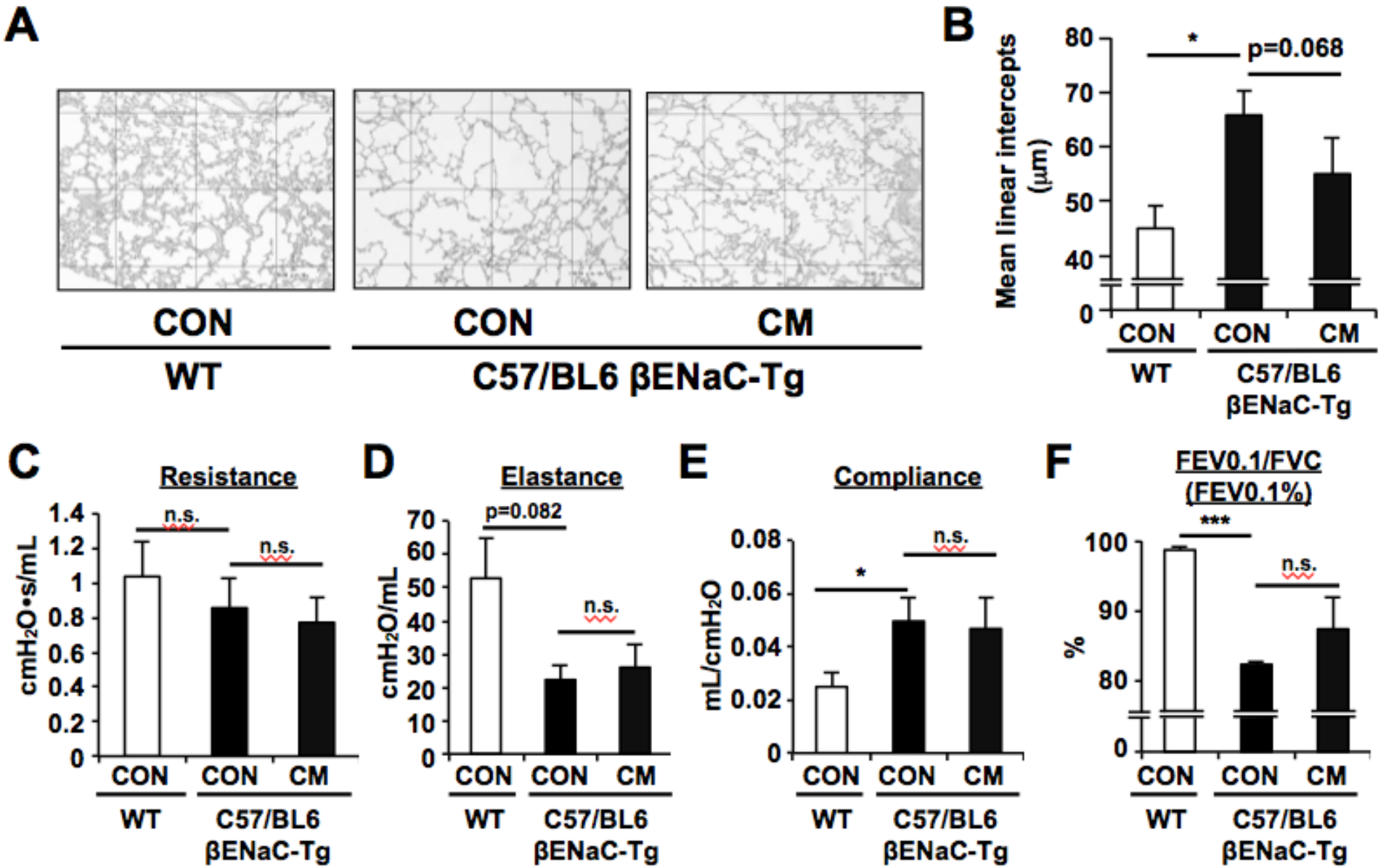


Therapeutic evaluation - Protease inhibitors



	Camostat mesilate (CM)	ONO-3403
Features	A synthetic orally-treatable serine protease inhibitor Clinically used for the treatment of pancreatitis and reflux esophagitis	A derivative of camostat mesilate A higher protease-inhibitory activity
Duration	**3 weeks**	**2 weeks**
Dose	**100 mg/kg (twice/day)**	**20 or 100 mg/kg (twice/day)**
Method	**oral**	**oral**

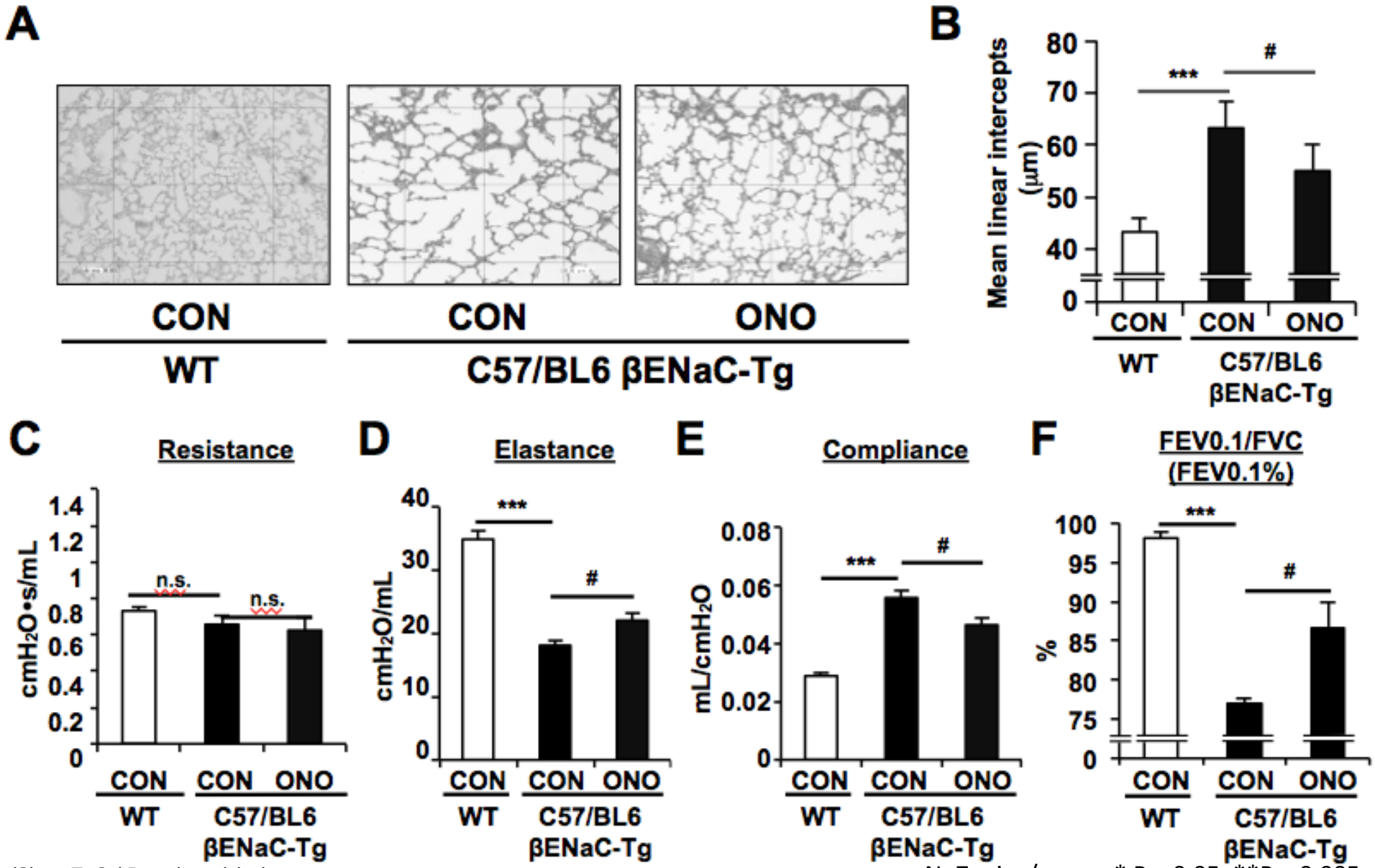
Effect of Camostat mesilate (CM) on the pulmonary emphysema and dysfunction in C57/BL6-βENaC-Tg mice



(Shuto T, *Sci Rep.*, in revision)

N=7 mice/group, * P < 0.05, **P < 0.005

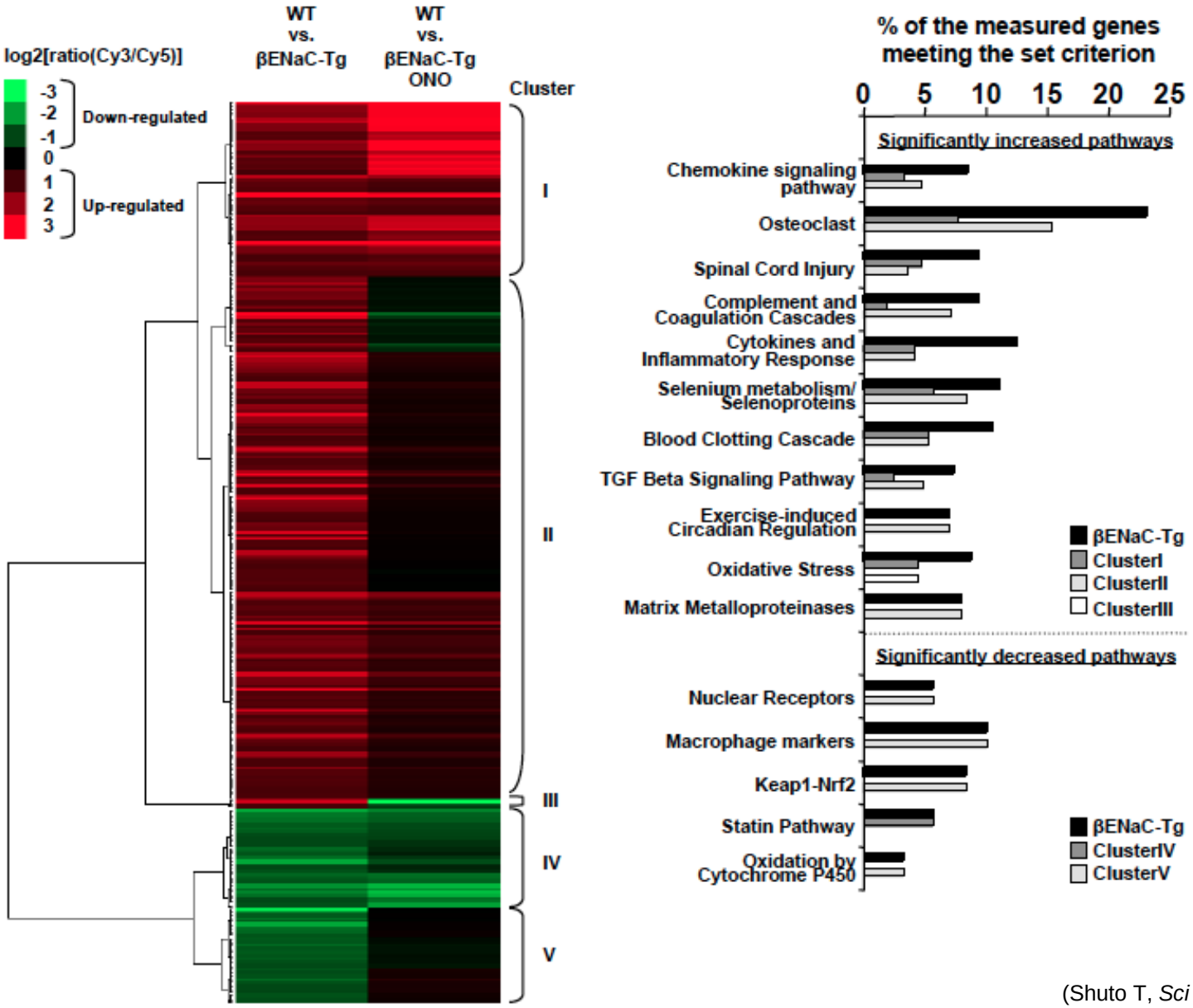
Effect of ONO-3403 on the pulmonary emphysema and dysfunction in C57/BL6-βENaC-Tg mice



(Shuto T, *Sci Rep.*, in revision)

N=7 mice/group, * P < 0.05, **P < 0.005

ONO3403 treatment dampens excessive activation of protease and oxidative stress pathways



(Shuto T, *Sci Rep.*, in revision)

Brief Summary 1

	Camostat mesilate (CM)	ONO-3403
Duration	3 weeks	2 weeks
Dose	100 mg/kg (twice/day)	20 or 100 mg/kg (twice/day)
Method	Mucin genes expression, Emphysema-like phenotype, Lung function	
	Slight improvement	**Improvement**

Inhibiting protease activity, especially by ONO-3403, improves the mucus hypersecretory and emphysema-like phenotypes in the airway-specific ENaC-Tg mice

Potential usefulness of the ENaC-Tg mice for **therapeutic evaluation** of pulmonary phenotypes of CF

Therapeutic approach – ENaC inhibitor, P-1037

PARION SCIENCES

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« Topical epithelial sodium channel blocker targets ocular surface hydration

Parion Sciences Announces Key Management Promotion »

Parion Sciences Announces Initiation of Phase 2 Clinical Study of P-1037 for the Treatment of Cystic Fibrosis

Published May 6, 2015 | By Paul Boucher

First Patient with Cystic Fibrosis Enrolled in CLEAN-CF Phase 2 Clinical Trial

Durham, NC (May 6, 2015) – Parion Sciences, a company dedicated to the development of novel treatments for pulmonary and ocular diseases, announced today it has begun enrollment of a phase 2 clinical trial of P-1037 in patients with Cystic Fibrosis (CF).

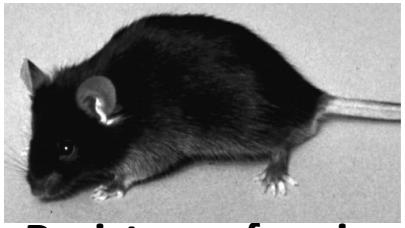
The trial has been named the "CLEAN-CF" trial which refers to "Clearing Lungs with ENaC inhibition in Cystic Fibrosis". The CLEAN-CF study will include CF patients regardless of an individual's genetic mutation. Inhibiting the epithelial sodium channels (ENaC) in the airways with P-1037, an "ENaC blocker," is expected to re-hydrate the mucus layers, thus restoring airway clearance, improve lung function and, ultimately, reduce exacerbations. P-1037 has demonstrated to be long acting in preclinical models and to have a favorable safety and tolerability profile in the completed Phase 1 studies. The initiation of the Phase 2 clinical trials was supported by an award from Cystic Fibrosis Foundation Therapeutics Inc. (CFFT), the nonprofit affiliate of the Cystic Fibrosis Foundation.

Pulmonary phenotypes of low-mortality βENaC-Tg mice

C3B6 line

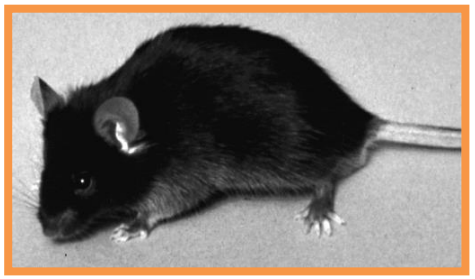
High-mortality mice
(Mall *et al.*, *Nat Med* 2004)

C57BL/6 line



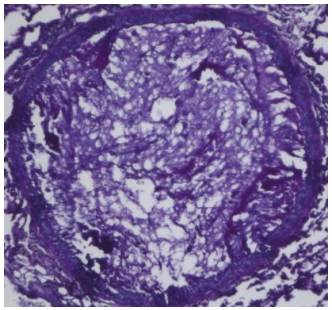
Resistance for airway imflammation mice
(Churg and Wright, *Karger* 2007)

Crossing



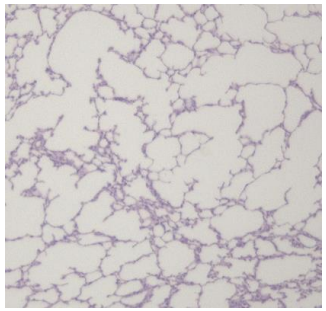
low-mortality βENaC-Tg mice
(C57BL6-βENaC-Tg mouse)

Mucus plug



Bronchia

Emphysema



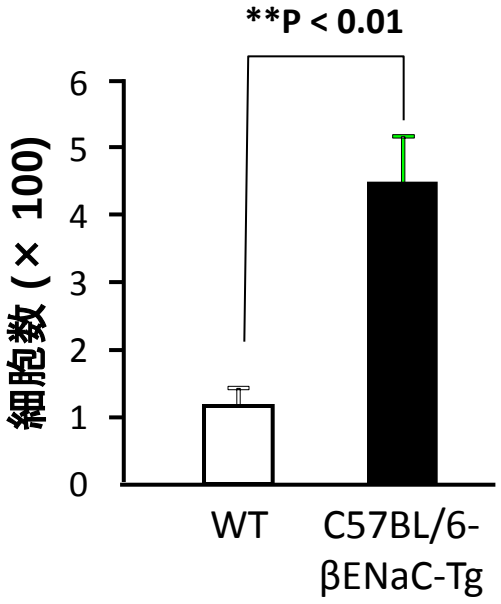
Pulmonary alveolus

Microarray

- Proteases
  -antiproteases imbalances
- **Oxidative Stress**

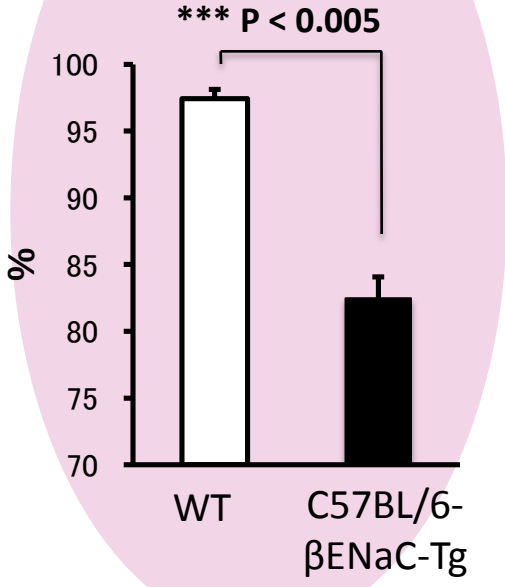
Inflammation

Neutrophils



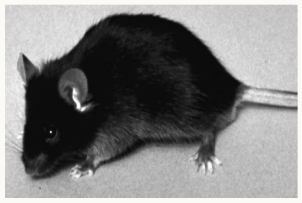
Lung function

FEV0.1%
（Forced expiratory volume % in 0.1 second）



Therapeutic evaluation – anti-oxidant, N-acetylcystein (NAC)

C57BL/6-
βENaC-Tg mice



Birth

14 - 16 weeks

Disease development

Treatment

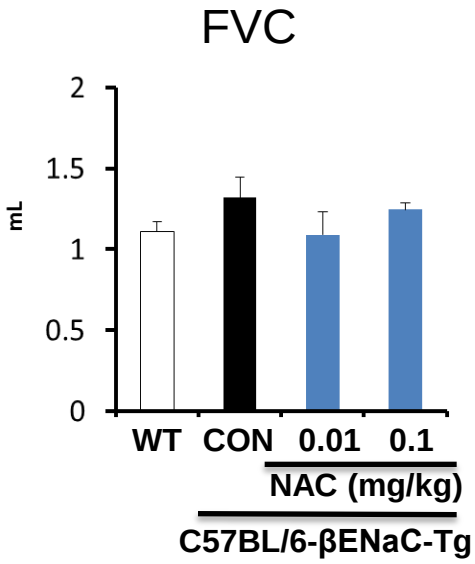
Emphysema

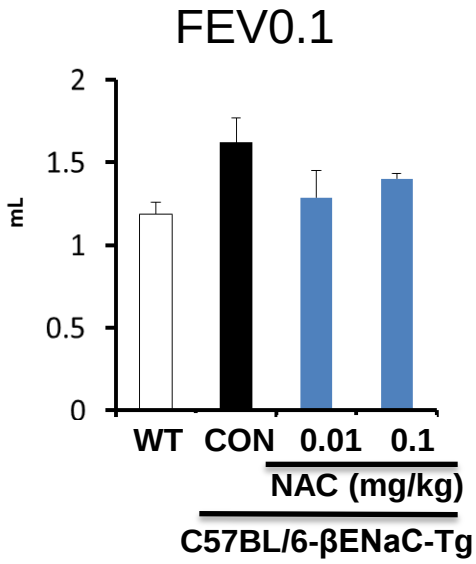
Lung function

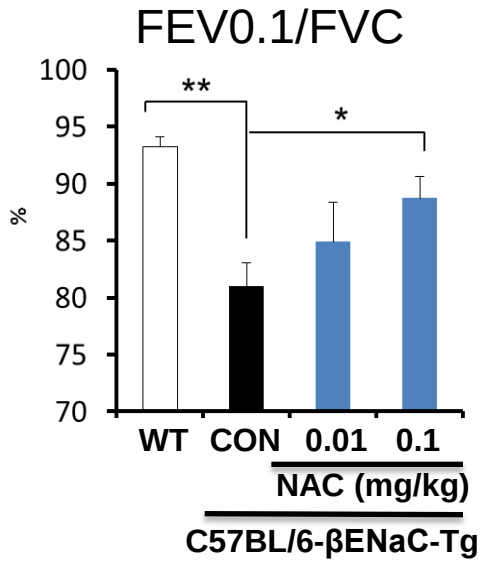
	N-acetylcystein (NAC)	ambroxol	carbocysteine
Feature	NAC serves as a precursor to the antioxidant, glutathione Known as a mucolytic agent (expectorant)	One of the expectorants Secretolytic actions It stimulates release of surfactant	One of the expectorants A mucolytic that reduces the viscosity of sputum
Duration	**2 weeks**	**2 weeks**	**3 weeks**
Dose	**0.01 – 0.1 mg/kg (once/day)**	**0.3-10 mg/kg (twice/day)**	**300 mg/kg (twice/day)**
Method	**i.t.**	**i.p.**	**oral**

Effect of N-acetylcystein on pulmonary phenotype in βENaC-Tg mice

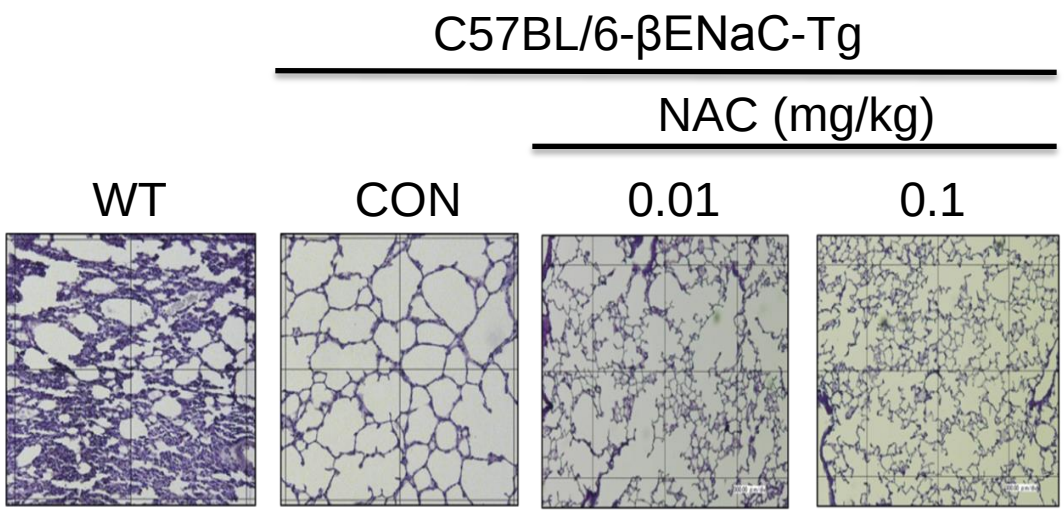
Lung function (0.01 – 0.1 mg/kg, i.t.)

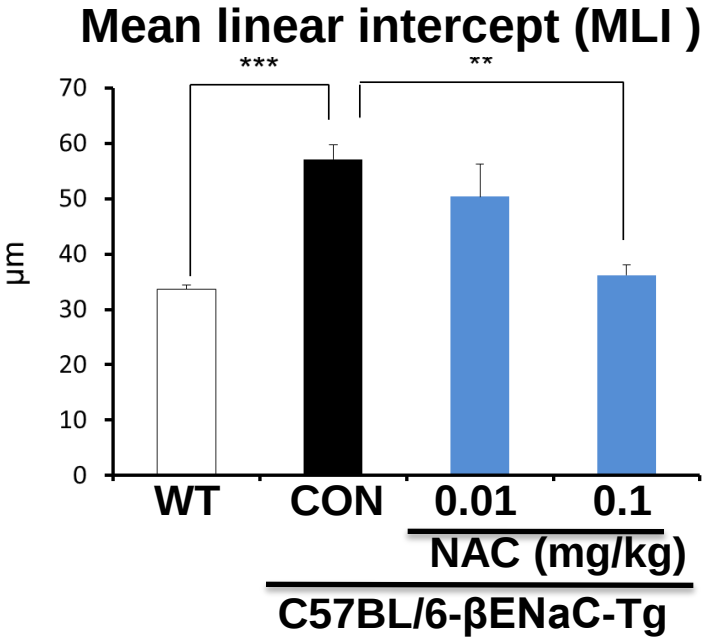






Emphysema (MLI) (0.01 – 0.1 mg/kg, i.t.)



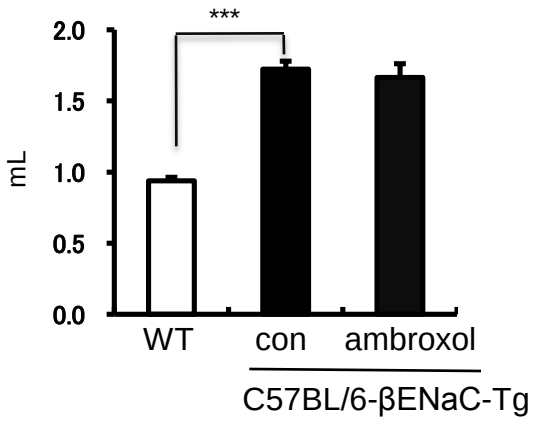


(Shuto T, *Sci Rep.*, in revision)

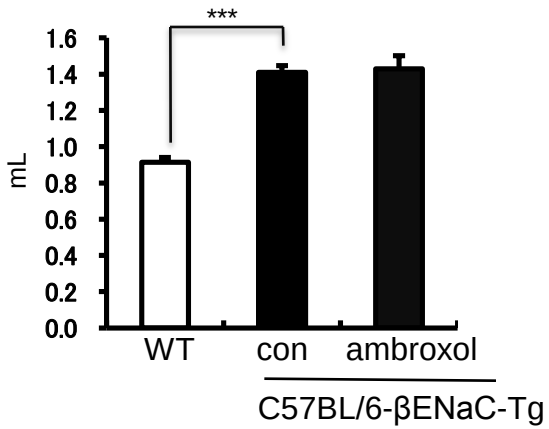
Expectorant ambroxol does not rescue pulmonary phenotype

Lung function (1mg/kg, i.p.)

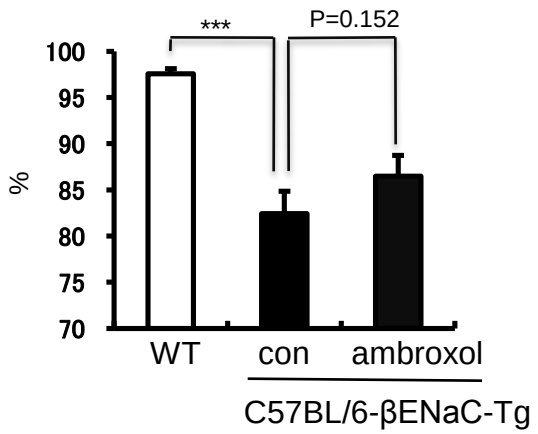
FVC



FEV0.1



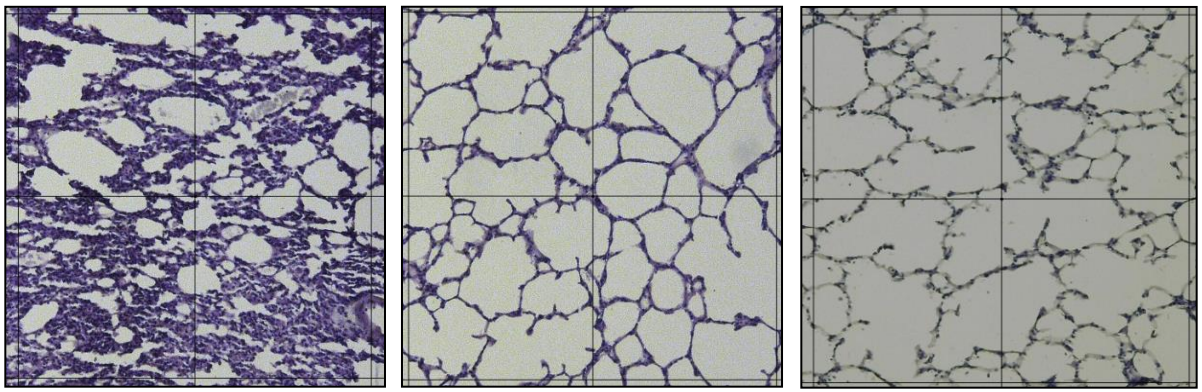
FEV0.1/FVC



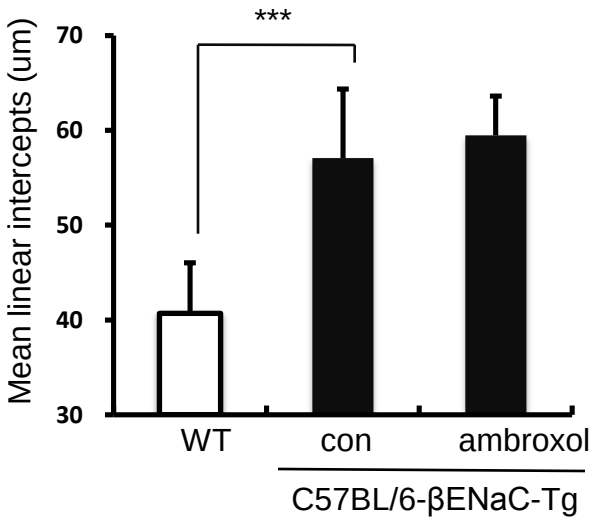
Emphysema (MLI) (1mg/kg, i.p.)

C57BL/6-βENaC-Tg

WT | CON | ambroxol

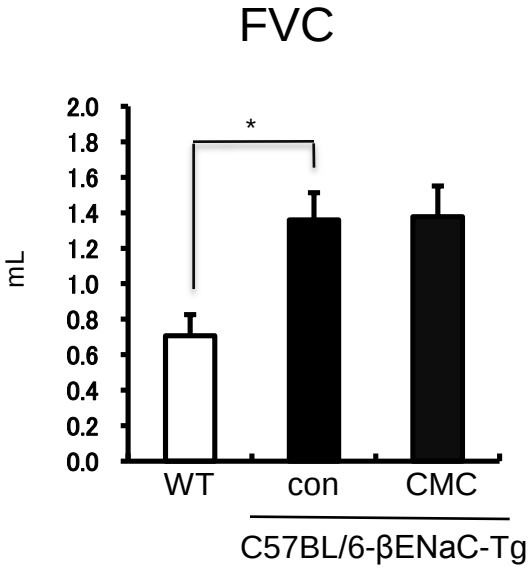


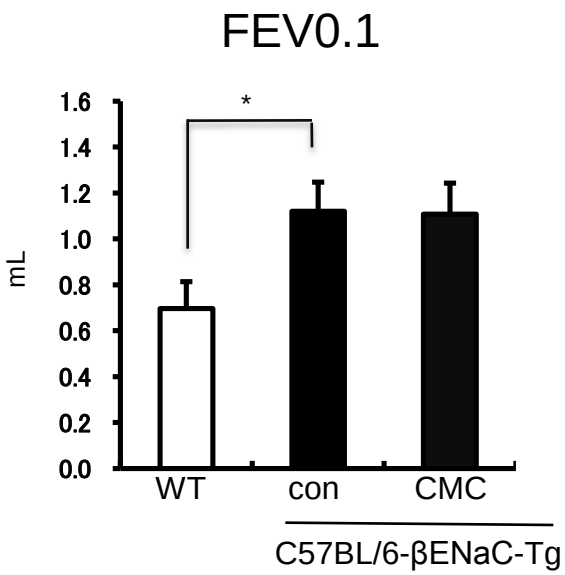
Mean linear intercept (MLI)

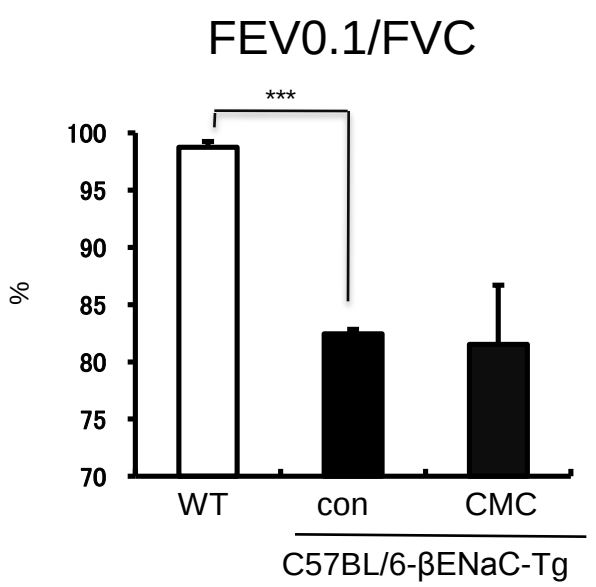


Expectorant carbocysteine does not rescue pulmonary phenotype

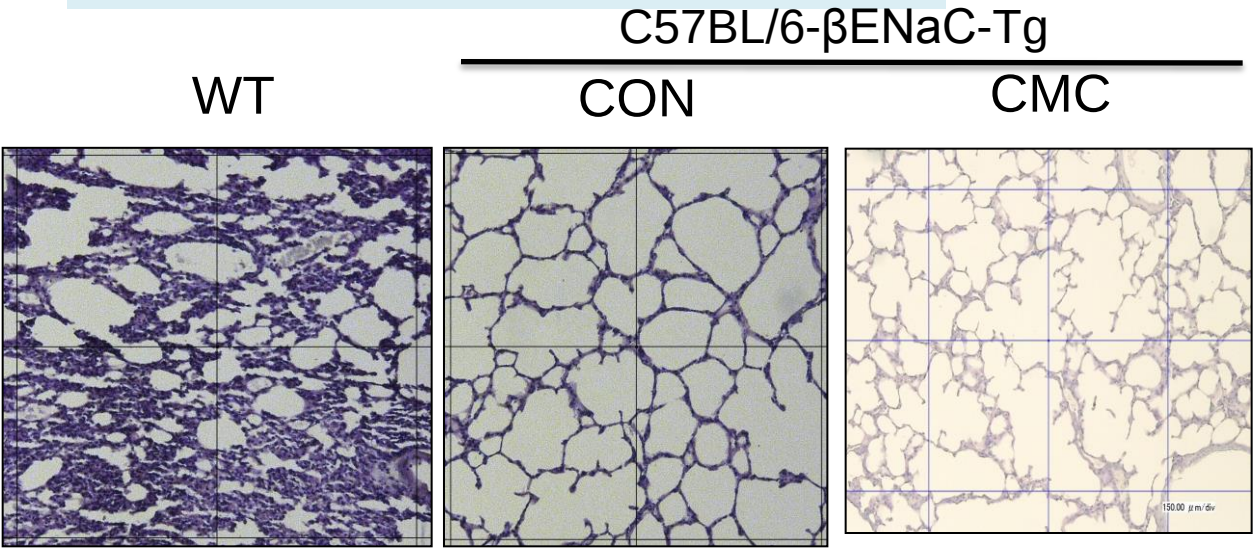
Lung function (300 mg/kg, p.o.)

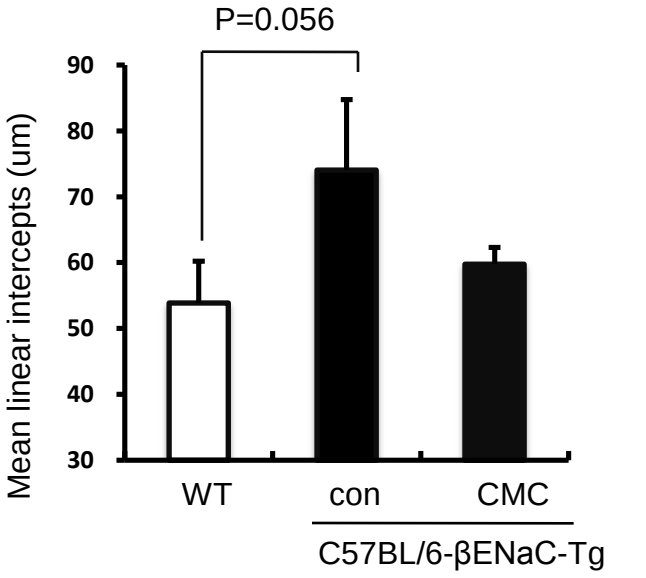






Emphyesema (MLI) (300 mg/kg, p.o.)





Brief Summary 2

Class	Anti-oxidant, Expectorant	Expectorant	Expectorant
Name	N-acetylcystein (NAC)	ambroxol	carbocysteine
Duration	2 weeks	2 weeks	3 weeks
Dose	0.01 – 0.1mg/kg (once/day)	0.3-10 mg/kg (twice/day)	300 mg/kg (twice/day)
Method	i.t.	i.p.	oral

Improvement of FEV 0.1%, MLI

No therapeutic effect

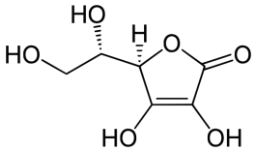
NAC (i.t.) has a beneficial role to improve obstructive lung phenotype in βENaC-Tg mice possibly by its anti-oxidative function

High-dose oral *N*-acetylcysteine, a glutathione prodrug, modulates inflammation in cystic fibrosis (Kanter et al., 2006; Dawson et al., 1989)

High-dose NAC resulted in significantly improved small airways function and decreased exacerbation frequency in patients with stable COPD (Tse et al., 2013)

Anti-oxidant, Vitamin C, and COPD·CF

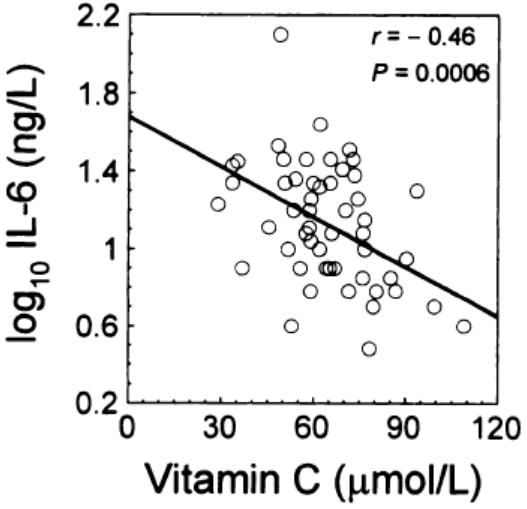
Vitamin C

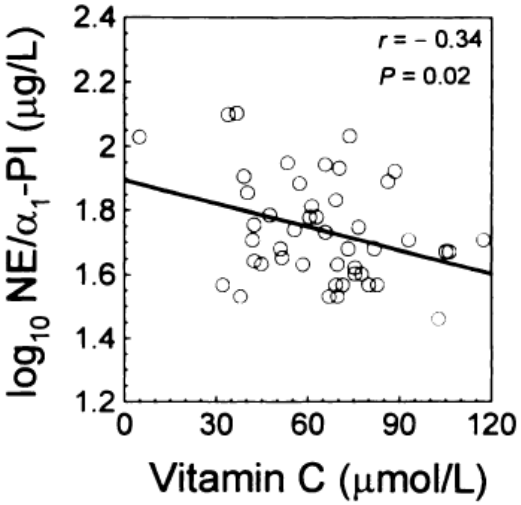


- Anti-oxidant
- **Essential vitamin in human**
  (Human cannot synthesize Vitamin C in the body)

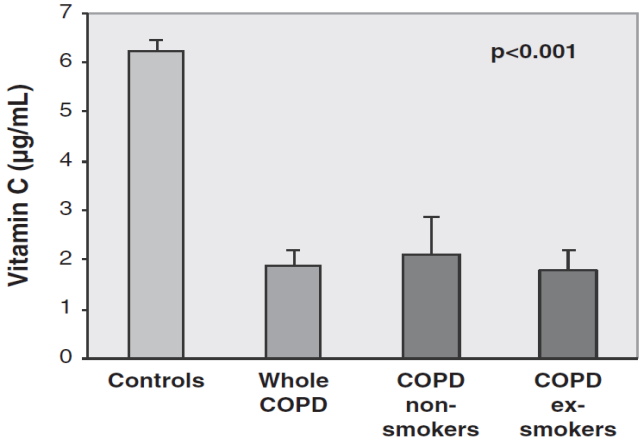
Mice could endogenously produce Vitamin C (Enzyme SMP30)

◎ Vitamin C (VC) is reduced in the plasma in CF and COPD.





Plasma vitamin C concentrations in patients with cystic fibrosis: evidence of associations with lung inflammation (Winklhofer-Roob et al., 1997)



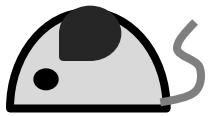
Decreased plasma concentration of VC in COPD patients (Cristóvão et al., 2012)

Does endogenous VC has any impact on the pulmonary phenotypes of the ENaC-Tg mice?

Experimental procedures

Does endogenous Vitamin C contribute to the protection against exacerbation of pulmonary disease in ENaC-Tg mice?

C57BL/6 βENaC-Tg mice



SMP30 knockout (KO) mice

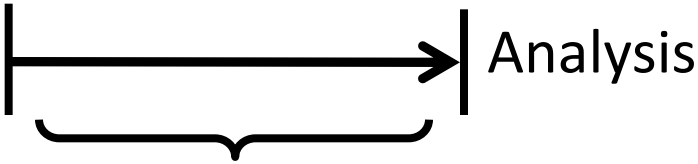
crossing

C57BL/6 βENaC-Tg -SMP30 KO mice

Critical enzyme that can endogenously produce Vitamin C (Gluconolactonase)

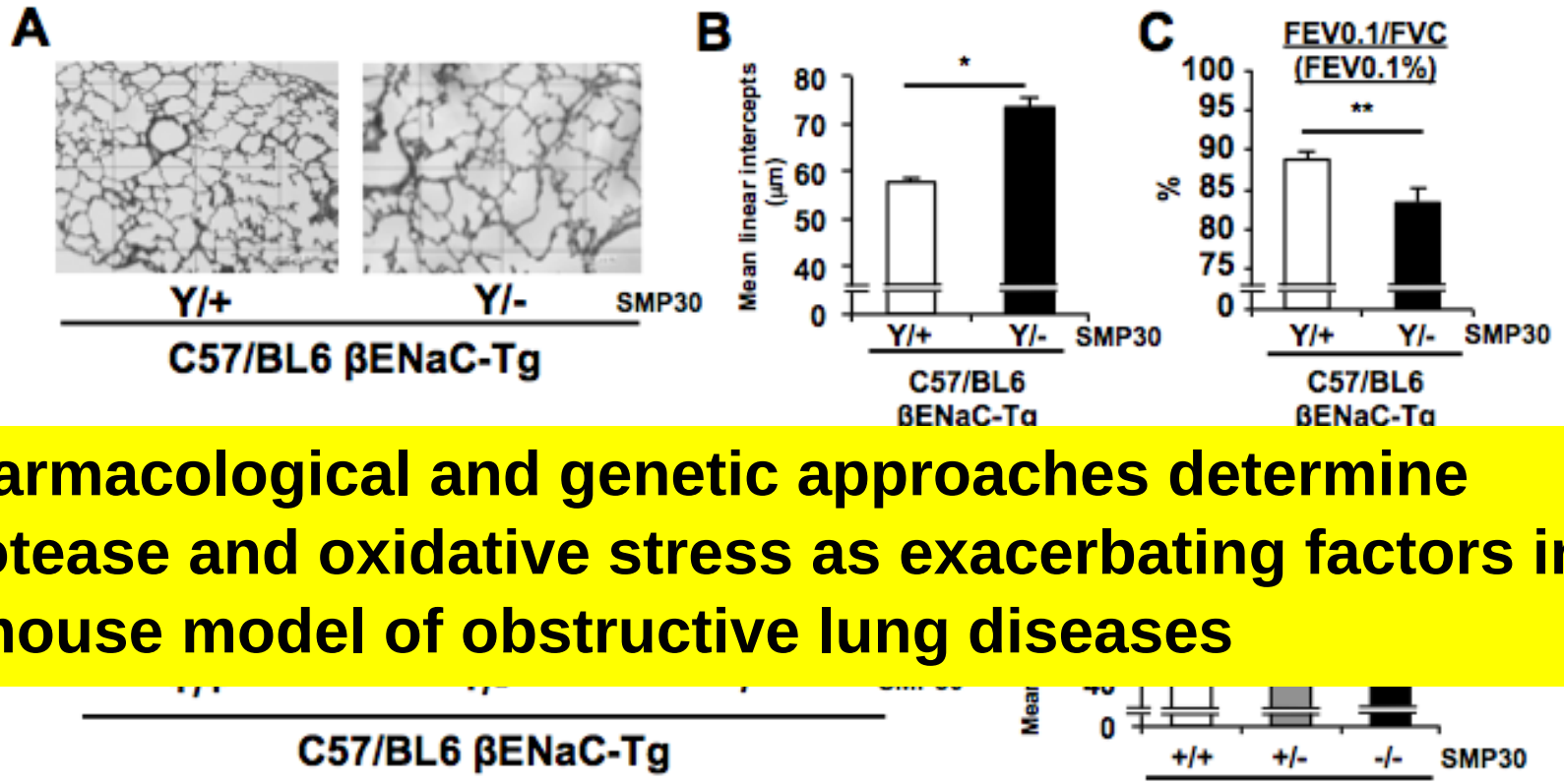
♂ C57BL6 βENaC-Tg mice
- SMP30 Y/+ (WT)
- SMP30 Y/- (KO)

♀ C57BL6 βENaC-Tg mice
- SMP30 +/+ (WT)
- SMP30 +/- (Hetero)
- SMP30 -/- (KO)



Vitamin C deficiency induction

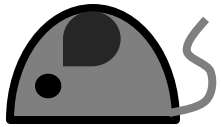
SMP30 deficiency exacerbates pulmonary emphysema and dysfunction in C57/BL6-βENaC-Tg mice



Pharmacological and genetic approaches determine protease and oxidative stress as exacerbating factors in a mouse model of obstructive lung diseases

(Shuto T, *Sci Rep.*, in revision)

Significance



C57BL/6 ENaC-Tg mice

Endogenously produces VC

≠

Human
COPD/CF patients

Similarity

=

C57BL/6-ENaC-Tg-SMP30 KO mice

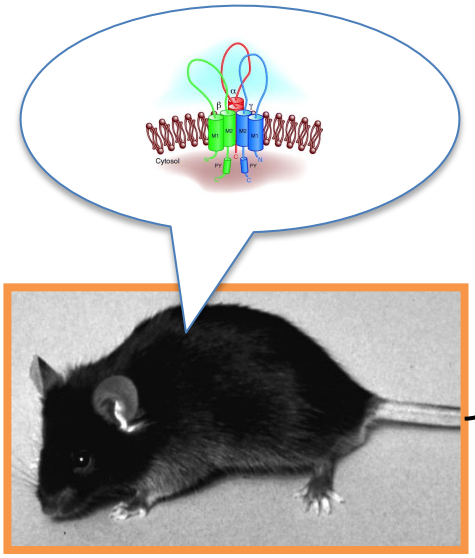
Cannnot produce VC endogenously

Model mouse	Respiratory function (FEV 0.1%)	Emphysema MLI	Others
Tabaco-induced model	90-95 %	65 μm	inflammation
β-ENaC Tg	85-90 %	55 μm	Inflammation, mucus retention
SMP30 KO-β-ENaC Tg	75-85 %	70 μm	Inflammation, mucus retention

ENaC-Tg-SMP30 KO mice is a novel model that may resemble the human obstructive pulmonary diseases.

Summary

Epithelial Sodium channel(ENaC)



C57BL/6 βENaC-Tgmice

(Shuto T, *Sci Rep.*, in revision)

Novel human-like animal model

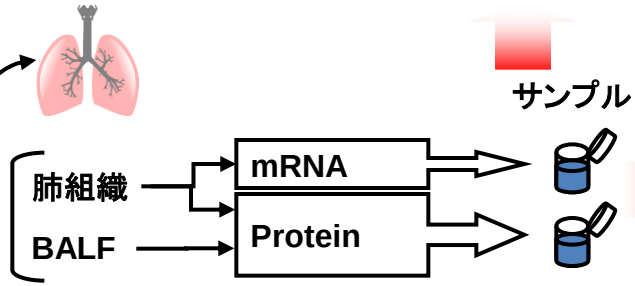
C57BL/6 βENaC-Tgmice (CF/COPD-like mice)

SMP30 knockout (KO) mice

VC deficient foods

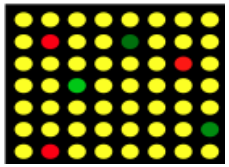
C57BL/6 βENaC-Tg -SMP30 KOmice

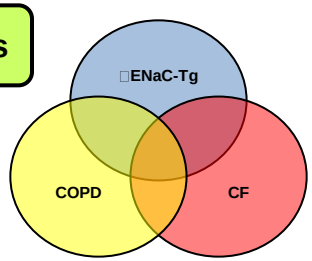
Novel model of CF/COPD that has no endogenous VC



Comparison analysis

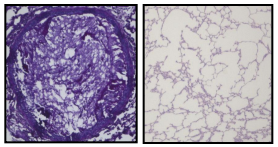
DNA microarray



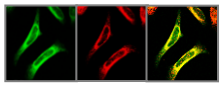


(Ono T., et al., *Ped. Pulmonol.*, S34, 277-278. 2011)

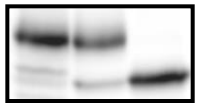
Pathogenesis, molecular analysis



Tissue analysis



Immunofluorescence



Western blotting

Pulmonary function (flexiVent)

(Ono T., et al., *Ped. Pulmonol.*, S34, 277-278. 2011)

CF-like pulmonary diseases

Therapeutic evaluation

C57 BL/6 βENaC-Tg mice

Pulmonary phenotypes

(Matsumoto C., et al., *Ped. Pulmonol.*, S34, 278. 2011)

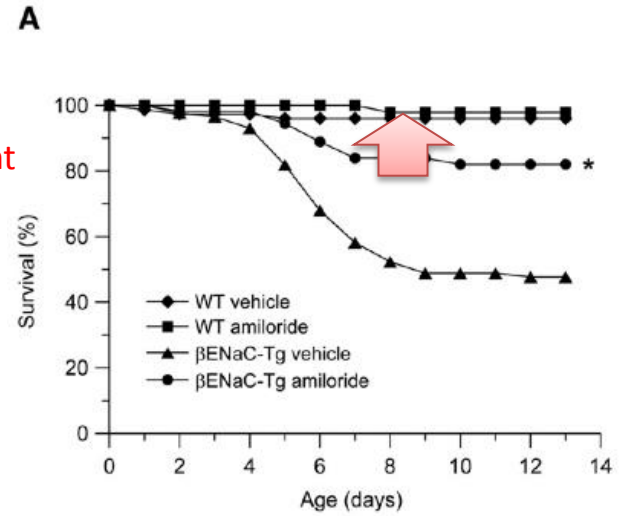
Protease inhibitors（Camostat、ONO3403）
Anti-oxidant, NAC

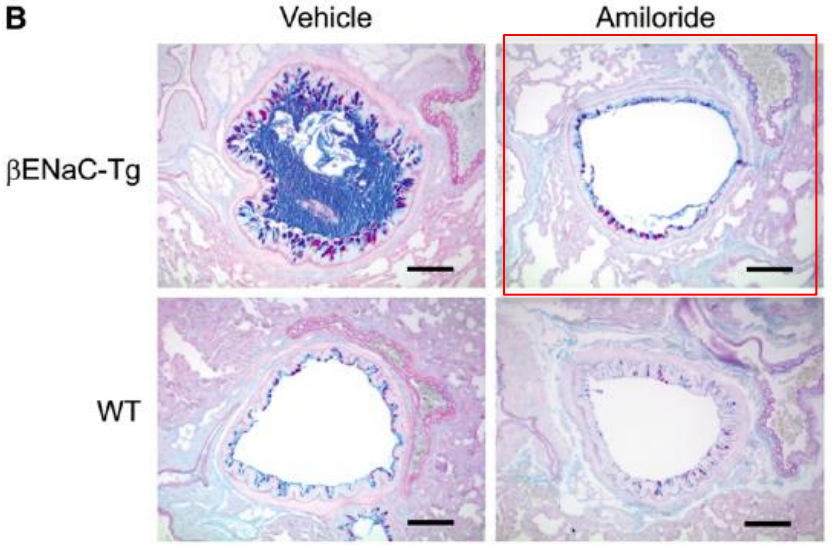
Pulmonary phenotypes of βENaC-Tg mice are therapeutically targeted?

【Amiloride (ENaC inhibitor) treatment (C3B6 βENaC-Tg mice)】

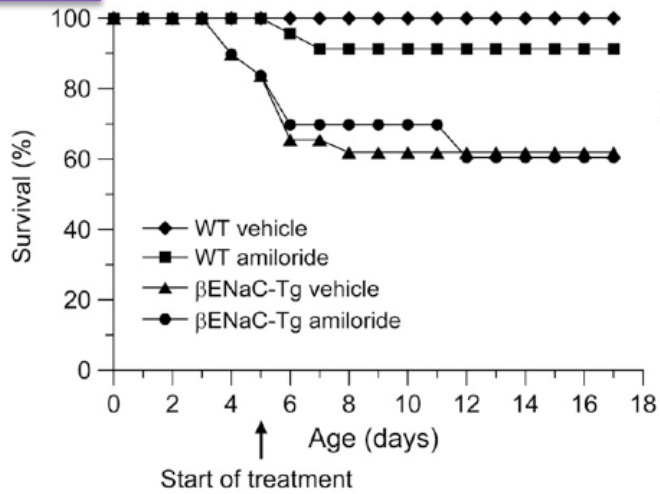
After birth

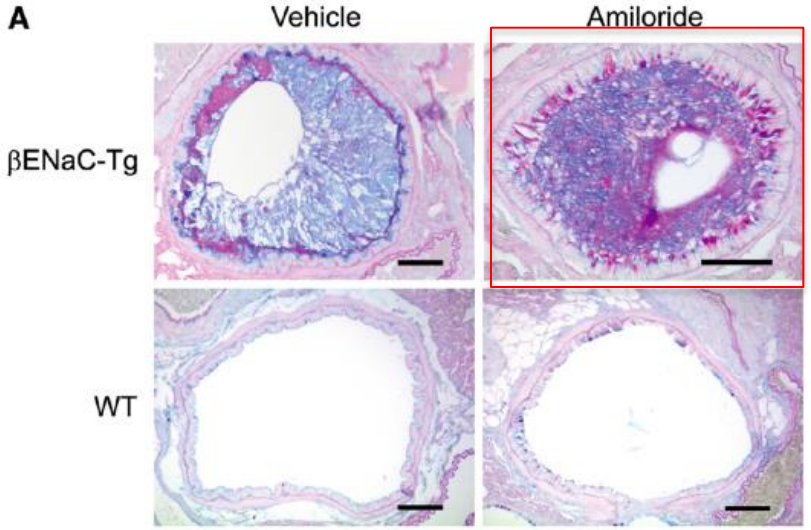
Need to treatment foreverto keep treatment





5 days after birth



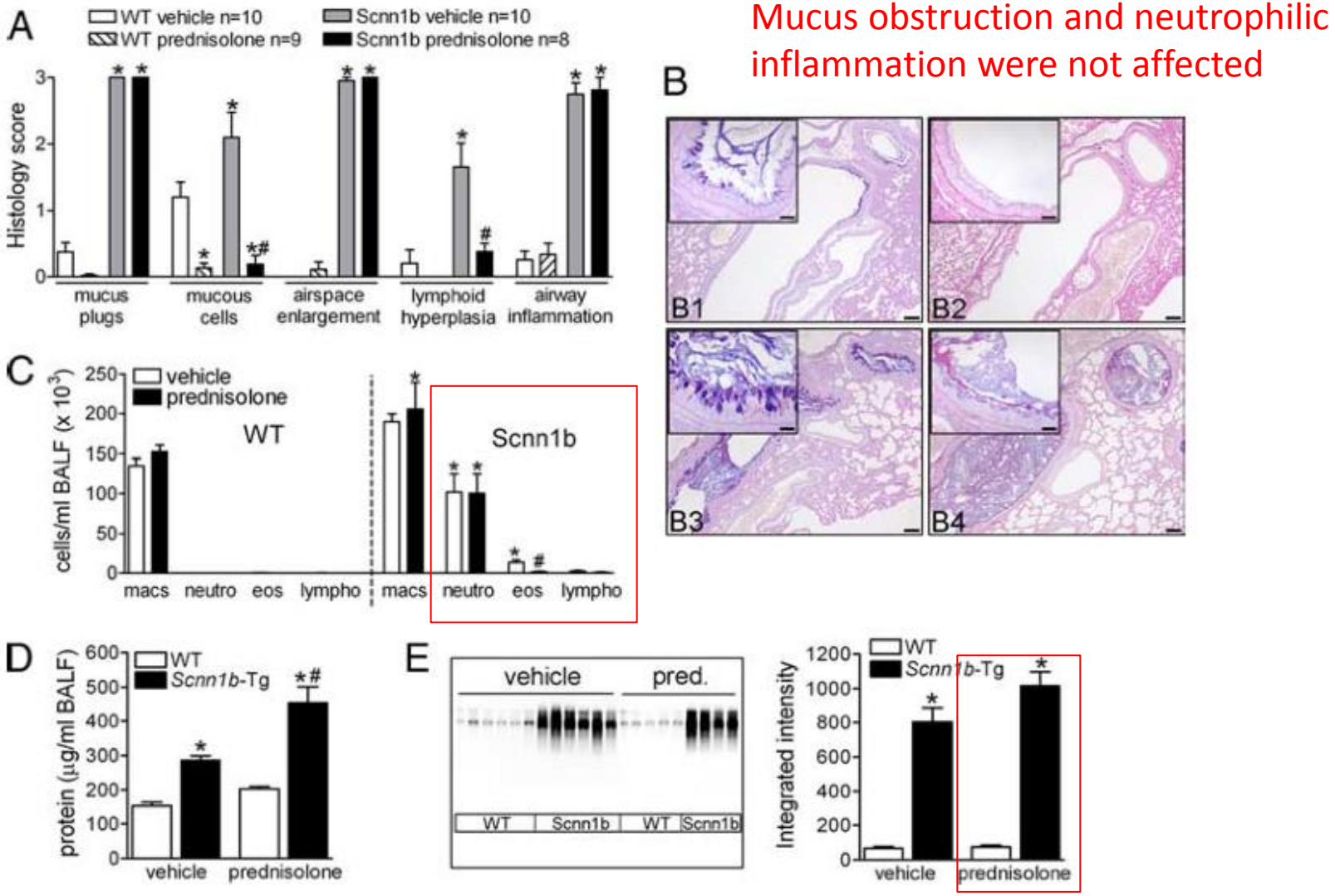


14 days (3 times/day), 10 mmol/L (i.n.)

Zhou Z et al., *Am J Respir Crit Care Med.* **2008**

Pulmonary phenotypes of βENaC-Tg mice are therapeutically targeted?

【Prednisolone (Steroid) treatment (C57BL/6 βENaC-Tg mice)】



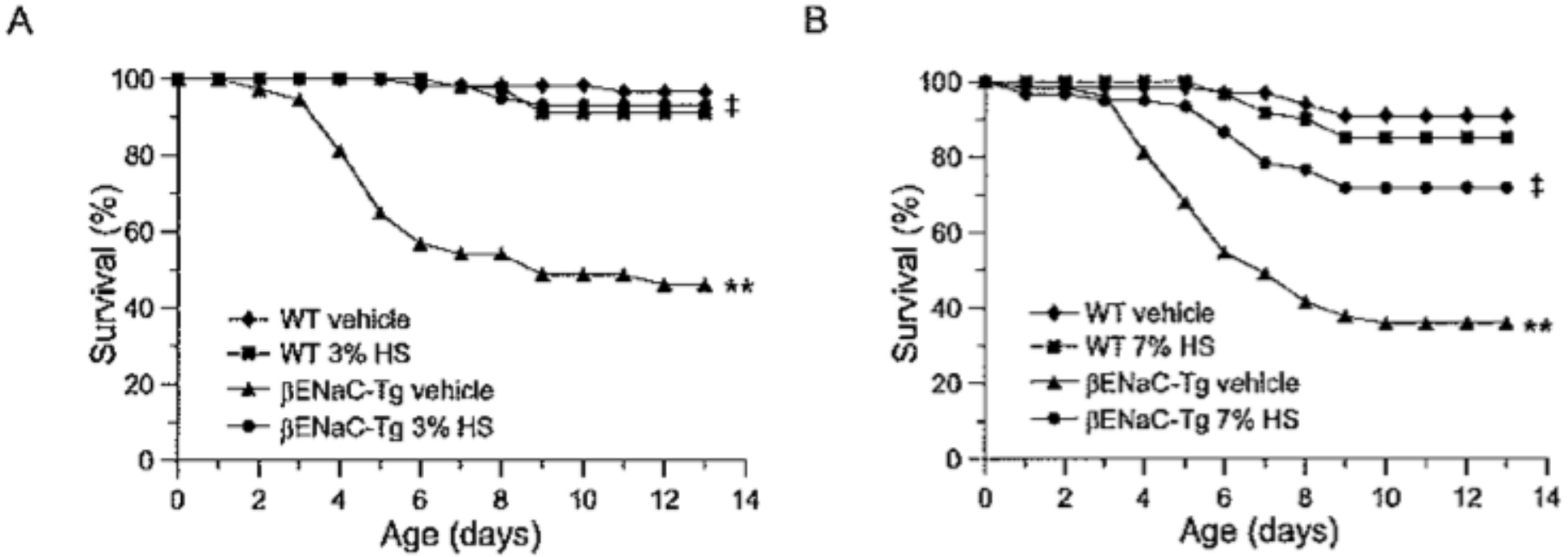
Mucus obstruction and neutrophilic inflammation were not affected

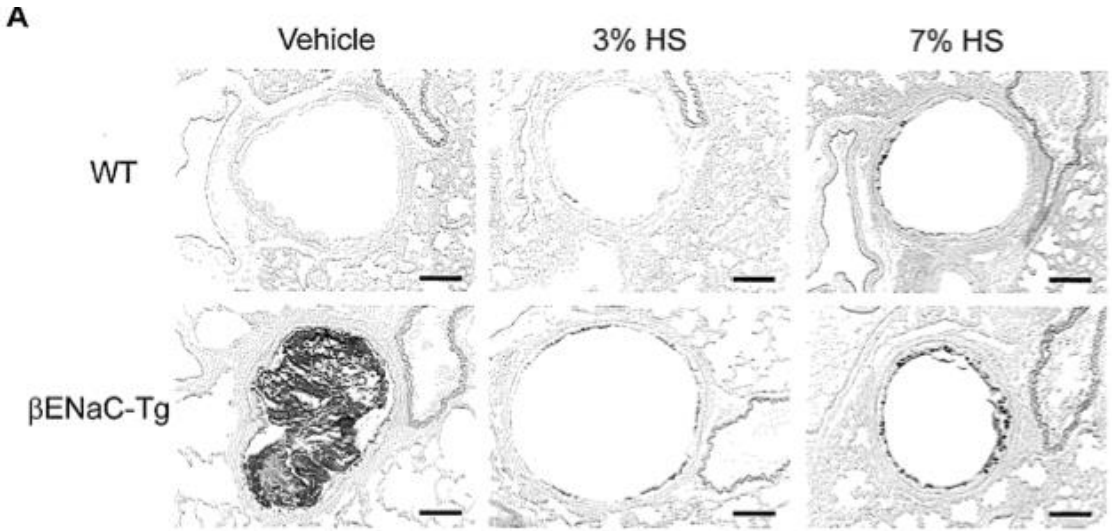
2 weeks, 20 mg/kg/day (i.p.)

Livraghi A et al., *J Immunol.* **2010**

Pulmonary phenotypes of βENaC-Tg mice are therapeutically targeted?

【Hypertonic saline (高張液) treatment (C3B6 βENaC-Tg mice)】





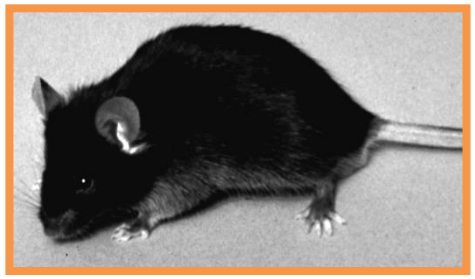
Survival and mucus obstruction were corrected

Inflammation were not affected

14 days (3 times/day),
1 μl/g/day (i.n.)

Graeber SY et al., *AJRCMB.* **2008**

Identification of Pulmo-modulatory factors using C57/BL6-βENaC-Tg mice

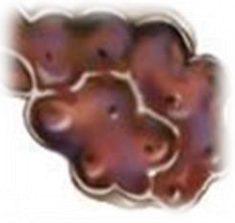


C57BL/6 βENaC-Tg mice
(COPD model mice)

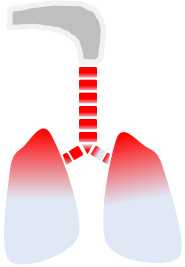
Novel
Pulmo-modulatory factors

Protective？
Negative？

Mucus obstruction



Emphysema



Pulmonary defect

【Goal】
Identification of Novel Pulmo-modulatory factors using C57/BL6-βENaC-Tg mice

Importance of metabolic factors in the pathogenesis of COPD

Title: Plasma uric acid as a protective factor of respiratory dysfunction and emphysema in female mice and human with obstructive pulmonary diseases

Haruka Fujikawa, Kumamoto University, Japan

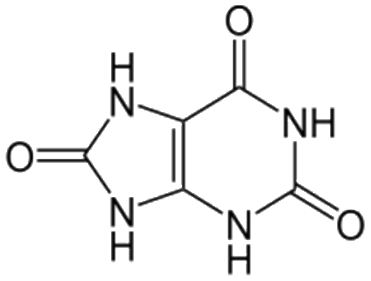
Title: GLP-1 receptor agonist exacerbates mucus hyper secretory phenotype in βENaC-transgenic mouse with obstructive lung diseases

Hirofumi Nohara, Kumamoto University, Japan

Joshi, et al., Indian Journal of Endocrinology and Metabolism,18, 2014, 608-616

Uric Acid (UA)

Uric acid



- A product of purine metabolism
- One of the strongest antioxidant
- Present in the epithelial lining fluid (EFL) to protect the exposed surface of the lung from the environment

A previous report about the relation between COPD and UA

On this cohort study

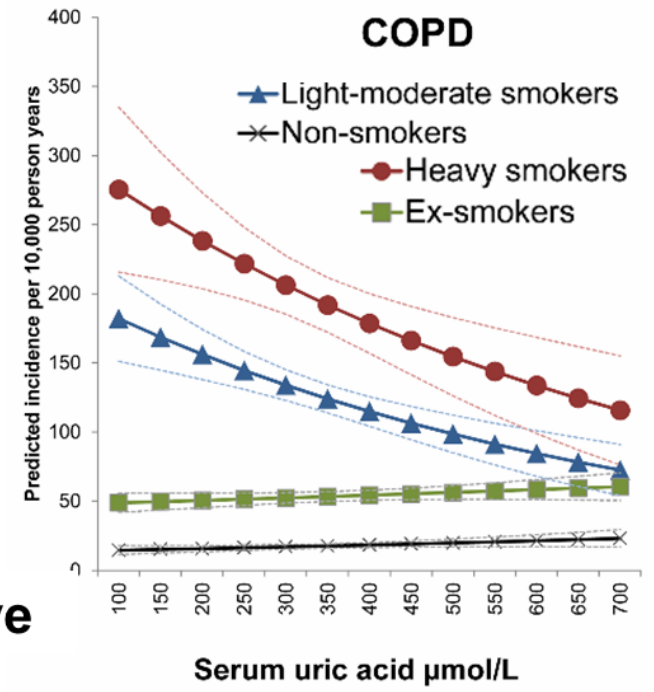
UA → The risk of COPD

(Horsfall LJ, *et al.*, ***Thorax*** 2014)

UA might suppress the pathogenesis of COPD

But

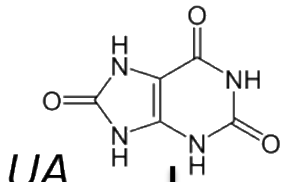
the experimental evidence remains inconclusive



Purpose

To clarify how serum UA levels affect the pulmonary phenotypes of COPD

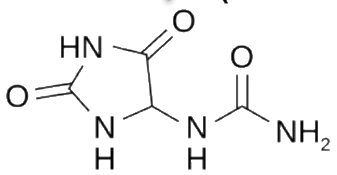
- The difference of purine metabolism between human and mouse



UA

Absent in human

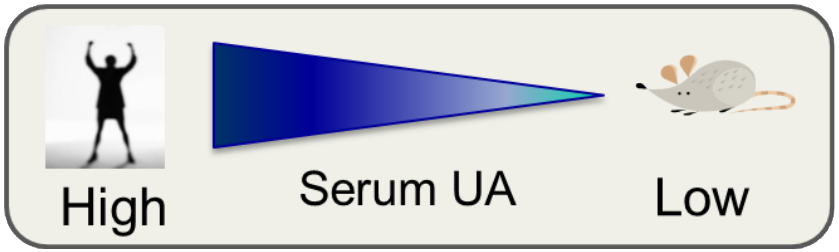
Uricase (Urate Oxidase)



Allantoin

This means …

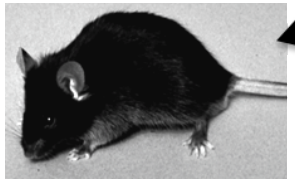
UA level in mice is not similar to that in human



In this study, we treated with uricase inhibitor, Oxonate, the ENaC-Tg mice to increase blood concentration of UA

Methods

βENaC-Tg mice



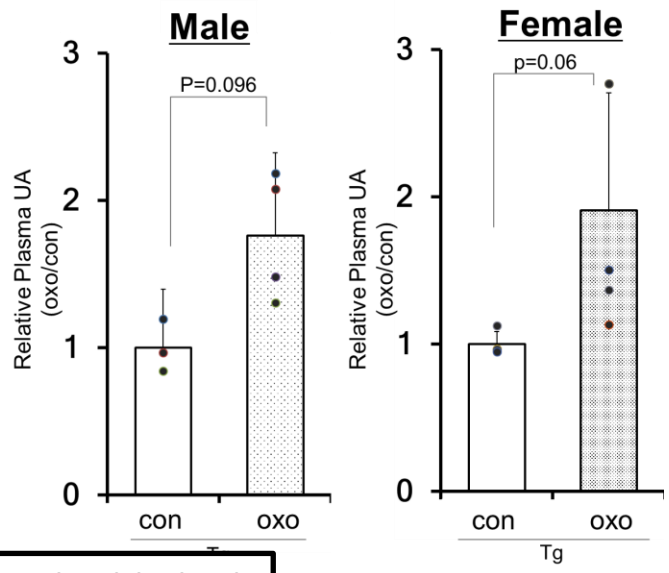
Oxonate Potassium (500 mg/kg/day, *p.o.*, 4-5 weeks)

→ Serum UA ↑ → Pulmonary phenotype

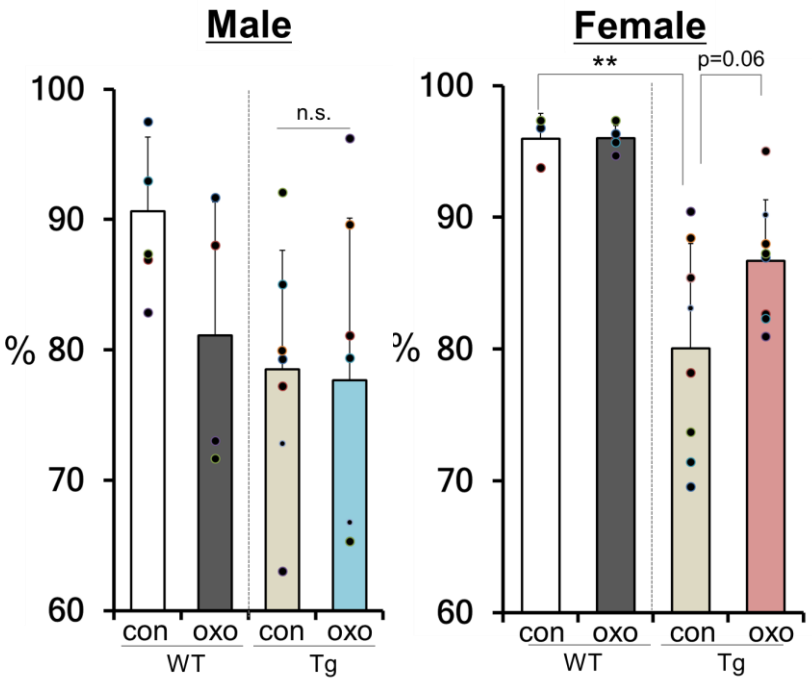
UA level and pulmonary function in mice and human

Mice treated with oxonate

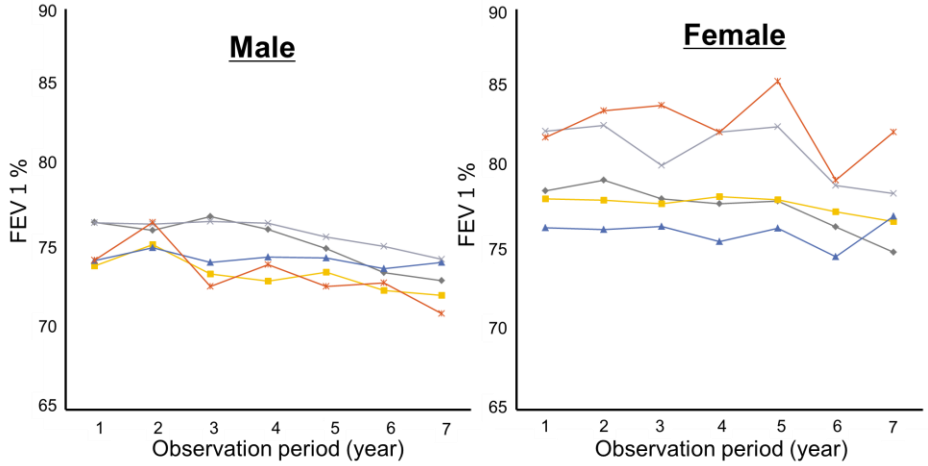
Plasma UA level

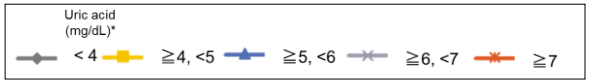


Forced expiratory volume in 0.1 second (FEV0.1%)



Human in health check





✓ **Multiple regression analysis regarding the value of FEV1%**

	Male (n = 196)				Female (n = 131)			
Uric acid level	n	B	SE	*P*	n	B	SE	*P*
< 4 (mg/dL)*	17	0	-	-	32	0	-	-
≥ 4, <5 (mg/dL)*	37	-1.80	1.71	0.30	57	0.15	1.18	0.90
≥ 5, <6 (mg/dL)*	64	-0.60	1.73	0.73	34	-0.39	1.34	0.77
≥ 6, <7 (mg/dL)*	60	0.57	1.68	0.74	**5**	**4.31**	**1.78**	**0.02**
≥ 7 (mg/dL)*	18	-2.06	2.29	0.37	**3**	**9.33**	**2.42**	**< 0.01**

B: partial regression coefficient adjusted by age, BMI and ever-smoking status ; SE: Standard error.
*Mean value of uric acid throughout the observation period.

Conclusions

Plasma UA is a protective factor of respiratory dysfunction and emphysema in female mice and human with obstructive pulmonary diseases

- The effect of the level of UA on respiratory function (FEV1%) in human

  Female : High level of UA (6-7mg/dl) improves respiratory function

  Male : No relation between UA level and respiratory function

- The effect of the level of UA on respiratory function and emphysema of βENaC-Tg mice

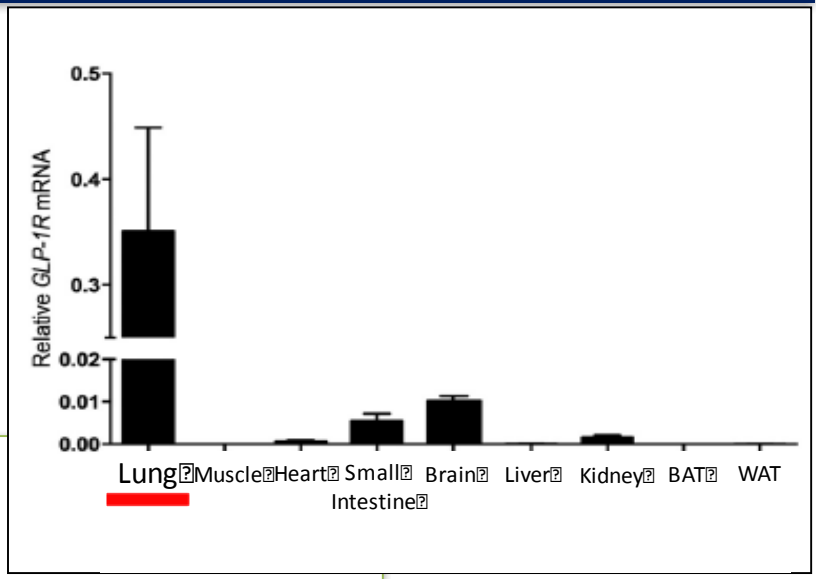
	ENaC-Tg ♂	ENaC-Tg ♀
The level of UA	↗ (p=0.096)	↗ (p=0.06)
Respiratory function (FEV0.1%)	→	↗ (p=0.06)
Emphysema (MLI)	→	↘ (p=0.08)

Glucagon-like peptide-1 (GLP-1)

GLP-1 receptor agonist

Exendin-4

Liraglutide



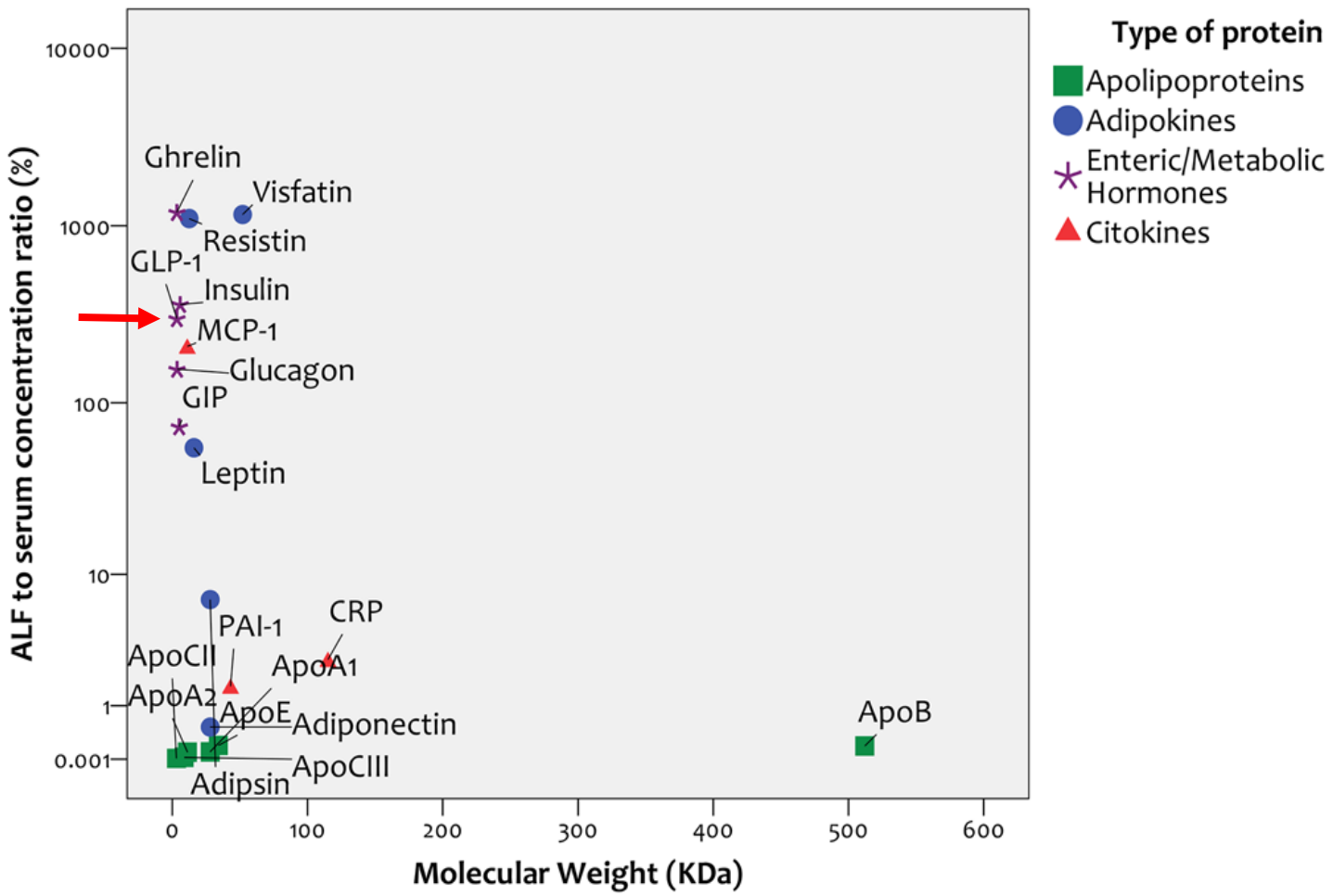
- **Drugs for the treatment of type 2 diabetes**
- **Pleiotropic effects on multiple organs**
  - Neuroprotective effect *(Lciar et al., JCI 2013)*
  - Intrahepatic glucogenesis repression *(Miller RA et al., Nature 2013)*
  - Myocardial protective effect *(Ban K et al., Endocrinology 2010)*
  - Renoprotective effect *(Fujita et al., Kidney-international 2013)*

→ **But, little is known about the physiological and pathophysiologiocal roles of GLP-1 in lung**

Purpose

To identify the role of GLP-1 signals in the lung, we sought to **determine the effect of GLP-1 agonist on the pulmonary phenotypes of COPD.**

GLP-1 expression in BALF



Correlation between protein molecular weight and alveolar lining fluid / serum ratio.（Carlos O. Mendivil, et al., PLoS ONE, 2015より引用）

COPD models

C57BL/6-βENaC-Tg mice

Airway-specific βENaC (epithelial Na+ channel β subunit)-transgenic mice

(Modified mice from Mall et al., Nat Med 2004)

- Concentration of fucose in BALF is increased
- Mucous-related genes expression is up-regulated
- Alveolar mean linear intercept (MLI) is exacerbated (emphysema)
- FEV0.1% is decreased

(FEV0.1/FVC; forced expiratory volume in 0.1 second/forced vital capacity)

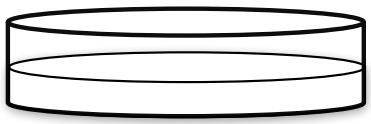
βENaC is increased in bronchial epithelium cells from COPD patients

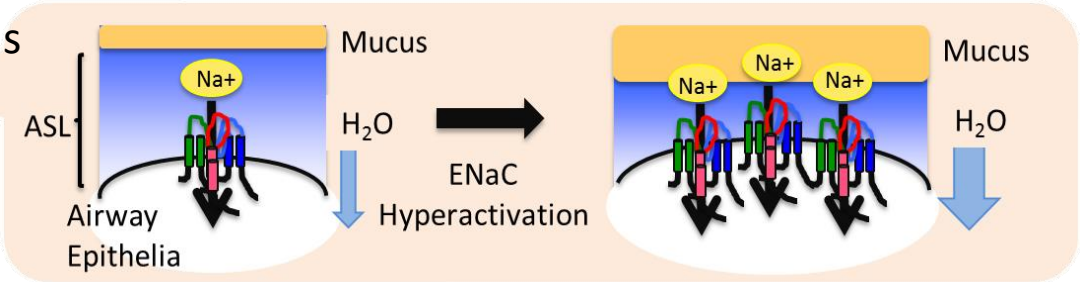
(BKM Chan, et al., 2008)

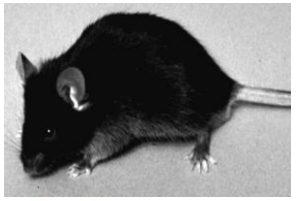
Human airway epithelial cells

β/γENaC-overexpressing models

(β/γENaC-16HBE14o-)







GLP-1 receptor agonist Exendin-4 exacerbates emphysema in βENaC-Tg mice

Schedule

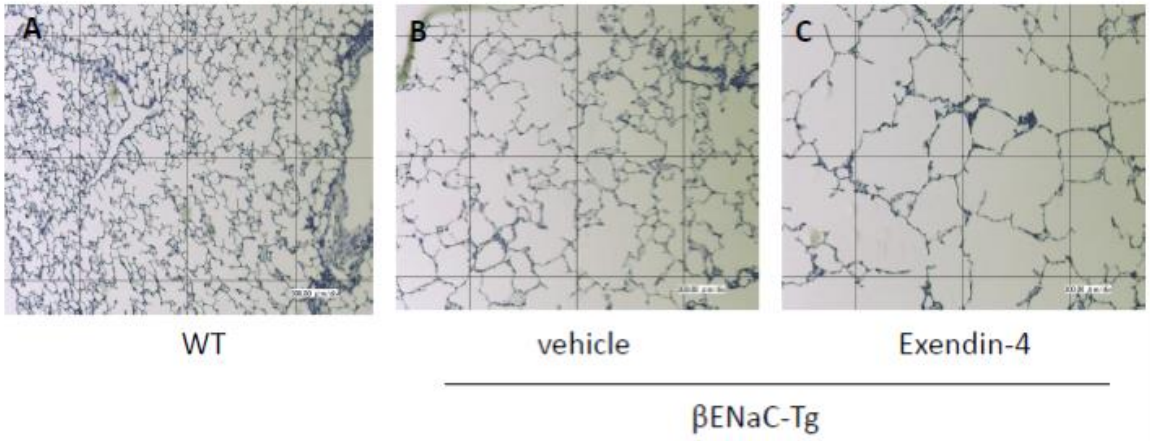
Birth — 12-14 wks old — Analysis

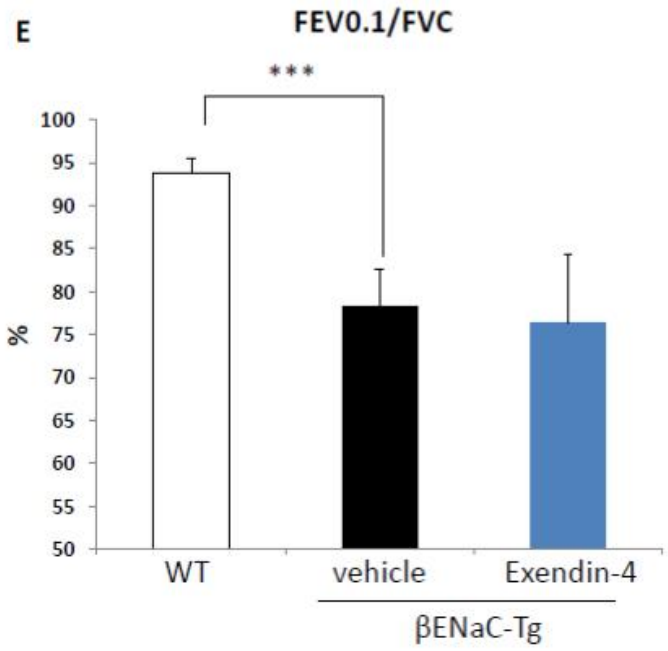
Pathology onset by ENaC activation

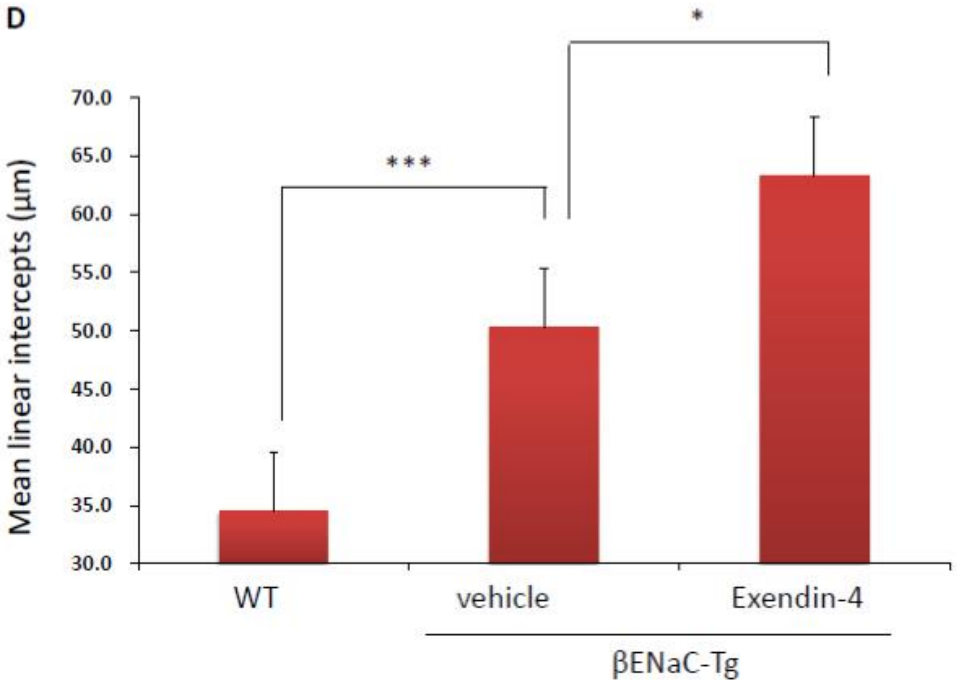
Exendin-4 treatment (2 wks)

Method : intratracheal treatment

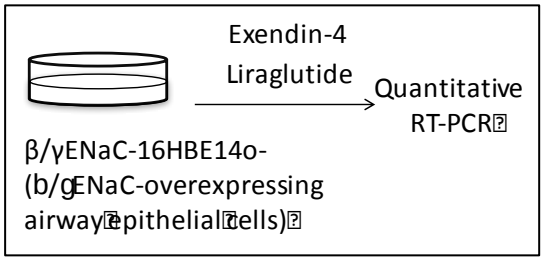
Dose : 10 pmol/day



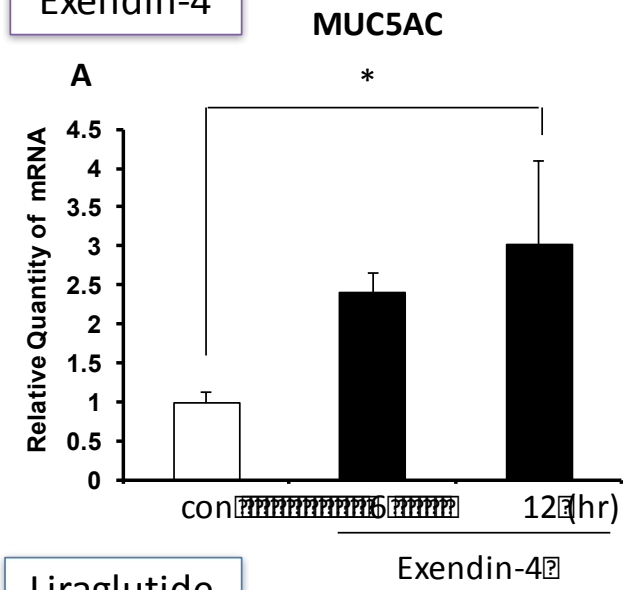


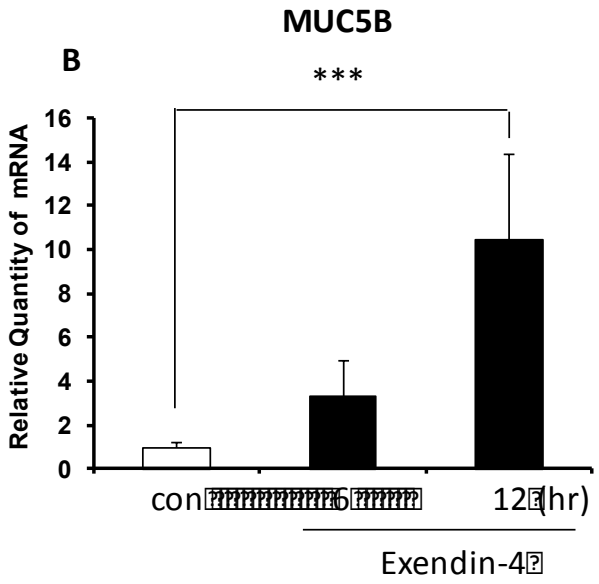


GLP-1 receptor agonist (Exendin-4, Liraglutide) up-regulates mucus production in COPD model cells

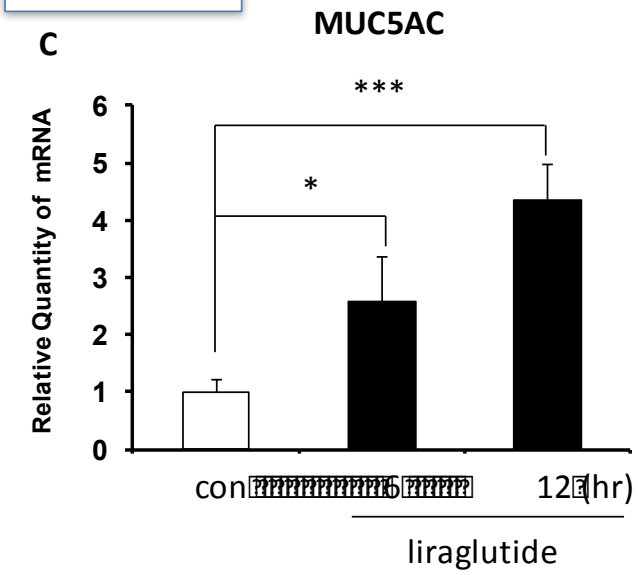


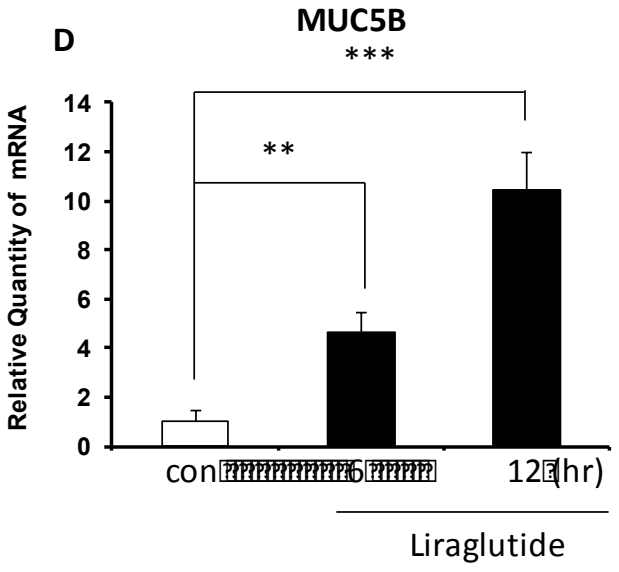
Exendin-4

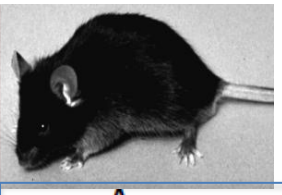




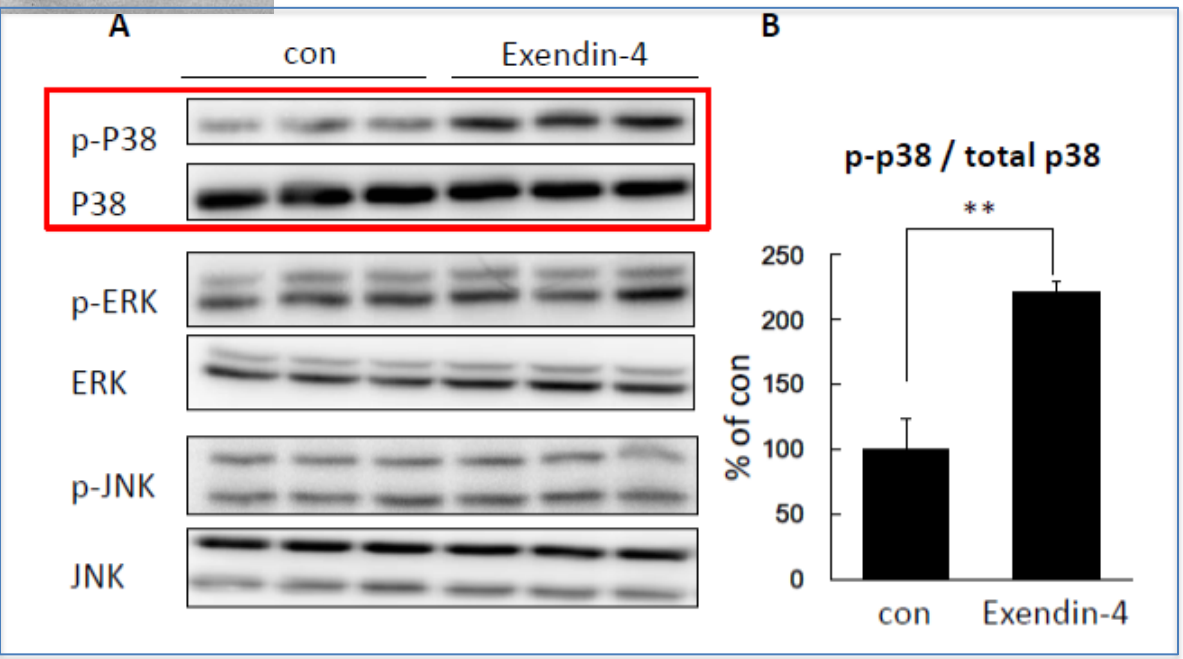
Liraglutide

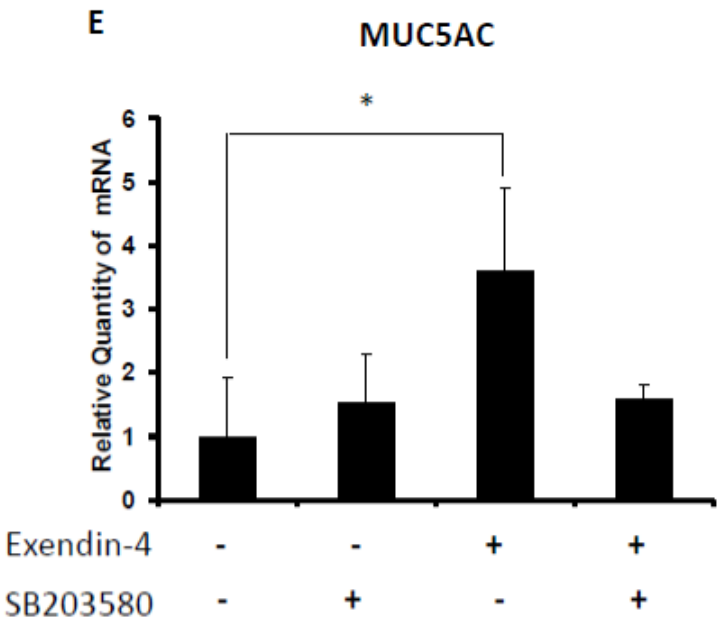


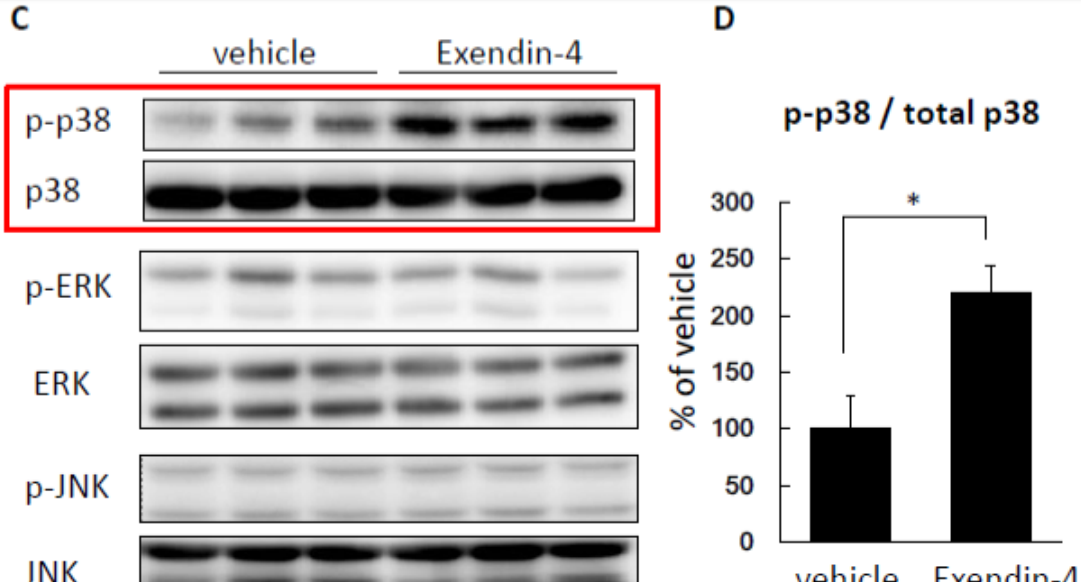


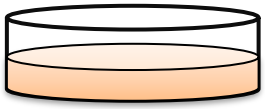


Exendin-4 up-regulates mucus production trough p38 signaling activation



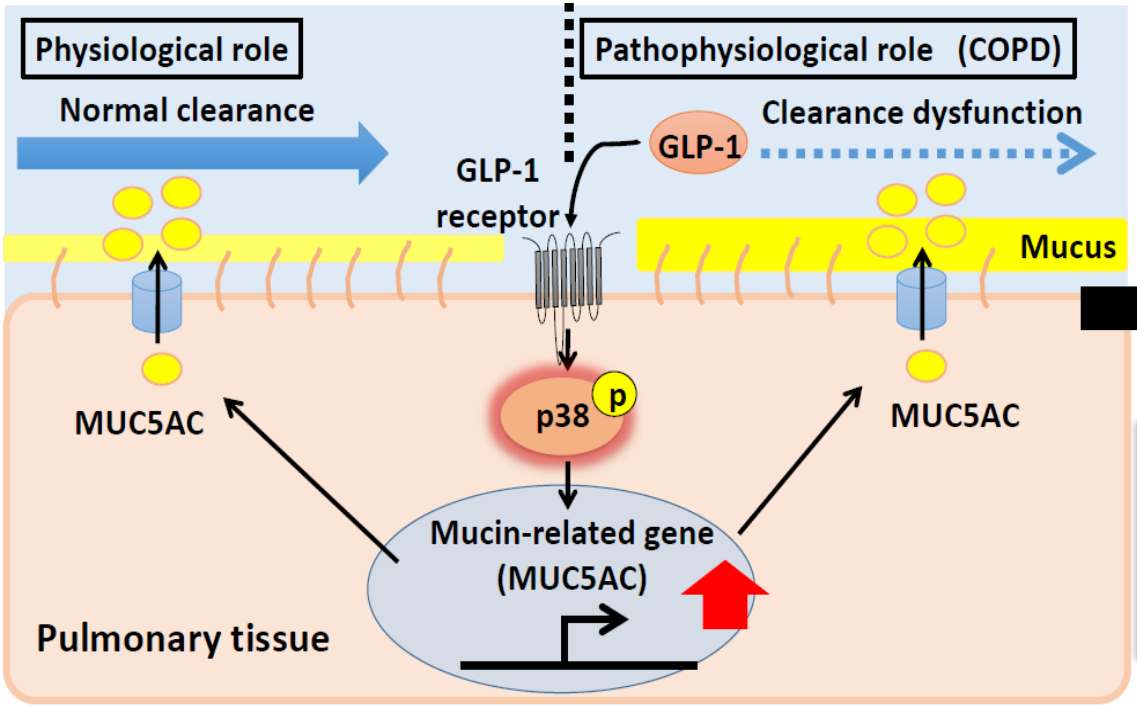






β/γENaC-16HBE14o- (β/γENaC-overexpressing airway epithelial cells)

Conclusion



COPD exacerbation

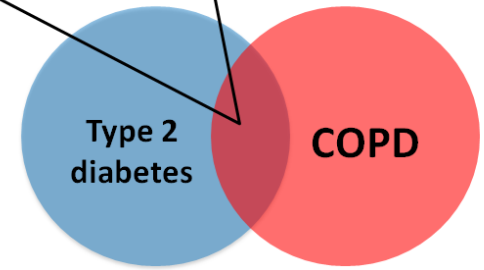
- Mucus accumulation
- Emphysema

Pulmonary GLP-1 signal exacerbates the phenotypes of COPD mice at least partly *via* p38 MAPK-dependent mucin induction

Significance

Recent studies suggest that COPD is associated type 2 diabetes…

- More than 10% of type 2 diabetes patients have COPD.
- 15% smokers have type 2 diabetes.



COPD patients may have chance to use GLP-1 receptor agonist during the treatment.

Our data may caution against the clinical use of inhaled GLP-1 receptor agonist in COPD patients.

Acknowledgement

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Kasumi Maruta (lncRNAs-CF)

Ryunosuke Nakashima (GLP-1 agonist)

Kumamoto University

科研費 KAKENHI

熊本大学薬学部／大学院薬学教育部
School of Pharmacy / Graduate school of Pharmaceutical Sciences

Program for Leading Graduate Schools
HIGO program
Health life science: Interdisciplinary and Glocal Oriented

Aging Regulation, Tokyo Metropolitan Institute of Gerontology
(SMP30 KO mice)

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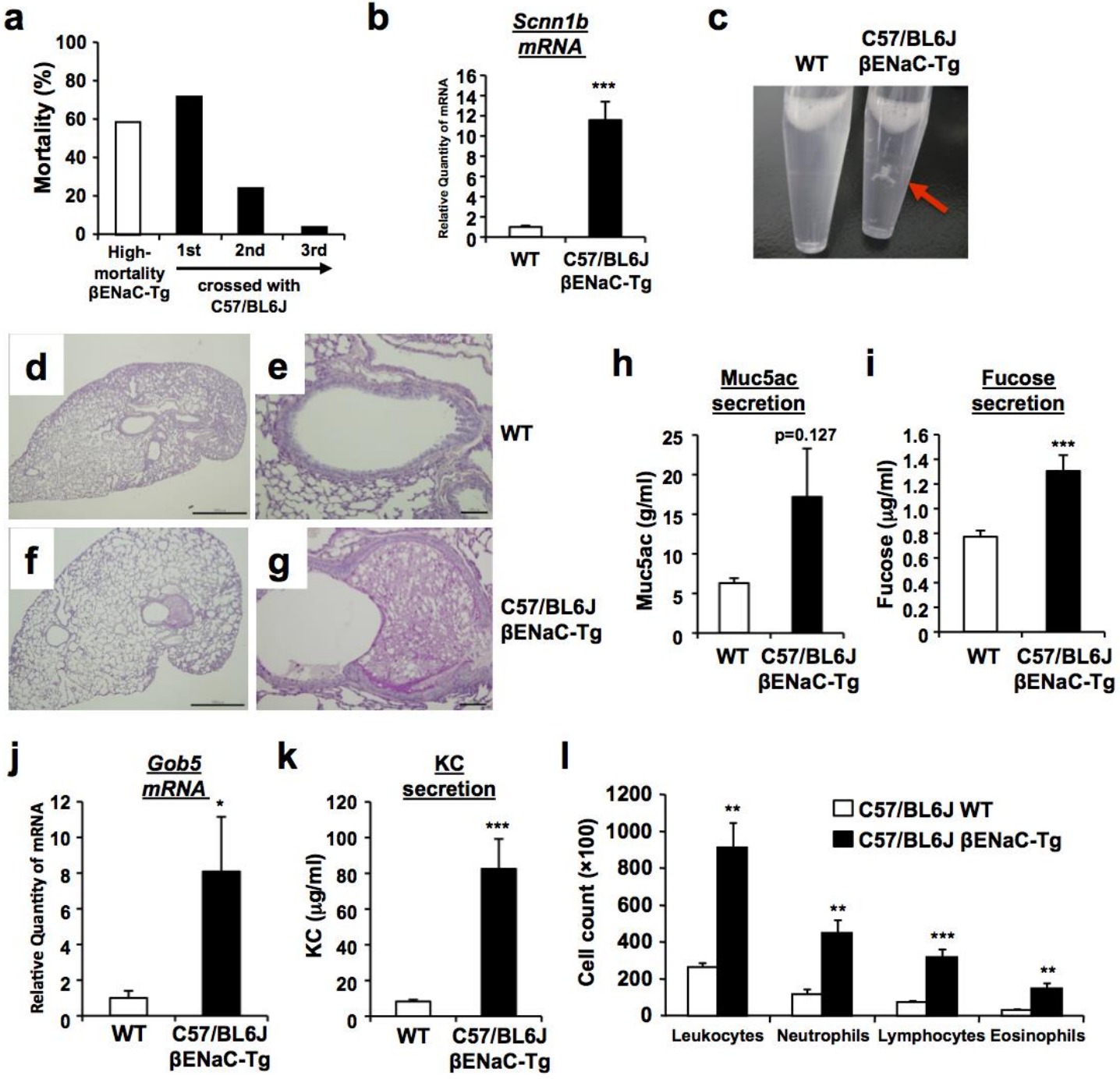
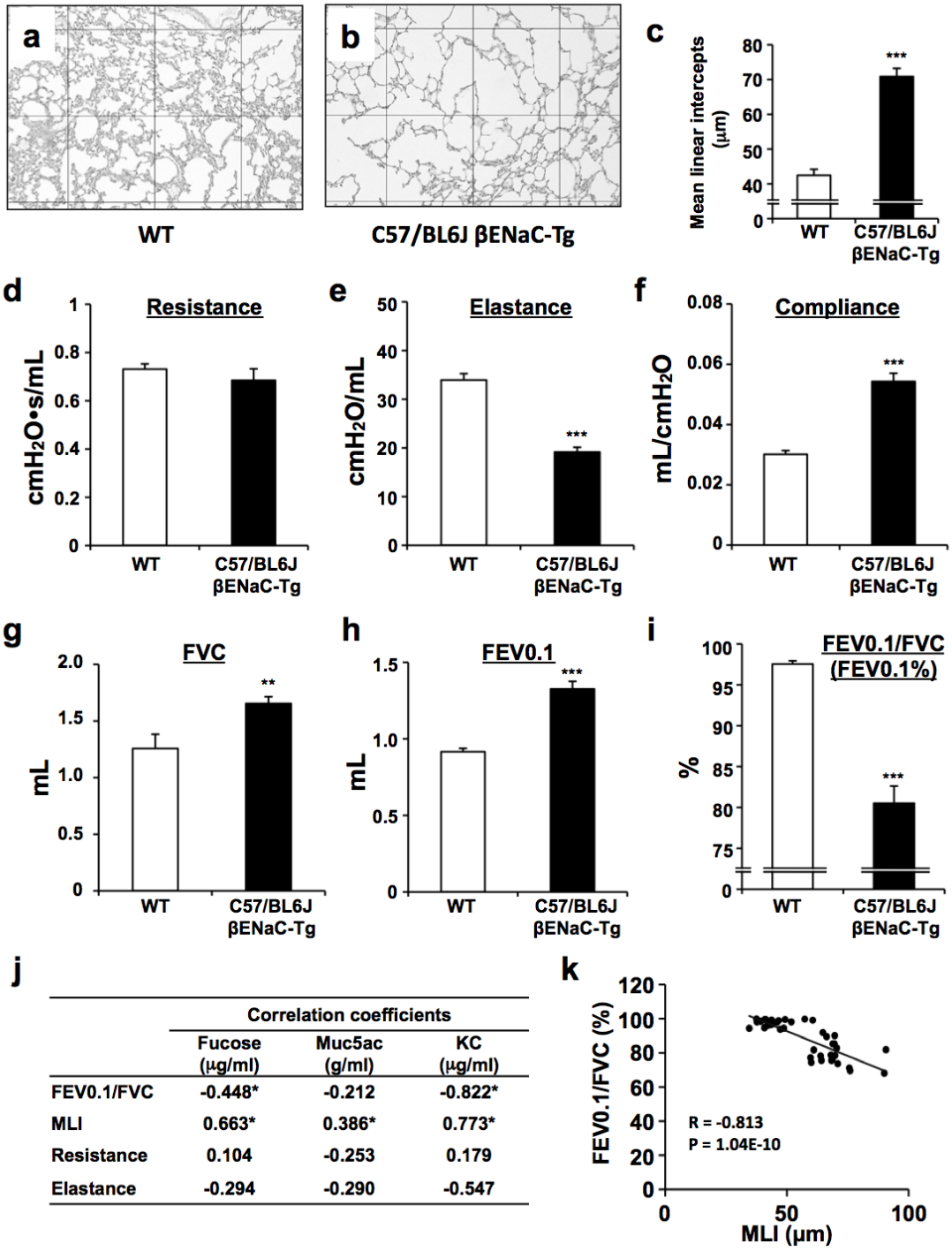
Molecular Regulation of Aging


a
Mortality (%)
100
80
60
40
20
0
High-mortality βENaC-Tg
1st
2nd
3rd
crossed with C57/BL6J
b
Scnn1b mRNA
Relative Quantity of mRNA
16
14
12
10
8
6
4
2
0
***
WT
C57/BL6J βENaC-Tg
c
WT
C57/BL6J βENaC-Tg
d
e
f
g
WT
C57/BL6J βENaC-Tg
h
Muc5ac secretion
Muc5ac (g/ml)
25
20
15
10
5
0
p=0.127
WT
C57/BL6J βENaC-Tg
i
Fucose secretion
Fucose (μg/ml)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
***
WT
C57/BL6J βENaC-Tg
j
Gob5 mRNA
Relative Quantity of mRNA
12
10
8
6
4
2
0
*
WT
C57/BL6J βENaC-Tg
k
KC secretion
KC (μg/ml)
120
100
80
60
40
20
0
***
WT
C57/BL6J βENaC-Tg
l
Cell count (×100)
1200
1000
800
600
400
200
0
C57/BL6J WT
C57/BL6J βENaC-Tg
**
**
***
**
Leukocytes
Neutrophils
Lymphocytes
Eosinophils



j

	Correlation coefficients		
	Fucose (μg/ml)	Muc5ac (g/ml)	KC (μg/ml)
FEV0.1/FVC	-0.448*	-0.212	-0.822*
MLI	0.663*	0.386*	0.773*
Resistance	0.104	-0.253	0.179
Elastance	-0.294	-0.290	-0.547

Lessons from Previous reports-薬物療法

薬物	マウス系統	投与条件	結果
アミロライド	C3B6	10mmol/L, 1μg/g (鼻腔投与) 14days (1日3回) Newborn 5 days old 4 weeks old	出生日から投与 ・生存率改善 ・粘液貯留症状改善 (染色, RNA) ・炎症細胞浸潤改善 ただし、持続的な投与が必要 !! 生後 5 日から投与 いずれの病態も改善効果無し
プレドニゾロン	C57BL/6	20mg/kg/day(i.p.) 2weeks 5-6 weeks old	リンパ球の浸潤や粘液産生細胞数は減少させるが, 粘液の蓄積や好中球数には影響無し
Hypertonic saline (高張液)	C3B6	1μl/g/day (鼻腔投与) 14days (1日3回) Newborn	Survivalを改善 粘液貯留を改善 炎症には影響なし ただし、病態発症後の投与では、3%HSは無効

・ステロイドによっては、粘液クリアランスは改善しない
・ENaCや水の動きをコントロールする薬剤により効果が顕著

Lessons from Previous reports-遺伝子改変

掛け合せ	結果
C57BL6-βENaC × hCFTR-Tg	病態改善効果なし
C57BL6-βENaC × TNFα KO	病態改善効果なし
C3:B6-βENaC × IL-4R KO	10日齢：生存率改善，粘液細胞，炎症細胞減少 5週齢：粘液細胞，炎症細胞数の差は消失
C57BL6-βENaC × MyD88 KO	生存率低下，バクテリア感染増加 粘液への影響無し
C57BL6-βENaC × CFTR KO	死亡率，粘液量上昇

・CFTRを補っても、ENaCによる粘液クリアランス障害は解消しない
・部分的な炎症関連因子を欠損させても、粘液クリアランス障害は解消しない
・CFTRの欠損で，さらに病態は悪化

本分野が見出したC57BL/6 βENaC-Tg-マウスやCFに関する知見

薬物	投与条件	結果
メシル酸カモスタット ONO-3403 （プロテアーゼ阻害）	20 or 100 mg/kg（強制経口投与） 2-3 weeks(1日2回) 14 weeks old	呼吸機能，肺気腫病態，粘液関連遺伝子の発現に対する影響 メシル酸カモスタット ・改善傾向 ONO-3403 ・有意な改善
アンブロキソール カルボシステイン （去痰）	0.3-10 mg/kg (i.p.) 300 mg/kg（強制経口投与） 2-3 weeks(1日2回) 14 weeks old	呼吸機能，肺気腫病態に対する影響 ・どちらも無効
N-アセチルシステイン (NAC) （抗酸化）	0.01 – 0.1mg/kg (経気管投与) 2 weeks(1日2回) 14 weeks old	呼吸機能，肺気腫病態に対する影響 ・有意な改善
Exendin-4 (GLP1受容体作動薬）	0.1 pmol/day(経気管投与) 2 weeks (1日1回) 14 weeks old	粘液産生・肺気腫病態に対する影響 ・粘液産生を亢進 ・肺気腫病態を悪化 ＊ただし、正常マウスにおいても粘液亢進をするが、肺病態を引き起こさない
オキソン酸 （ウリカーゼ阻害剤） →血清尿酸値を上昇させる	500 mg/day（強制経口投与） 4-5 weeks (1日1回) 7 weeks old	尿酸値に対する影響 ・上昇傾向 呼吸機能，肺気腫病態に対する影響 ・メスにおいてのみ改善傾向

Up- and down-regulated genes in airway-specific βENaC-Tg mice

125 (up : βENaC (Cy3) ≥ 20 , down : WT (Cy5) ≥20) 34

Name	ENaC-Tg/WT
Il8ra	23.6
Gp2	15.8
Cxcl5	13.1
Ear11	12.9
Ccl20	11.9
Saa3	11.2
Slc39a2	10
Cxcl1	9.3
Gpnmb	6.9
Cd200r2	6.9
EDRF	6.6
Marco	6.4
Chi3l4	6.2
Ctsk	6.2
Ly6i	5.9
Fst	5.5
Fgg	5
Hspa1a	4.9
Chi3l3	4.9
Cxcl2	4.8
Slc26a4	4.7
EG546038	4.9
Col8a1	4.2
Ccl3	4.1
Trem2	3.9
Chit1	3.8
Ch25h	3.8
Oas2	3.7
Cd200r4	3.6

Name	ENaC-Tg/WT
Lilrb4	3.3
Kynu	3.2
Mmp12	3.2
Cdc2l6	3.2
Lrp12	3.2
Clec5a	3.1
B3gnt7	3.1
Cxcl12	3.1
Csf2	3
Retnla	3
Acp5	3
Gdf15	3
Mmp8	3
Cd200r1	2.9
Lcn2	2.9
Igf1	2.9
Hsp110	2.9
Itih4	2.9
Chordc1	2.9
Itgax	2.8
Npy1r	2.8
Mark1	2.8
Il33	2.8
Ccl12	2.8
Tdo2	2.8
Fabp5	2.7
Ms4a7	2.6
Cd68	2.6
Mfsd2	2.5

Name	ENaC-Tg/WT
Mest	2.5
Tcfec	2.5
Stip1	2.5
P4ha2	2.4
Clec4d	2.4
Banp	2.4
Adamts15	2.4
Lhfpl2	2.4
Gpr137b	2.4
Reg3g	2.4
Syngr1	2.3
Ctsd	2.3
Fcgr3	2.3
Fcgr3a	2.3
Speer4d	2.3
Lrg1	2.3
Ankrd37	2
Noxo1	2.3
Ltf	2.3
Gap43	2.2
Fos	2.2
Armet	2.2
Dnaja1	2.2
Hmox1	2.2
Pparg	2.2
Dnajb9	2.2
Ly75	2.2

Name	ENaC-Tg/WT
Egr1	2.2
Hspa4l	2.2
Adfp	2.2
Tbxas1	2.2
BC057170	2.2
Mal	2.2
Cth	2.2
F7	2.1
Ms4a8a	2.1
Fabp4	2.1
Serpina3g	2.1
C3ar1	2.1
Slpi	2.1
Sec14l2	2.1
Pira3	2.1
P2ry13	2.1
S100a8	2.1
Cfb	2.1
Nr1d1	2.1
Jag2	2.1
Hspb1	2.1
Ms4a6d	2.1
Ccl2	2.1
Hspa8	2
Ap2b1	2.2
Cacybp	2
Amd1	2.3
C6	2

Name	ENaC-Tg/WT
Cyp2a5	0.5
Wdr19	0.5
Gsta2	0.5
Cd300e	0.5
Ms4a1	0.5
Thbs1	0.5
Cnfn	0.5
Tmsb4x	0.5
Lyzs	0.5
Wdr31	0.4
Ccr7	0.4
Bex2	0.4
Lypd2	0.4
Nr4a2	0.4
Nppa	0.4
Cyp2f2	0.4
Cnn1	0.4
Sox11	0.4
Scgb3a2	0.4
Faim3	0.4
Ifitm6	0.4
Agtrl1	0.4
Cbr2	0.4
Hba-a1	0.4
Grp	0.3
Apoc2	0.3
Hbb-b1	0.3
Igfbp3	0.3
Plunc	0.3
Nr4a1	0.2
Esm1	0.2

Alteration of gene expression similar to those observed in COPD and CF

COPD & CF (up)

Gene symbol	Gene name	ENaC-Tg/WT
Il8ra	interleukin 8 receptor, alpha	23.6
Saa	serum amyroid A	11.2
Mmp8	matrix metallopeptidase 8	3
Lcn2	lipocalin 2	2.9
Edn1	endothelin 1	2.5
Ltf	lactotransferrin	2.3
Cp	Cp ceruloplasmin	2.2
Slpi	secretory leukocyte peptidase inhibitor	2.1

CF (up)

Gene symbol	Gene name	ENaC-Tg/WT
Ccl3	chemokine (C-C motif) ligand 3	4.1
Ctsd	cathepsin D	2.3
Hmox1	heme oxygenase (decycling) 1	2.2
S100a8	S100 calcium binding protein A8 (calgranulin A)	2.1

COPD (up)

Gene symbol	Gene name	ENaC-Tg/WT
Cxcl1	Cxcl1 chemokine (C-X-C motif) ligand 1	9.3
Trem2	triggering receptor expressed on myeloid cells 2	3.9
Chit1	chitinase 1 (chitotriosidase)	3.8
Mmp12	matrix metallopeptidase 12	3.2
Igf1	insulin-like growth factor 1	2.9
Spp1	secreted phosphoprotein 1	2.2
Egr1	early growth response 1	2.2

CF (down)

Gene symbol	Gene name	ENaC-Tg/WT
Cbr2	carbonyl reductase 2	0.4
Igfbp3	insulin-like growth factor binding protein 3	0.3

∗ Picked up the genes by previously-reported publication

flexiVent system (SCIREQ, inc.)

flexiVent is at its core a computer-controlled precision piston pump that can intersperse mechanical ventilation with a variety of volume and pressure controlled maneouvres to obtain accurate, reproducible measurement of respiratory mechanics.

Forced oscillation technique (FOT), Snap shot, P-V loop

- R, Resistance : Dynamic resistance quantitatively assesses the level of constriction in the lungs.
- C, Compliance : Dynamic compliance captures the ease with which the lungs can be extended.
- E, Elastance : Dynamic elastance captures the elastic rigidity of the lungs.

Negative pressure-driven forced expiratory manoeuvres (NPFE)

• FEV0.1	Forced expiratory volume in 0.1 second.
• FVC	Forced vital capacity.
• FEV0.1/FVC (FEV0.1%)	Forced expiratory volume % in 0.1 second.