

2023.1 학기
전북대학교
약학대학
신약개발연구소
정례 세미나



전북대학교
JEONBUK NATIONAL UNIVERSITY

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2023.03.15.

**2023년 1학기 신임 교수
연구 내용 발표**

**전북대 약대
강지현 교수님**

Respiratory drug delivery of pirfenidone for idiopathic pulmonary fibrosis treatment

Jeonbuk National University

Ji-Hyun Kang

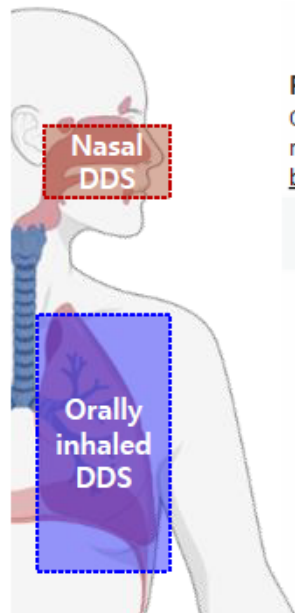


Chapter I

Respiratory drug delivery systems (RDDs)



Orally Inhaled and Nasal Drug Product (OINDPs)



Respiratory drug delivery systems (RDDs)...

OINDPs to deliver a metered or pre-metered **aerosolized** medication by means of the human respiratory tract including nasal cavity, tracheal, bronchial and alveolar sites

Disperse medium	Dispersed phase		
	Gas	Liquid	Solid
Gas	All gases are miscible in each other so no colloid	Liquid Aerosols (Fog, Mist, Hair sprays)	Solid Aerosols (Smoke, Clouds, Air particles)
Liquid	Foam (Creams (Whipped cream, Shaving cream))	Emulsions (Mayonnaise, Milk, Hand creams)	Sol (Blood and pigmented ink, Milk of magnesia)
Solid	Solid foam (Pumice stone, Styrofoam, Aero gel, foam sponge)	Gel (Gelatin, Jams, Jellies, Silica gel, agar)	Solid Sol (Metals alloys, Pearl, Cranberry glass)

• ISO 20072:2009 Aerosol drug delivery device design verification — Requirements and test methods
• <https://app.biorender.com/biorender-templates/60087/med7130600a41258e1-head-and-neck-anatomy>



Orally Inhaled Drug Delivery

For locally acting drugs...

- 1) Direct delivery to the Action site
- 2) Rapid onset of action
- 3) Non-invasive administration
- 4) Reducing the dose required
- 5) Reducing the systemic side-effects
- 6) For treatment of asthma, COPD, CF..



Seretide
(GSK)



Spiriva
(Boehringer Ingelheim)

For systemically acting drugs...

- 1) **Alternative administration route**
- 2) Avoidance of first-pass metabolism
- 3) Large absorptive surface area (up to 100 m²)
- 4) Extremely thin (0.1 µm – 0.2 µm) absorptive mucosal membrane
- 5) Rapid absorption and onset of action
- 6) For peptide (or protein) delivery..



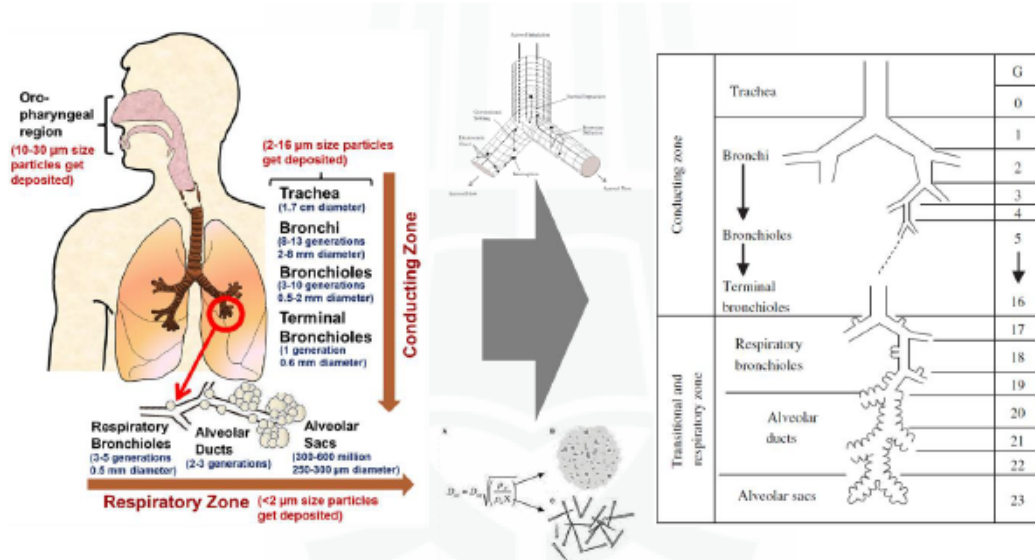
Exubera
(2006~2007, Pfizer)



Afrezza
(2014, MannKind/Sanofi)



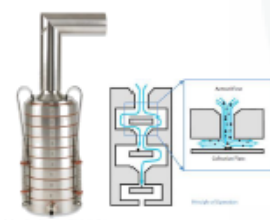
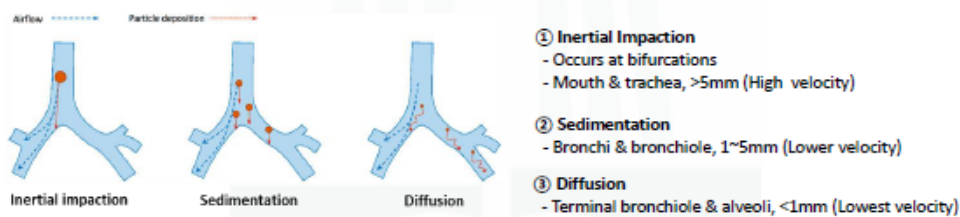
Aerodynamic Particle Size



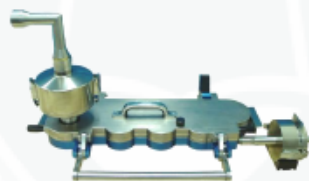
- European Journal of Pharmaceutical Sciences (2003)
- Weibel, E.R., Morph of human lung (1963)
- Handbook of Non-Invasive DDI, Chap 9 (2010)
- Respiratory Care (2005)



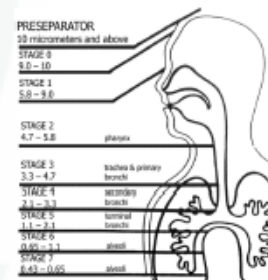
Deposition Mechanisms in the Respiratory Tract



Anderson cascade impactor



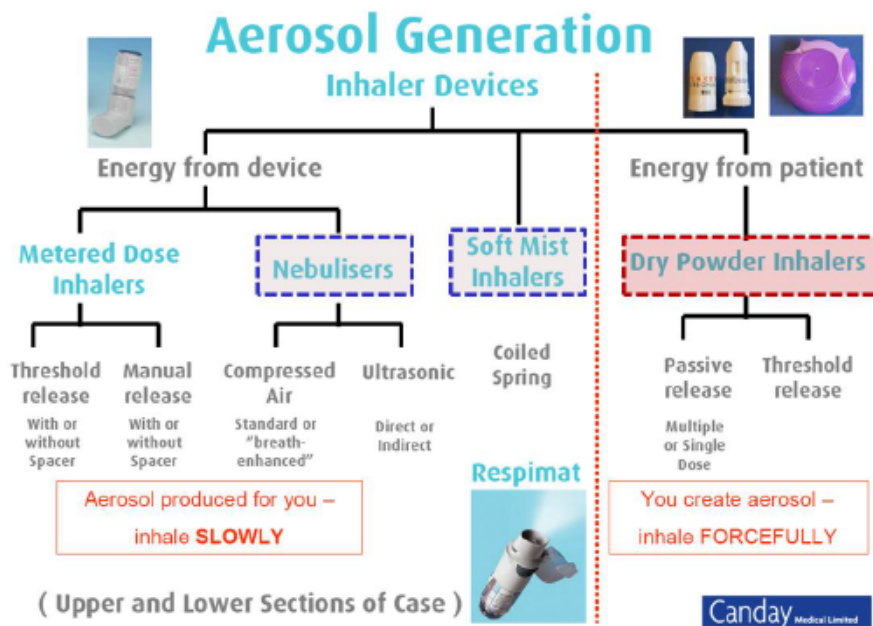
Next generation impactor



Simulation of human respiratory system



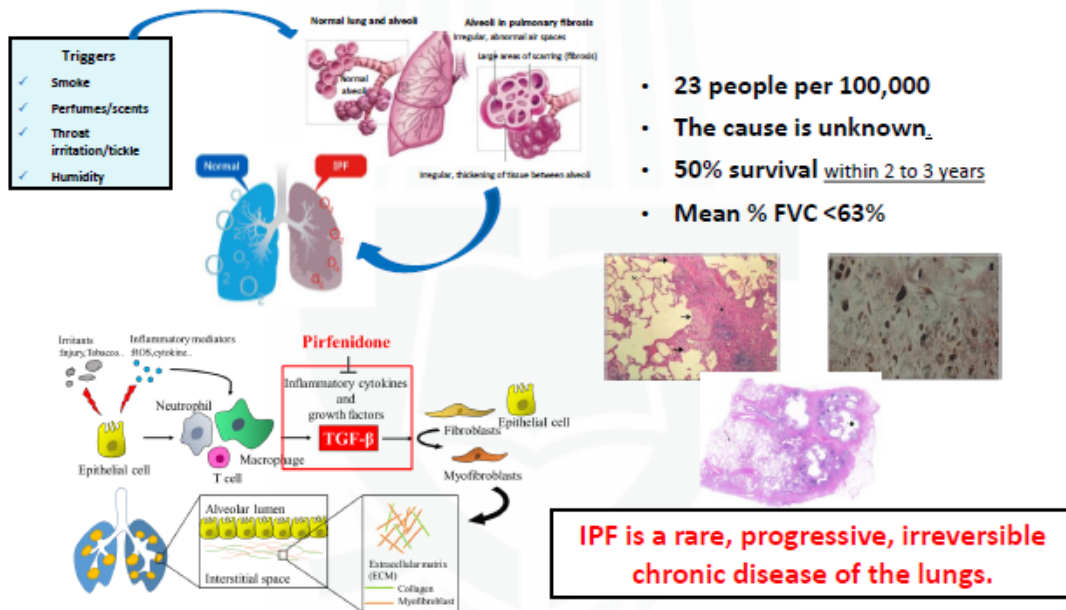
Pulmonary Drug Delivery: Inhaled Devices



Chapter 2

Case study #1: Nanoemulsion using eutectic mixture for soft mist inhalation

Idiopathic pulmonary fibrosis (IPF)

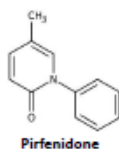


• NIH, NHLBI. What is Idiopathic Pulmonary Fibrosis? Accessed at: www.nih.gov/health/health-topics/topics/ipf/. Accessed August 2016.

• Song JW, et al. Acute Ischemic Stroke of Idiopathic Pulmonary Fibrosis: Incidence, Risk Factors and Outcome. *Ear Hear* 2011;32:356-363



Pirfenidone (PRF)



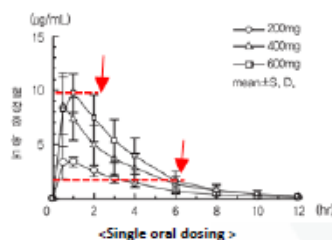
- Oral dosage form
- Half life : 2.4 hrs
- BCS class : I

Adverse effect

- Photosensitivity, nausea, tiredness, indigestion or stomach upset and others.



1st line medication of IPF



- **Fluctuation is too high** for a day
- Distribution
 - Plasma > Skin > Eyes > **Lung**

Too complex dosage

Week	1	2	3 (ouward)
Morning	200mg x 1	200mg x 2	200mg x 3
Afternoon	200mg x 1	200mg x 2	200mg x 3
Evening	200mg x 1	200mg x 2	200mg x 3
Total pills per day	200mg x 3	200mg x 6	200mg x 9



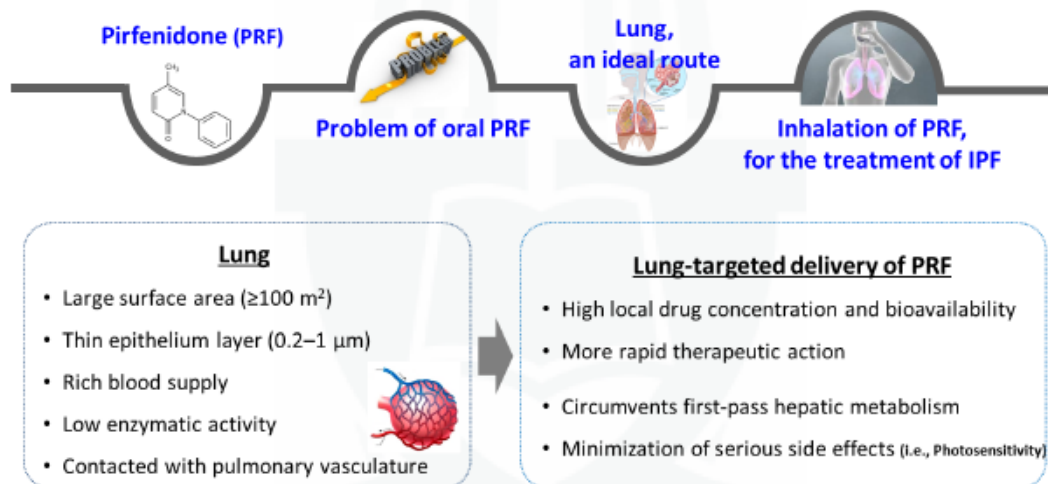
High dose and frequent number of dose
Too high C_{max} and short T_{max} cause side effects

• Onoue, S., Seto, Y., Kato, M., Aoki, Y., Kojo, Y., & Yamada, S. (2013). Inhalable powder formulation of pirfenidone with reduced phototoxic risk for treatment of pulmonary fibrosis. *Pharmaceutical research*, 30(6), 1586-1596.

• Raghu, G., Collard, H. R., Egan, J. J., Martinez, F. J., Behr, J., Brown, K. K., ... & Lynch, D. A. (2011). An official ATS/ERS/JES/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *American journal of respiratory and critical care medicine*, 183(8), 788-824.



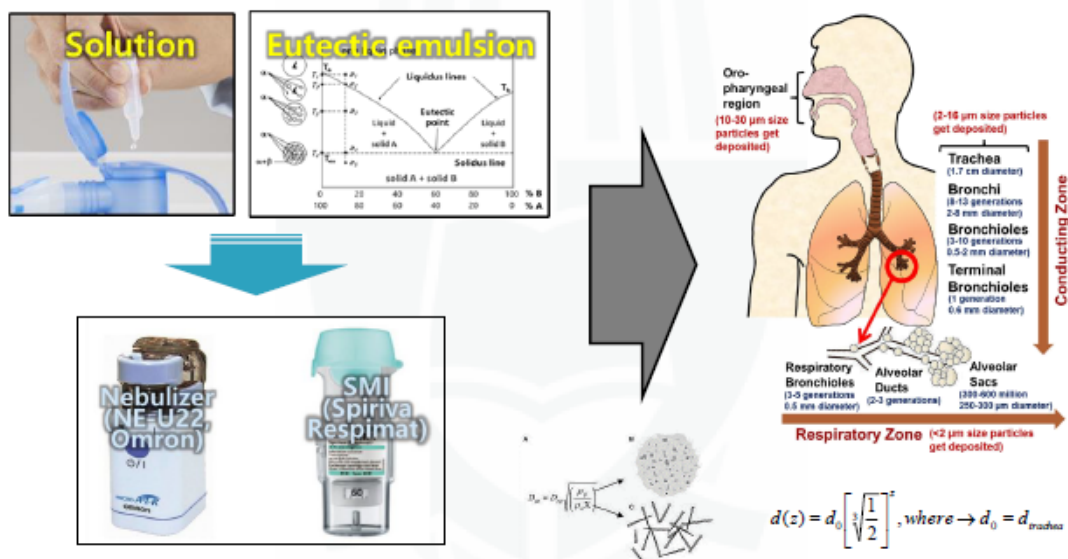
Inhalable PRF for lung-targeted DDS



- Yang, M. Y., Chan, J. O. Y., & Chen, H. K. (2014). Pulmonary drug delivery by powder aerosols. *Journal of Controlled Release*, 193, 228-240.
- Kumbhar, P., Marjappa, A., Shah, R., Jha, N. K., Singh, S. K., Das, K., ... & Patravale, V. (2021). Inhalation delivery of repurposed drugs for lung cancer: Approaches, benefits and challenges. *Journal of Controlled Release*.



Formulation Strategies for liquid preparation



Methods

Eutectic mixture (EM)

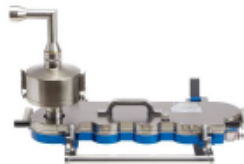
- Mix PRF and N-acetylcysteine(NAC) in proportions
 - 10:0:0:10, mol/mol
- 80 °C oven, for 3hr

Preparation of EM nanoemulsion (N.E.)

- Mix PRF and NAC in a ratio of 6:4 (mol/mol)
- 80 °C oven, for 3hr
- Add distilled water in proportions
 - 10:0:0:10, g/ml

In vitro aerodynamic performance

- Next generation impactor (NGI, Copley, UK)
- Breath simulator (BRS, Copley, UK)
 - Inhaler device
 - RS-01
 - Pump operation
 - 60 LPM, 4s (USP 601)



Next generation impactor



Breath simulator

In vivo pharmacokinetics

- Plasma
- Lung tissue



Microsprayer, PenWu Device

In vivo pharmacodynamics

- Lung function
- Cytokine and protein
- Micro-CT
- Histology



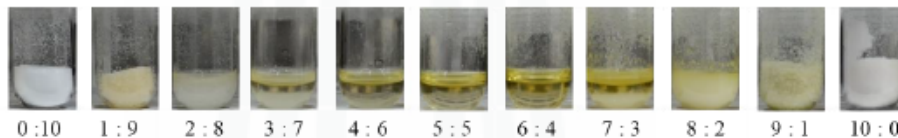
Group	Dosage form	Route	Dose	n
Negative control (NC)	-	-	-	6
Positive control (PC)	-	-	-	6
Oral/Sol	PRF solution	Oral	30mg/kg	6
ITI/Sol	PRF solution	Intratracheal instillation (ITI)	30mg/kg	6
ITI/N.E.	EM nanoemulsion (N.E.)	ITI	30mg/kg	6



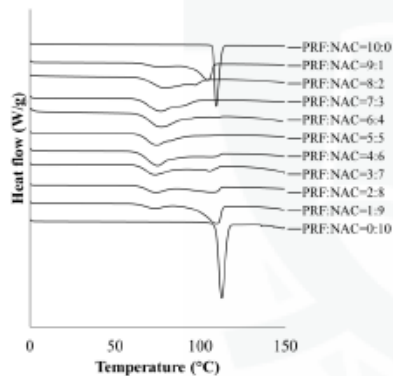
Results (physicochemical properties of EM)

At 80°C

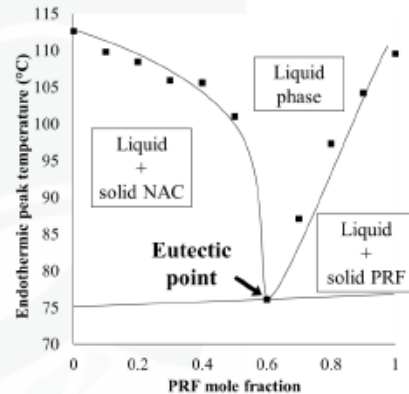
PRF : NAC



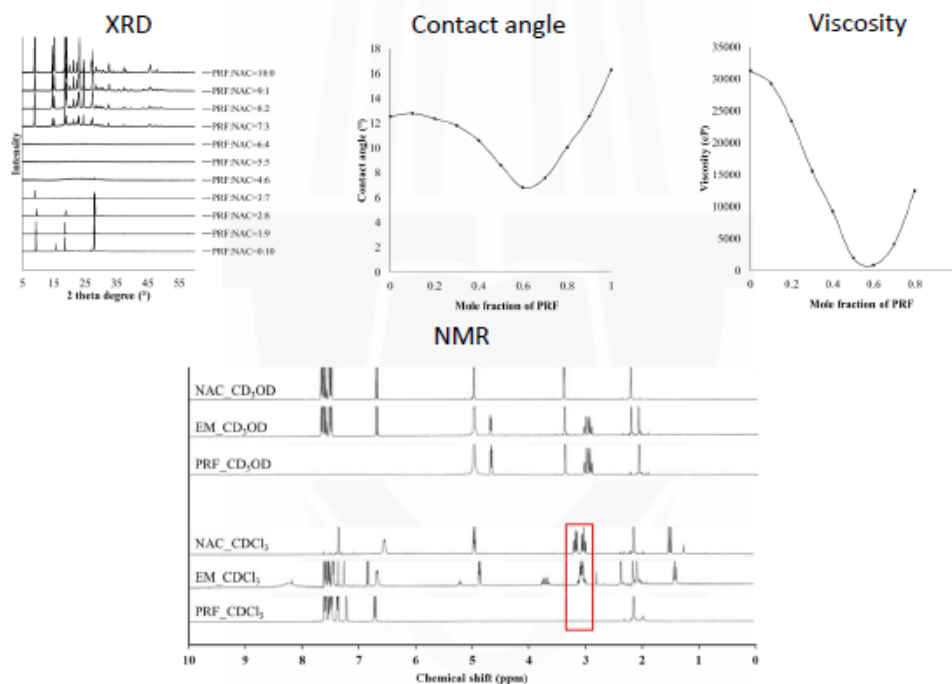
DSC



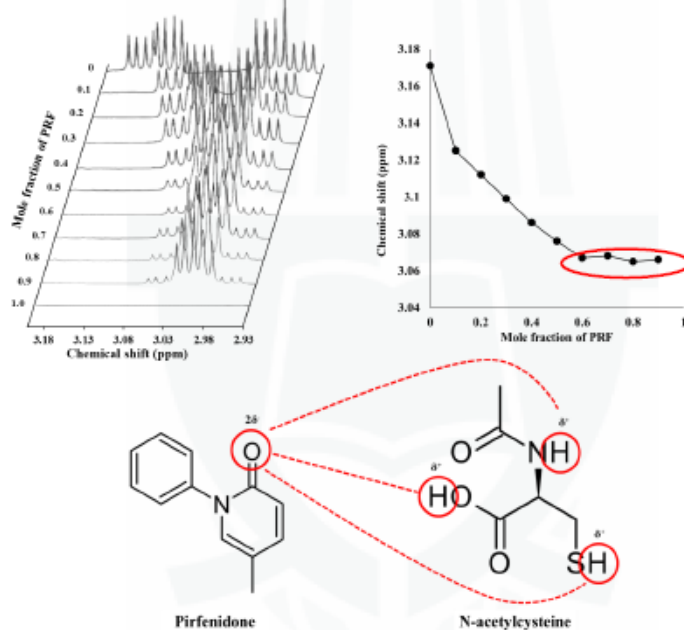
Phase diagram



Results (physicochemical properties of EM)



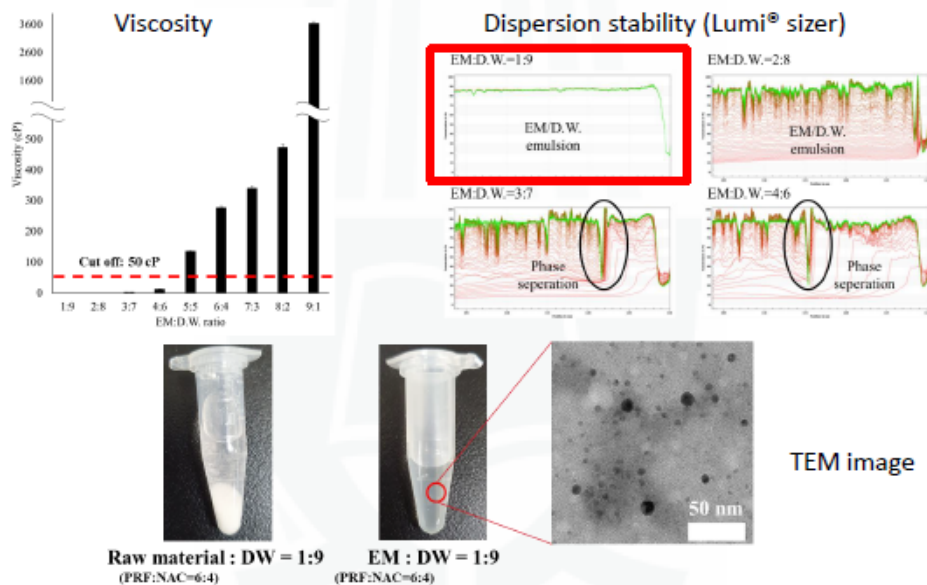
Results (physicochemical properties of EM)



Eutectic point → PRF:NAC=6:4



Results (physicochemical properties of Nanoemulsion)

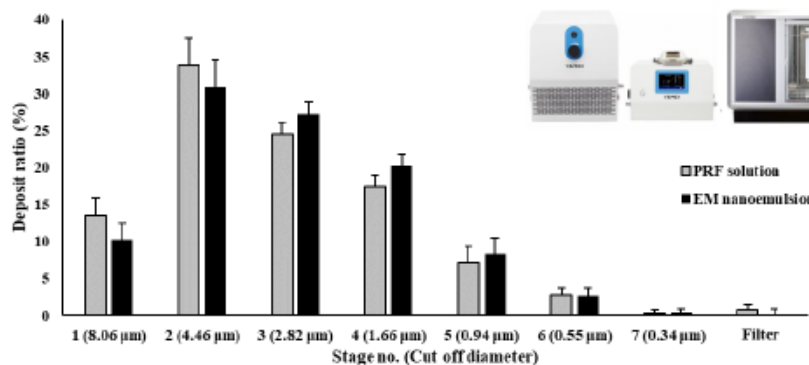


Optimal EM nanoemulsion (N.E.) → EM:DW=1:9

• McCallion, O. N., Taylor, K. M., Thomas, M., & Taylor, A. J. (1995). Nebulization of fluids of different physicochemical properties with air-jet and ultrasonic nebulizers. *Pharmaceutical research*, 12(11), 1682-1688.



Results (In vitro aerodynamic performance of N.E.)



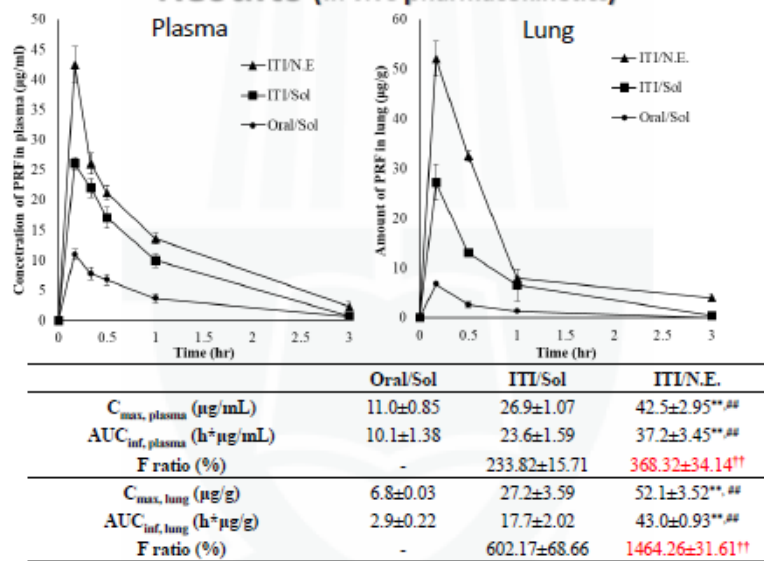
Parameter	PRF solution (10mg/mL)	EM nanoemulsion (66.7mg/mL)
FPF (%)	86.4±1.4	89.8±0.7
FPD (µg)	111.5±3.9	852.7±70.7**
MMAD (µm)	2.2±0.1	2.0±0.0
GSD	2.7±0.0	2.6±0.0

**Student t-test, p-value <0.005

Delivery efficiency : EM nanoemulsion > PRF solution (7.0-fold)



Results (In vivo pharmacokinetics)



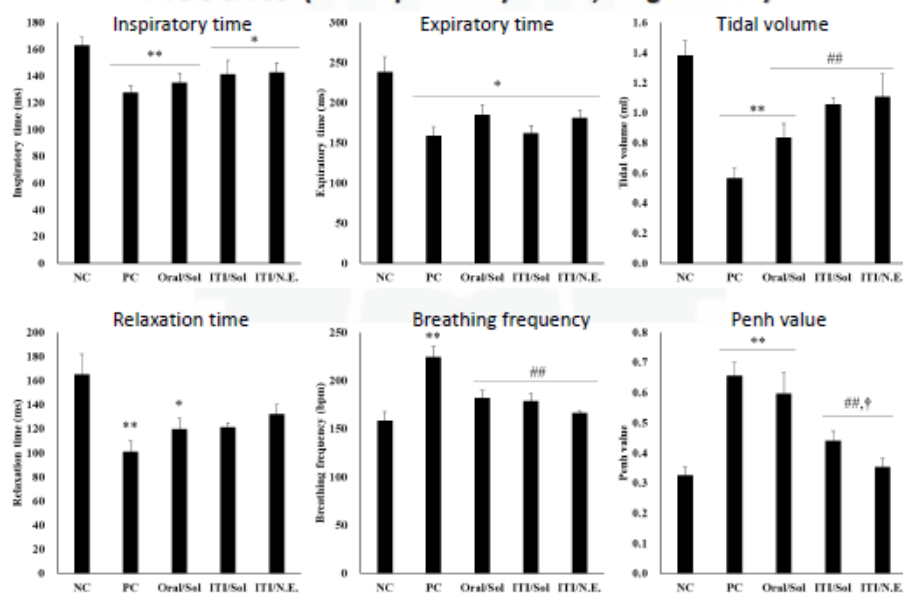
*,** ANOVA, p-value < 0.05, 0.005 compared with Oral/Sol
^{##},^{††} ANOVA, p-value < 0.05, 0.005 compared with ITI/N.E.

F ratio (%) Lung : ITI/N.E. > Oral/Sol (14 fold↑)

• Oral/Sol: PRF solution/Oral/30mpk; ITI/Sol: PRF solution/Lung/30mpk; ITI/N.E.: EM nanoemulsion/Lung/30mpk.



Results (In vivo pharmacodynamics, Lung function)

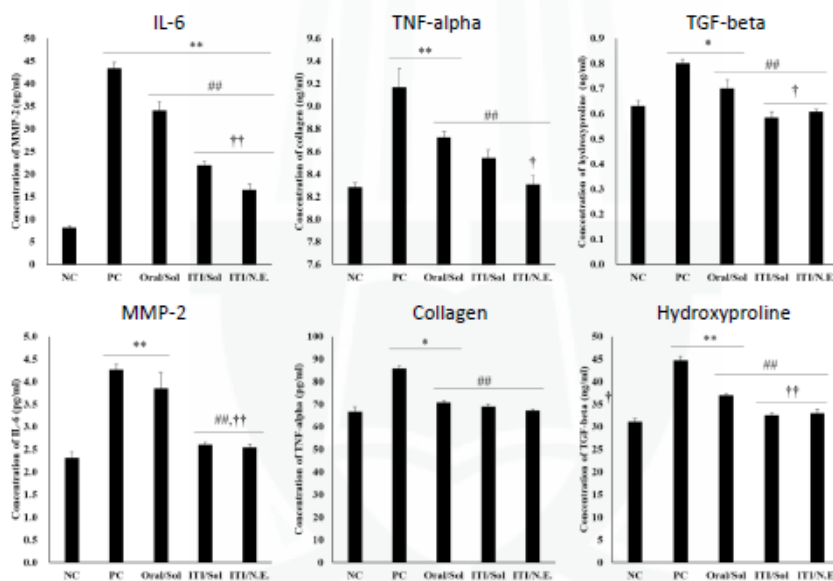


*,** ANOVA, p-value < 0.05, 0.005 compared with NC
^{##},[§] ANOVA, p-value < 0.05, 0.005 compared with PC
[†],^{††} ANOVA, p-value < 0.05, 0.005 compared with Oral

• Oral/Sol: PRF solution/Oral/30mpk; ITI/Sol: PRF solution/Lung/30mpk; ITI/N.E.: EM nanoemulsion/Lung/30mpk.



Results (In vivo PD, ELISA)

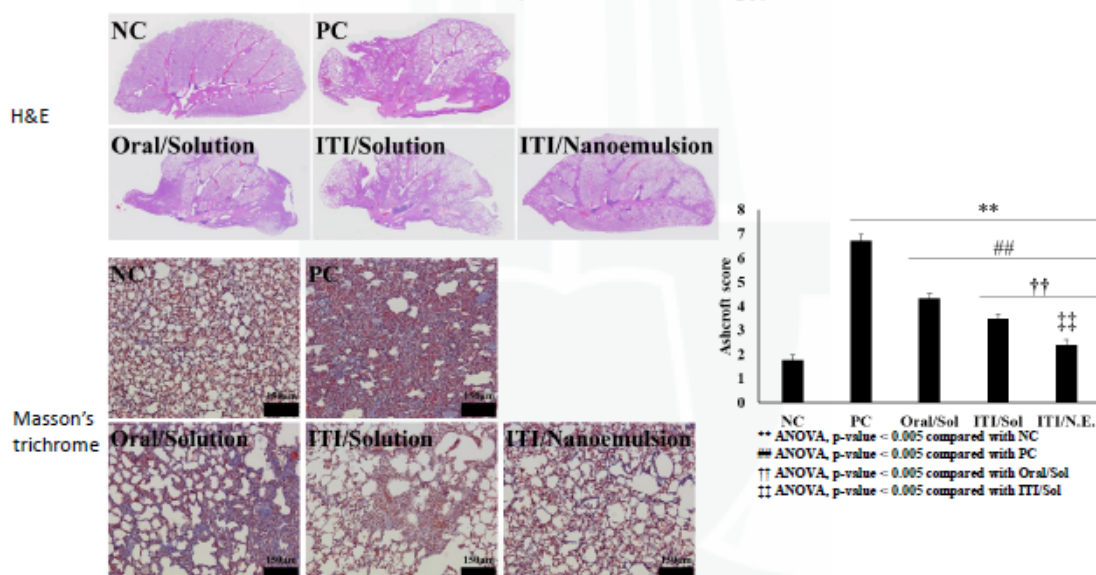


*** ANOVA, p-value < 0.05, 0.005 compared with NC
 ## ANOVA, p-value < 0.05, 0.005 compared with PC
 †† ANOVA, p-value < 0.05, 0.005 compared with Oral

* Oral/Sol: PBF solution/Oral/30mpk; ITI/Sol: PBF solution/Lung/30mpk; ITI/N.E.: EM nanoemulsion/Lung/30mpk.



Results (In vivo PD, Histology)



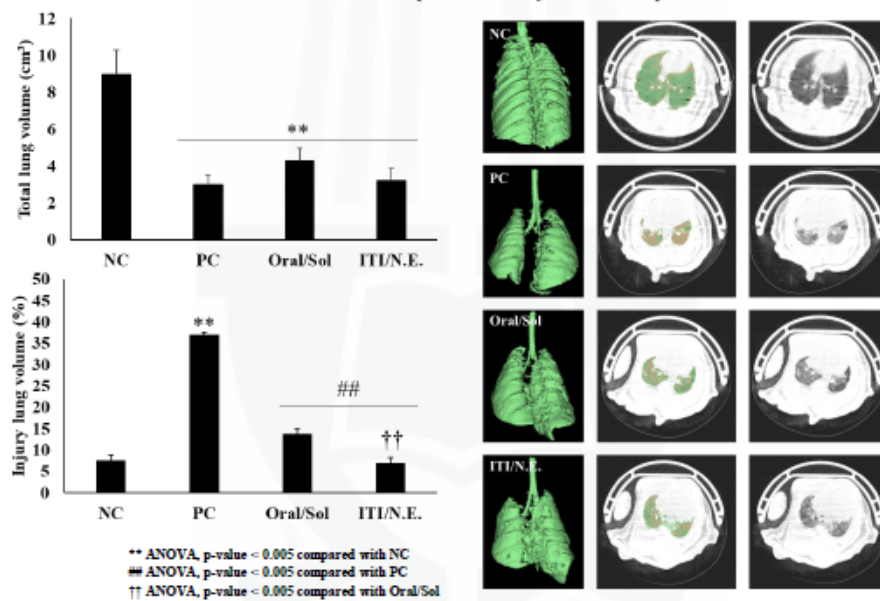
*** ANOVA, p-value < 0.005 compared with NC
 ## ANOVA, p-value < 0.005 compared with PC
 †† ANOVA, p-value < 0.005 compared with Oral/Sol
 ‡‡ ANOVA, p-value < 0.005 compared with ITI/Sol

IPF treatment efficiency: ITI/N.E. > Oral/Sol

* Oral/Sol: PBF solution/Oral/30mpk; ITI/Sol: PBF solution/Lung/30mpk; ITI/N.E.: EM nanoemulsion/Lung/30mpk.



Results (In vivo PD, Micro-CT)



The % injury lung tissue : ITI/N.E. > Oral/Sol

• Oral/Sol: PRF solution/Oral/30mg/kg; ITI/Sol: PRF solution/Lung/30mg/kg; ITI/N.E.: EM nanoemulsion/Lung/30mg/kg.



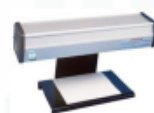
Methods

In vivo phototoxicity

Experimental group

Group	Route	Dose
Negative control	Oral	Saline
Oral (PRF solution, 160mg/kg)	Oral	160 mg/kg
ITI (EM nanoemulsion, 30mg/kg)	ITI	30 mg/kg

UV irradiance level

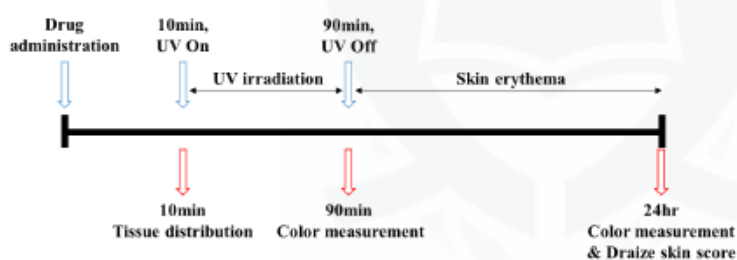


VL-6 LM

(Viber Lourmat, Mame-la-Vallée, France)

- UVA(365 nm), 6.5 mW/cm²
- Time: 80 min
- Area: 2*3 cm²
- Total: 31.2 J/cm²

Experiment Scheme

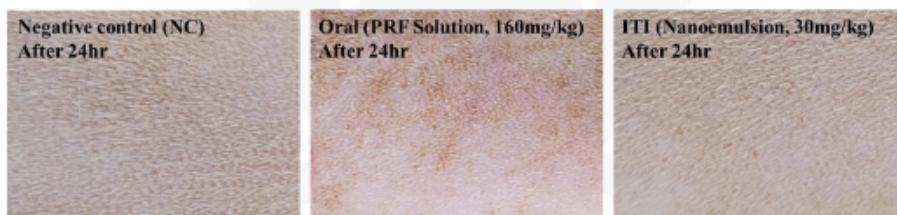
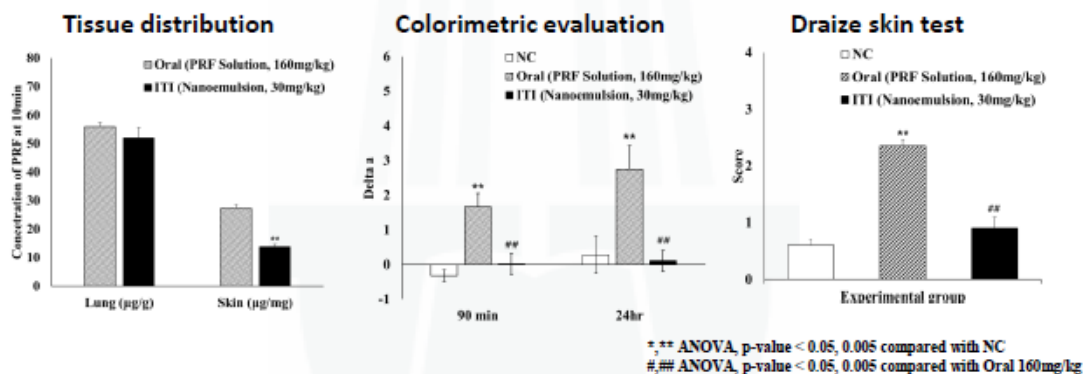


Evaluation

- Colorimeter
 - L*a*b space
- Draize skin test
 - Observation: 10 people
 - Blind test



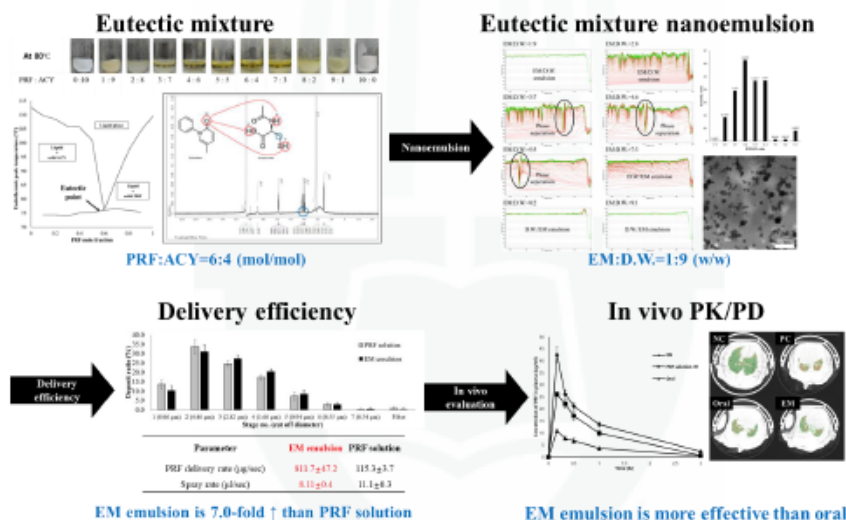
Results (In vivo phototoxicity)



ITI/N.E. : Skin distribution ↓ → Phototoxicity ↓



Overview



ITI/N.E. is more effective than Oral/Sol in IPF

- Korean patent number 10-2224539 (2021).
- Kang, J. H., et al. *Journal of Controlled Release**, 2022, 352: 570-585. (*IF: 11.467, PHARMACOLOGY & PHARMACY – SCIE 95.88%)



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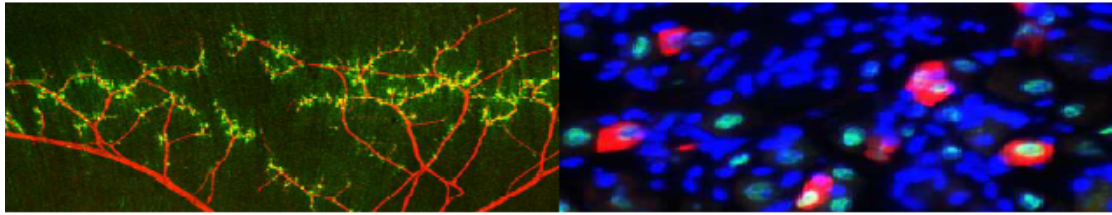
Thank you



2023.03.22.

우수연구자 초청 세미나

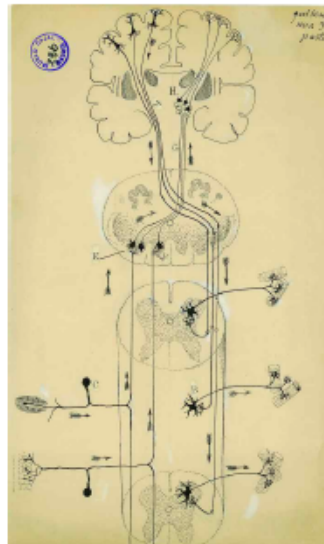
**광주과학기술원
송미령 교수님**



Understanding the Neural Control of Movement : How to Build a Sensorimotor Circuit



Gwangju Institute of Science and Technology
Mi-Ryoung Song

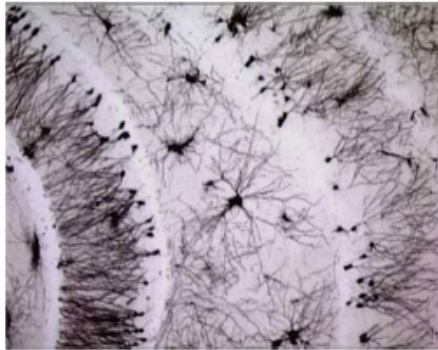


Santiago Ramón y Cajal

Golgi Staining

•Stains a limited number of cells at random in their entirety

1. Fix tissue blocks
2. 3% potassium bichromate for 3-7 days.
3. 2% silver nitrate solution for 1-3 day at RT.
4. Cut vibratome sections and mount.



Camillo Golgi



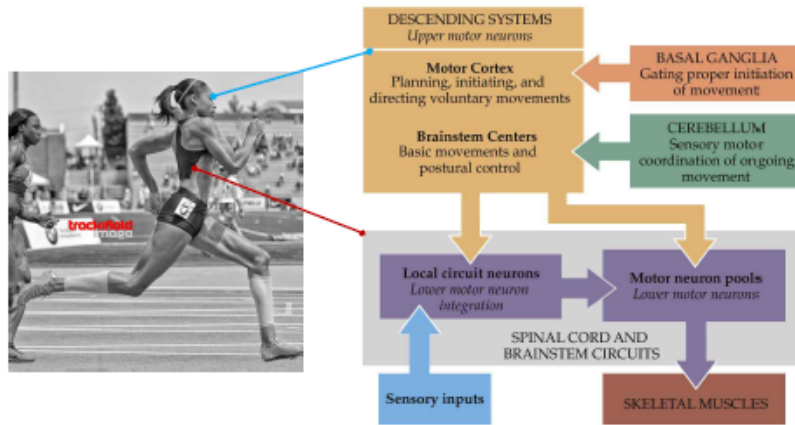
Santiago Ramón y Cajal



*Animation of the image stack of
Golgi-impregnated
pyramidal neuron
(300 μm -thick, 1 μm Z step)*

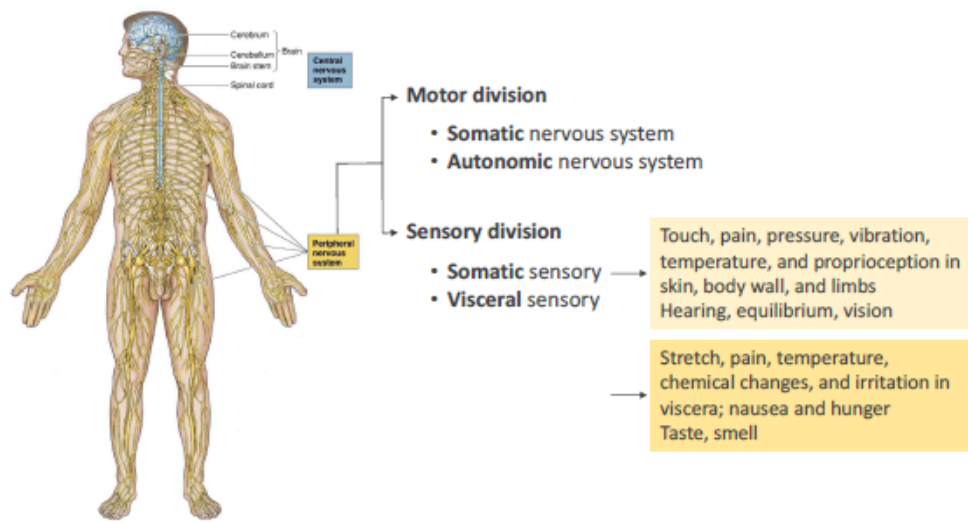
[neurodigittech](http://neurodigittech.com)

Motor circuits control movements



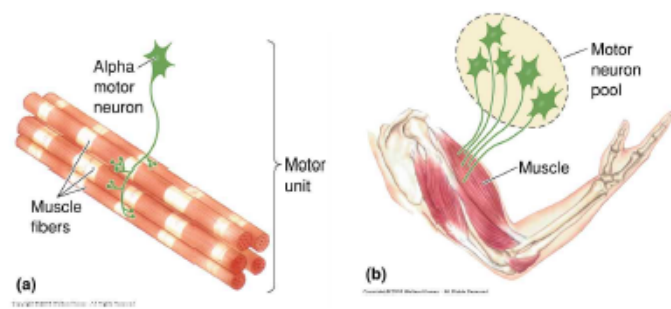
Introduction

Peripheral nervous system (PNS)



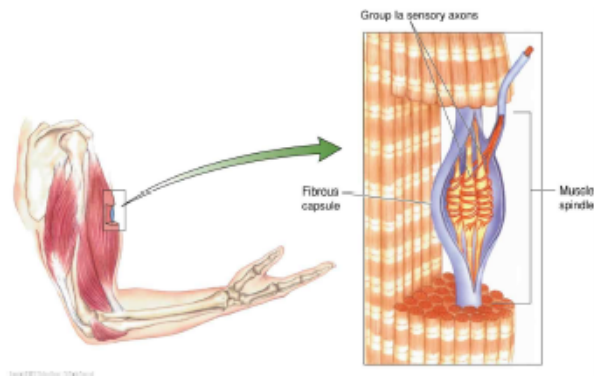
Alpha Motor Neurons

- Motor unit: motor neuron and all the muscle fibers it innervates
- Motor neuron pool: all the alpha motor neurons that innervate a single muscle

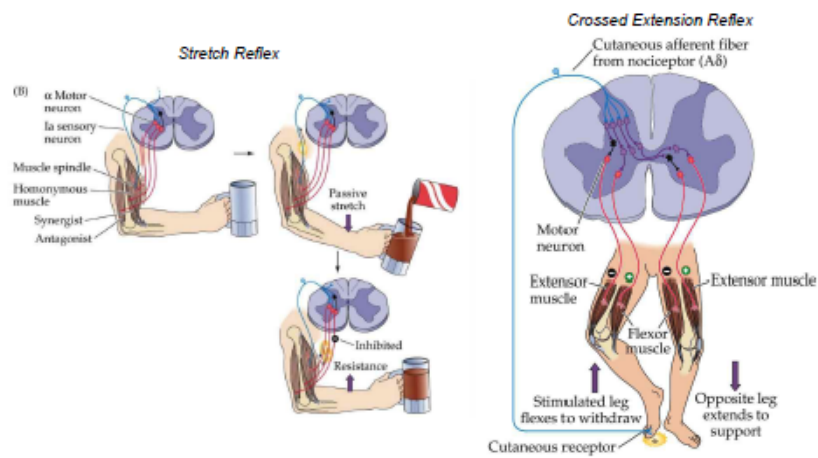


Spinal Control of Motor Units—Proprioceptors

- Sensory feedback from muscle spindles—stretch receptor

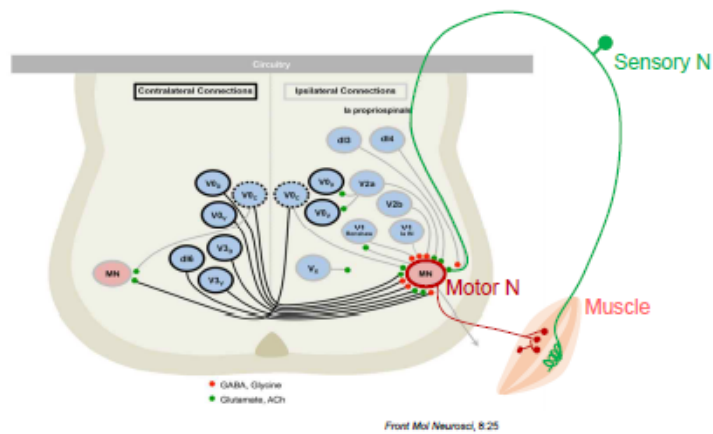


Spinal Reflexes



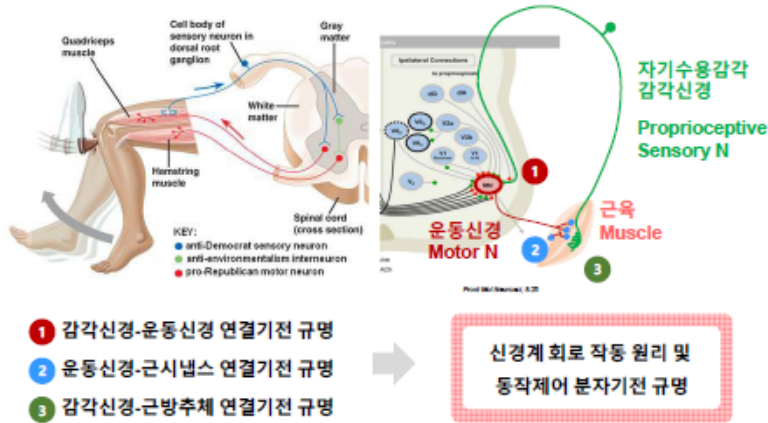
Motor circuit in the spinal cord

Neuronal circuit is a functional entity of interconnected neurons that influence each other



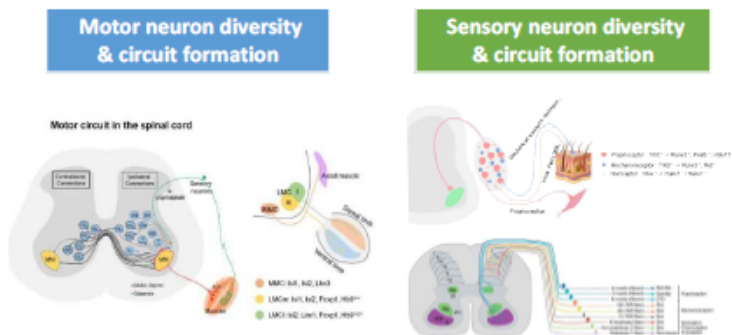
Closed-loop monosynaptic neural circuit

예) 무릎 밑 자극 → 감각신경 → 운동신경 → 근육 → 다리 움직임



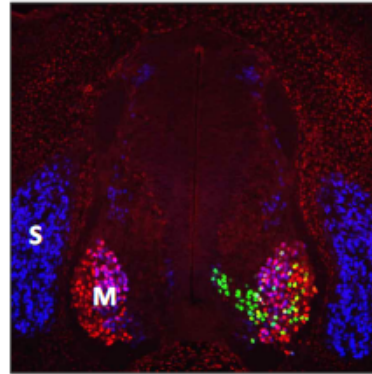
Laboratory of Neural Network and Transcriptomics

- ✓ To define gene regulatory networks during CNS development



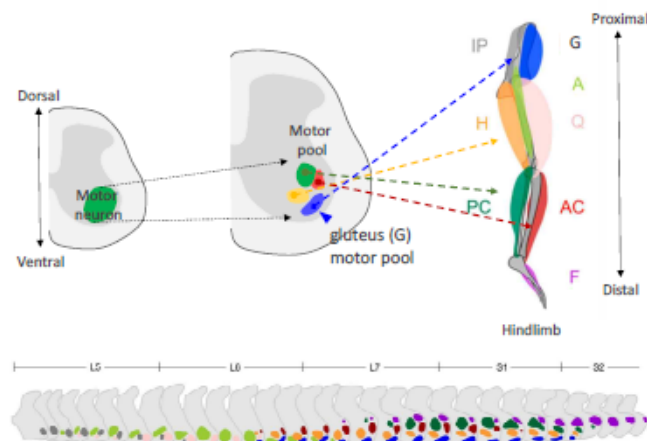
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- Introduction
- Results
 - Pt 1. Motor neurons**
 - Pt 2. Sensory neurons**
- Summary

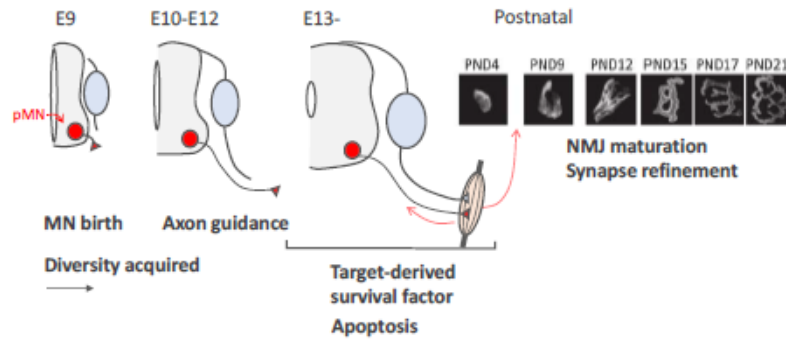


Motor pool and target hindlimb muscles

- ✓ A **motor pool** consists of all individual **motor neurons** that innervate a single muscle.

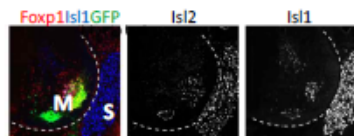


Motor neuron development

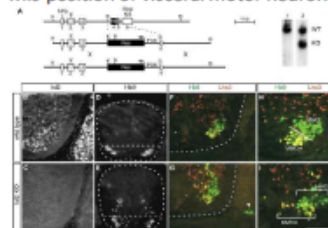


Isl2 is a member of LIM-HD transcription factor family

- ✓ A LIM-HD transcription factor with multimeric complex
- ✓ Selectively expressed in motor neurons and sensory neurons
- ✓ Controls motor neuron development: MN identity, differentiation, survival, axon pathfinding, neurotransmitter identity and etc.



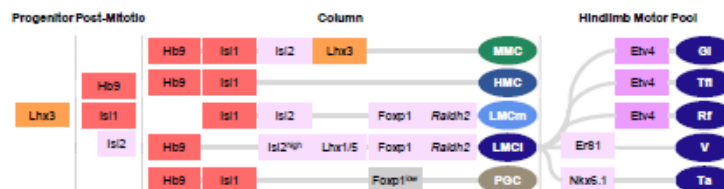
-Mis-position of visceral motor neurons



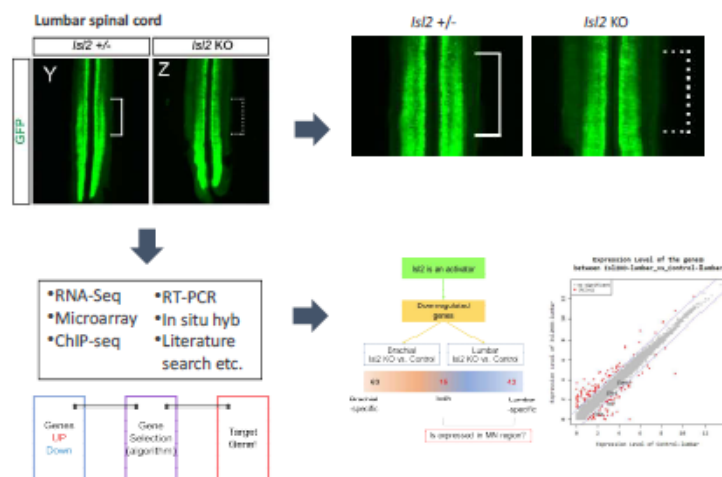
Neuron, Vol. 41, 337-350, 5, 2004

Isl2 is a member of LIM-HD transcription factor family

- ✓ A LIM-HD transcription factor with multimeric complex
- ✓ Selectively expressed in motor neurons and sensory neurons
- ✓ Controls motor neuron development: MN identity, differentiation, survival, axon pathfinding, neurotransmitter identity and etc.



Which genes are under the control of Isl2 transcription factor?



ALS animal models

D83G SOD1 mouse, 52 wks (HMG, 24, 1883)



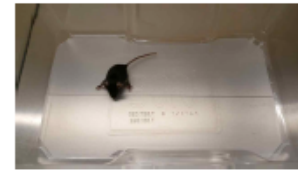
C9orf72 BAC mouse, 7 mo (Neuron, 90, 521)



G85R SOD1 mouse, 3 mo (PNAS, 111, 16883)

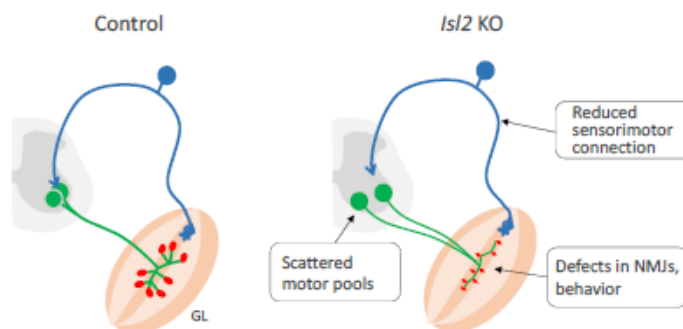


TDP-43 TG mouse, 3 wks (Nature, 544, 367)



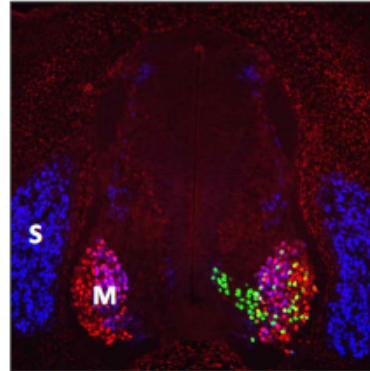
Summary

- ✓ Isl2 is required for *Pea3* (*Etv4*) transcription in motor neurons.
- ✓ Isl2 is important in development of proximal motor pool in the hindlimb.
- ✓ Isl2 may control motor-sensory neuron circuit formation.



CONTENTS

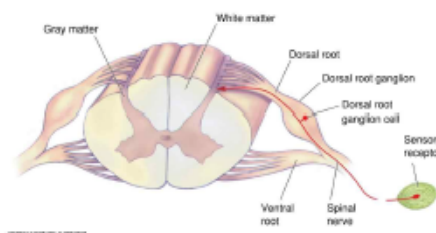
- Introduction
- Results
 - Pt 1. Motor neurons
 - Pt 2. Sensory neurons
- Summary



Introduction

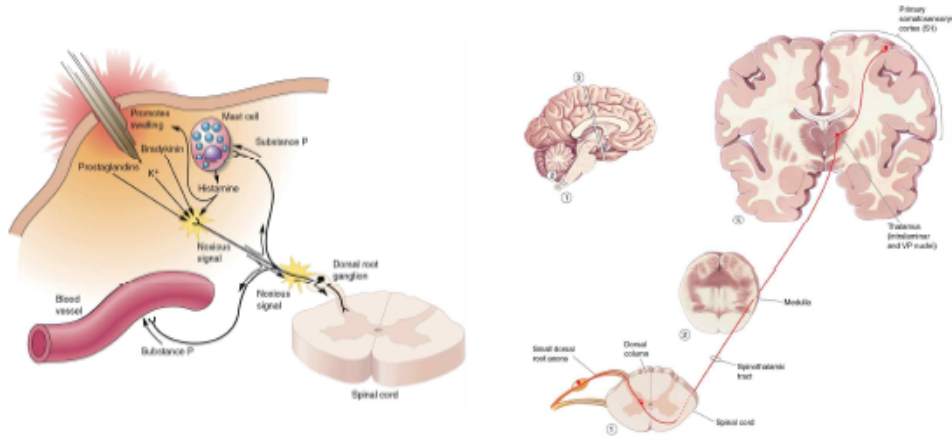
Somatic sensory system

- $A\alpha$, $A\beta$, $A\delta$, C axons
- C fibers mediate pain, temperature, and itch.
- $A\beta$ mediates touch sensations.



Axons from skin	$A\alpha$	$A\beta$	$A\delta$	C
Axons from muscles	Group I	II	III	IV
Diameter (μm)	15–25	5–12	1–5	0.2–1.5
Speed (m/sec)	80–120	35–75	5–30	0.5–2
Sensory receptors	Proprioceptors of skeletal muscle	Mechanoreceptors of skin	Pain, temperature	Temperature, pain, itch

Pain sensation (nociception)



Nobel prize in 2021: Discovery of receptors for temperature and touch

온도·촉각 등 '감각의 비밀' 밝힌 미국 두 과학자 노벨생리의학상

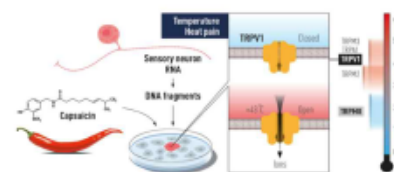
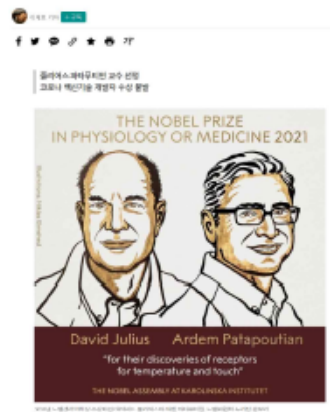


Figure 2. The discovery of TRPV1 using a gain of function screen of genes expressed in sensory neurons for reconstitution of capsaicin responsiveness in a non-responsive cell line. This paved the way to the unearthing of additional temperature-sensing TRP receptors, which together code for temperature sensation.

2023.03.29.

얹뭇학 전공

대학원생 발표 세미나

카말 학생



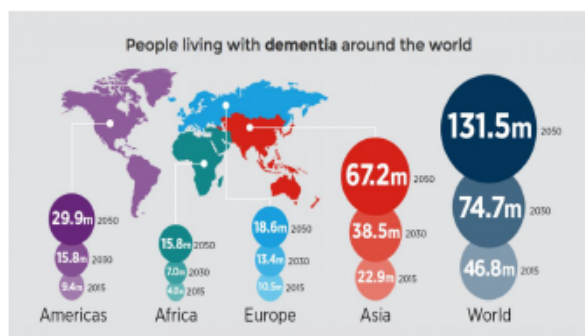
Identifying the probable synergistic mechanism of CBD and Taurine on Alzheimer's Disease

Presented By
Muhammad Kamal Hossain
 PhD Student
 Student ID- 202255221
 School of Pharmacy
 Jeonbuk National University (JBNU)



Alzheimer's is a progressive disease, where dementia symptoms gradually worsen over a number of years. Worldwide, **55 million** people are living with Alzheimer's and other dementias. Dementia is now the 7th leading cause of mortality globally. (Alzheimer's Disease International, World Alzheimer Report ; 2022)

Alzheimer's Symptoms



Source: World Alzheimer Report 2015



Etiology of AD

Although the etiology of AD remains unclear, multiple findings have revealed that oxidative stress is an early characteristic of the AD pathological process and also involved in the formation of A β and neurofibrillary tangles (NFTs)

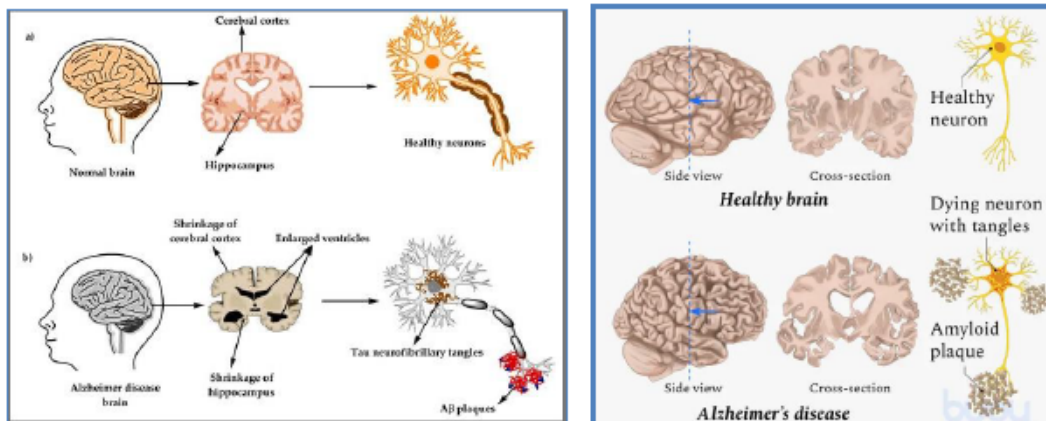


Figure Courtesy:
Zeinab Breijyeh et al.
Molecules 2020, 25(24), 5789

The role of plaques and tangles

Two abnormal structures called plaques and tangles are prime suspects in damaging and killing nerve cells. Plaques are deposits of a protein fragment called beta-amyloid that build up in the spaces between nerve cells.

Tangles are twisted fibers of another protein called tau that build up inside cells.



CBD, or cannabidiol, is the second most prevalent active ingredient in cannabis (marijuana). One of hundreds of components in marijuana, CBD does not cause a "high" by itself. According to World Health Organization, "In humans, CBD exhibits no effects indicative of any abuse or dependence potential (Non Psychoactive)"

Molecular formula: C₂₁H₃₀O₂

Molecular weight: 314.4 g/mol

In December 2015, the FDA eased the regulatory requirements to allow researchers to conduct CBD trials.

The evidence for cannabidiol health benefits

CBD has been touted for a wide variety of health issues.

Animal studies, and self-reports or research in humans, suggest CBD may also help with:

Anxiety. Several clinical trials are exploring the common report that CBD can reduce anxiety.

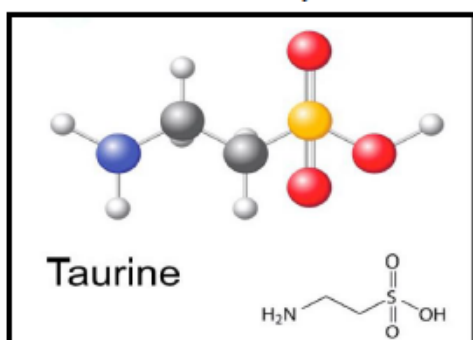
Insomnia. Studies suggest that CBD may help with both falling asleep and staying asleep.

Chronic pain. One animal study from the *European Journal of Pain* suggests CBD could help lower pain and inflammation due to arthritis when applied to skin. It may inhibit inflammatory and neuropathic pain, which are difficult to treat.

Addiction. Animal models of addiction suggest it may also help lessen cravings for alcohol, cannabis, opiates, and stimulants.

Epidiolex, which contains CBD, is the first cannabis-derived medicine approved by the FDA for these conditions.

Taurine is a naturally occurring sulfur-containing amino acid. It's particularly concentrated in brain, eyes, heart, and muscles. (Chian Ju Jong, *Molecules* 2021)



Molecular formula: $C_2H_7NO_3S$

Molecular weight: 125.15 g/mol

Taurine has important functions in the heart and brain. It helps support nerve growth. It might also benefit people with heart failure by lowering blood pressure and calming the nervous system.

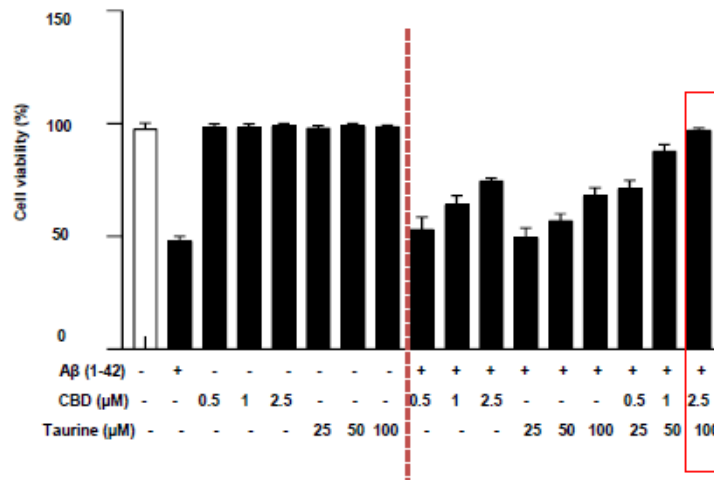
It is also used for obesity, athletic performance, fatigue, diabetes, and many other conditions, but there is no good scientific evidence to support these uses.

Why we interest on this Research Topic?

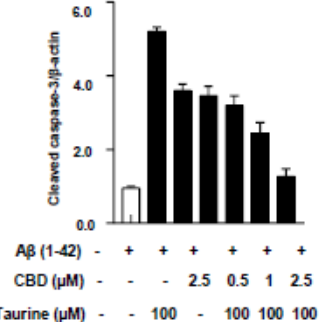
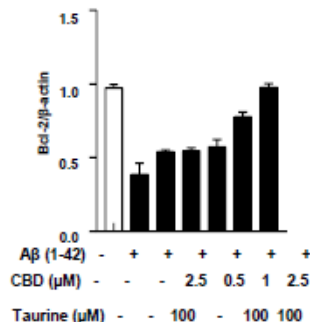
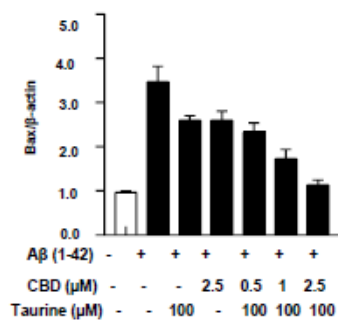
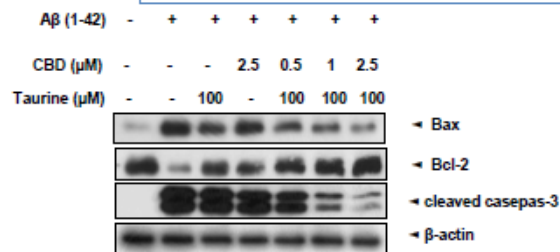
Identifying the probable synergistic mechanism of CBD and Taurine on Alzheimer's Disease

- Scientists do not know exactly what role plaques and tangles play in Alzheimer's disease. Most experts believe they somehow play a critical role in blocking communication among nerve cells and disrupting processes that cells need to survive.
- Several criteria have been proposed for a more accurate diagnosis of AD, including clinical biomarkers, bodily fluids, and imaging studies.
- The treatment of AD remains symptomatic, without alteration in the disease's prognosis.
- Limited AD therapy: Inhibitors to cholinesterase enzyme such as galantamine, donepezil, and rivastigmine, and NMDA antagonists such as memantine, improve memory and alertness but do not prevent progression.
- Future therapies such as disease-modifying treatment can alter the progression of AD by targeting the A β pathway, and many drugs have entered the clinical trials, like AN-1792, solanezumab, bapineuzumab, semagacestat, avagacestat, and tarenflurbil, but failed in demonstrating efficacy in the final clinical stages.
- Recent discoveries have demonstrated how the modulation of cannabinoid receptor 1 (CB1) and receptor 2 (CB2) can exert neuroprotective effects without the recreational and pharmacological properties of Cannabis sativa. But, Without sufficient high-quality evidence, It can't pinpoint effective doses of CBD, and because CBD currently is typically available as an unregulated supplement.

In vitro findings



Previous in vitro findings from our Lab



Screening test for scopolamine

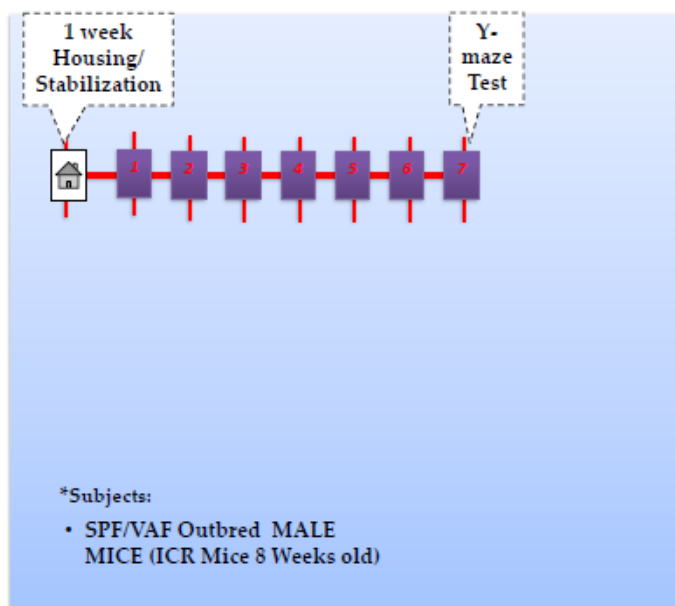
Data Analysis Assessment the dose effect of scopolamine for inducing Alzheimer's Disease (Y-maze Test)

Reference: Scopolamine induced memory Impairment

Dose	Strain	Schedule/ Days (treatment)	Test Performed	Behavioral Effects	Behavioral Implications	Reference
1 mg/kg	Sprague Dawley rats (180-200g; 6-8 weeks)	9 days	novel object recognition (NOR) Elevated plus maze (EPM) tests	Reduced the 'recognition index (%)' in novel-object recognition test. Slightly reduced the 'inflection ratio' during elevated-plus maze test.	Memory impairment Potential effects on anxiety level.	Bhuvamendran, S., Kumari, Y., Othuman, I., & Shaikh, M. F. (2018). Amelioration of cognitive deficit by embelin in a scopolamine-induced Alzheimer's disease-like condition in a rat model. Frontiers in pharmacology, 9, 665.
	ICR mice (25-30 g)	1 day (Single)	Passive avoidance task Response to electric shock Y maze Morris water maze	Reduced the 'latency time' in passive avoidance test. Reduced the 'spontaneous alternations' during the Y-maze test. Reduced 'visitation time' in the target quadrant during water- maze test.	Memory Impairment	Kim, D. H., Jeon, S. J., Son, K. H., Jung, J. W., Lee, S., Yoon, B. H., ... & Ryu, J. H. (2007). The ameliorating effect of oxosylin A on scopolamine- induced memory impairment in mice. Neurobiology of learning and memory, 87(4), 536-546.
	Mus musculus Swiss 2 months (25-30 g)	7 days	T-Maze NOR OFT	Increased the latency time of entry into the preferred arm Decreased the number of entries into the preferred arm Decreased the locomotors activity	Memory Impairment	Fanta Sabine Adeline Yadang, Yvette Nguizeye, Christelle Wayoue Kom, Patrick Herve Diboue Betote, Amina Mamat, Laure Rachel Yanthe Tchokouaha, Germain Sotoing Taiwé, Gabriel Agbor Agbor, Elisabeth Ngo Bum, "Scopolamine-Induced"

1.5 mg/kg	Swiss male mice	1 day (Single)	Y-maze Novel object recognition test (NOR) Morris water maze (MWM)	Lowered the percentage of spontaneous alternation Reduced mean latencies and swimming distances	Deficit in learning and spatial memory	Trang Huyen Xuan Hoang Duc Viet Ho, Kiem Van Phan, Quan Van Le, Ain Raal & Hoi Thi Nguyen (2020) Effects of Hippocampus reticulatum on memory, spatial learning and object recognition in a scopolamine-induced animal model of Alzheimer's disease, <i>Pharmaceutical Biology</i> Volume 58, 2020 - Issue 1, Pages 1107-1113
2 mg/kg	Swiss albino mice (25-30 g)	3 days	MWM PAT OFT	Increased time in locating the platform Slightly reduced the 'beam crosses'	Memory impairment Potential effects on motor activity and anxiety level.	Rachuvanshi, R., Nathakki, V. K., Singh, L., Singh, B., Bharate, S. S., Bhatti, R., & Bharate, S. B. (2021). Identification of Plant-based Multitargeted Leads for Alzheimer's Disease: In-vitro and In-vivo Validation of Woodfordia fruticosa (L.) Kurz . <i>Phytomedicine</i> , 153659.

Protocol spatial learning and memory.



08 Mice/ Group

Group- I: Vehicle

Group- II: Scopolamine 1 mg/kg

Group- III: Scopolamine 2 mg/kg

Group- IV: Scopolamine 5 mg/kg

Scopolamine Vehicle- Saline

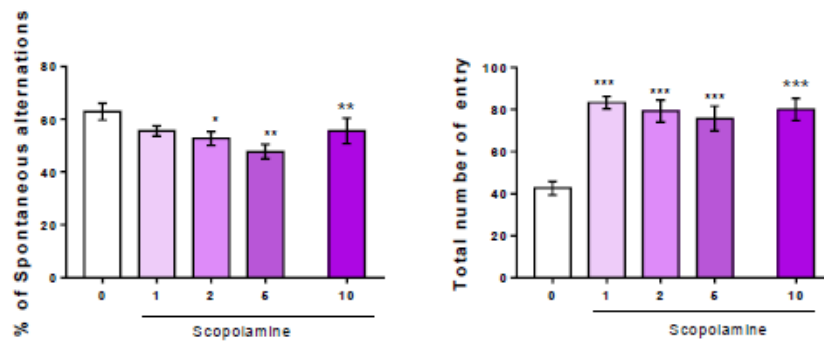
Dose-

1 day Single treatment

Route of Administration: IP

Y- maze test

- spatial learning and memory.
 - Number of Total Entry
 - Spontaneous alteration (%)
 - Duration 10 mins



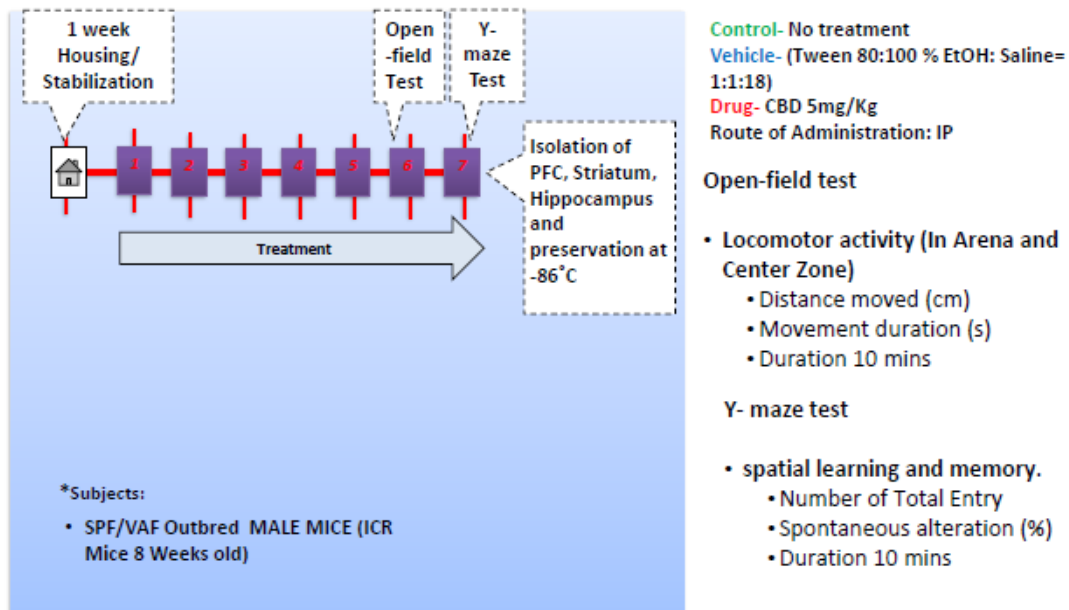
10mg/kg : DW(for 6Ds) + scopolamine (from previous data)
 0-5 mg/kg : Single scopolamine treatment

Data Analysis

Assessment the effect of CBD (5 mg/kg) , IP on ICR Mice (OFT and Y-maze Test)

Protocol

Locomotor sensitization and spatial learning and memory.



Open-field Test

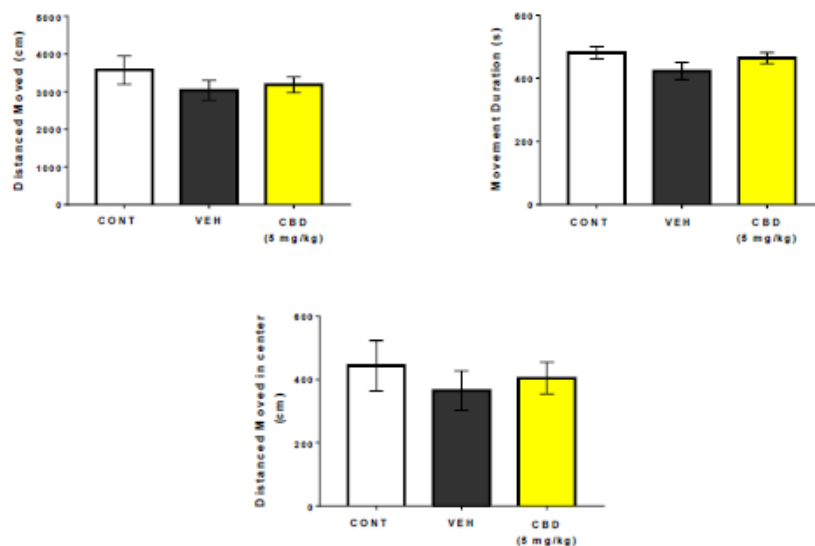


Fig. 1 Effect of CBD on the locomotor activity 8-week old ICR male mice **after a 6-day treatment**. Each bar represents the mean ± S.E.M. of the distance moved, movement duration, ICR in OFT. **n = 10** animals per group.

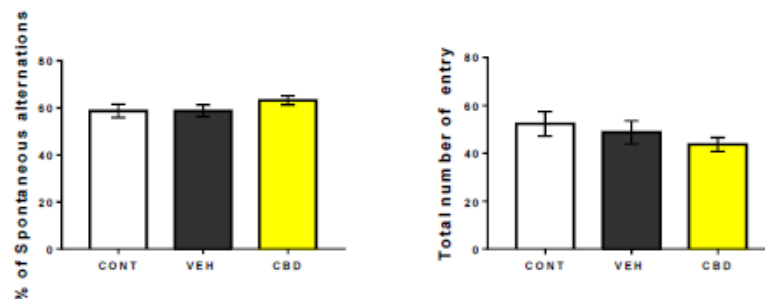


Fig. 2 Effect of CBD on the cognitive function of 8-week old ICR male mice after a 7-day treatment. Each bar represents the mean $\pm$ S.E.M. of the distance moved, movement duration. $n = 10$ animals per group.

Data Analysis

Assessment the effect of CBD at different doses
(1, 3, 10 30 and 100 mpk), i. p on ICR Mice

Y-maze Test after Acute Treatment

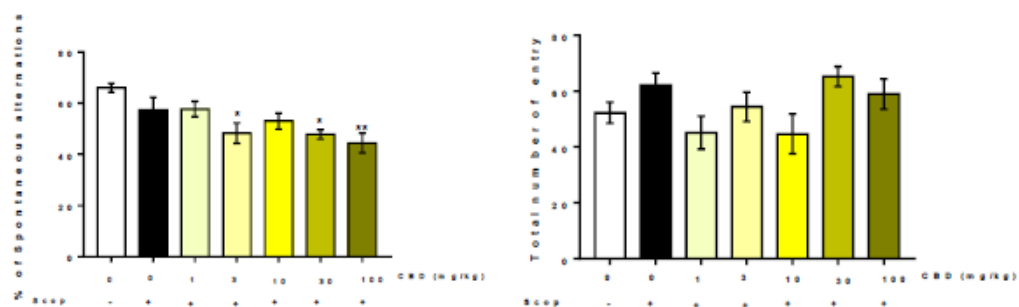


Fig. 12 Effect of CBD at different doses (1, 3, 10, 30, 100 mg/kg) on the cognitive function of Scopolamine (2 mg/kg) induced Alzheimer's 8-week old ICR male mice after single treatment. Each bar represents the mean $\pm$ S.E.M. of the % spontaneous alteration and total number of entry, $n = 4$ to 10 animals per group.

CBD (I/P administration)





Data Analysis

Assessment the effect of CBD at different doses
(1, 3, 10 and 30 mg/kg), p.o ; on ICR Mice

Y-maze Test after Acute Treatment

(Fed state)

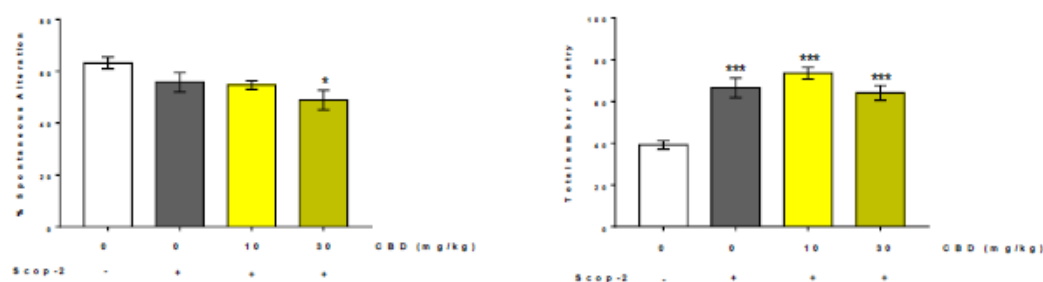


Fig. 12 Effect of CBD oral administration (Fed state) at different doses (10 and 30 mg/kg) on the cognitive function of Scopolamine (2 mg/kg) induced Alzheimer's 8-week old ICR male mice after single treatment. Each bar represents the mean $\pm$ S.E.M. of the % spontaneous alteration and total number of entry, $n = 5$ (Veh) to 10 (CBD) animals per group.

(Fasting state)

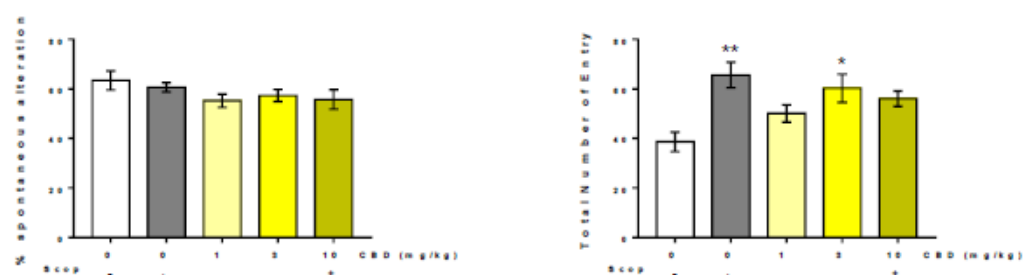


Fig. 12 Effect of CBD oral administration (Fasting state) at different doses (1, 3 and 10 mg/kg) on the cognitive function of Scopolamine (2 mg/kg) induced Alzheimer's 8-week old ICR male mice after single treatment. Each bar represents the mean $\pm$ S.E.M. of the % spontaneous alteration and total number of entry, $n = 5$ (Veh group) to 10 animals (CBD) per group.

Data Analysis

Assessment the effect of Taurine at different doses (10, 30, 60 and 100 mpk), p. o on ICR Mice

Y-maze Test after Acute Treatment

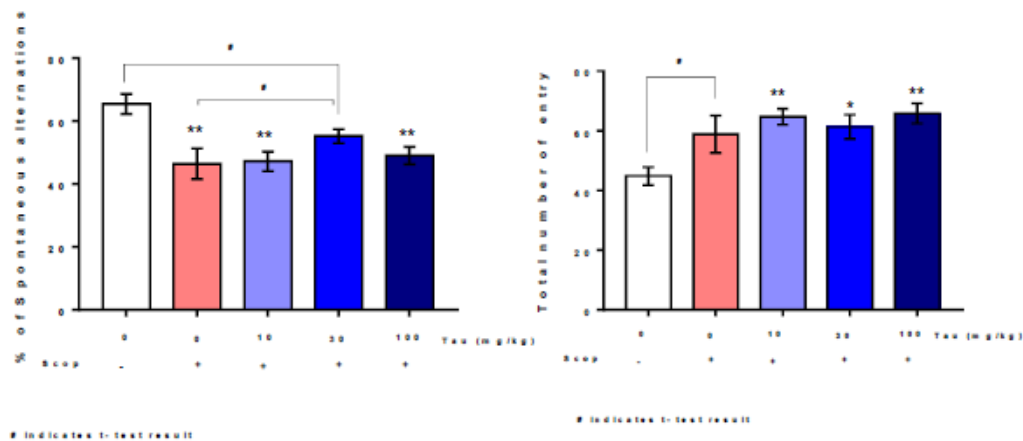


Fig. 11 Effect of Taurine at different doses (10, 30, 100 mg/kg) on the cognitive function of Scopolamine (2 mg/kg) induced Alzheimer's 8-week old ICR male mice after single oral treatment. Each bar represents the mean $\pm$ S.E.M. of the % spontaneous alteration and total number of entry, ICR in Y- Maze. $n = 5$ to 10 animals per group.

Combined with previous Data

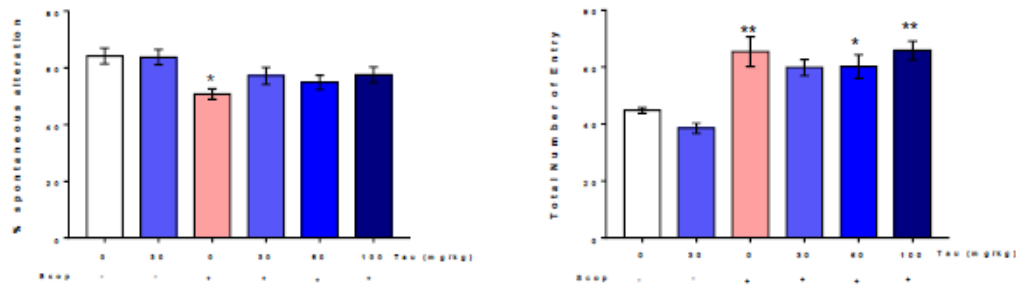
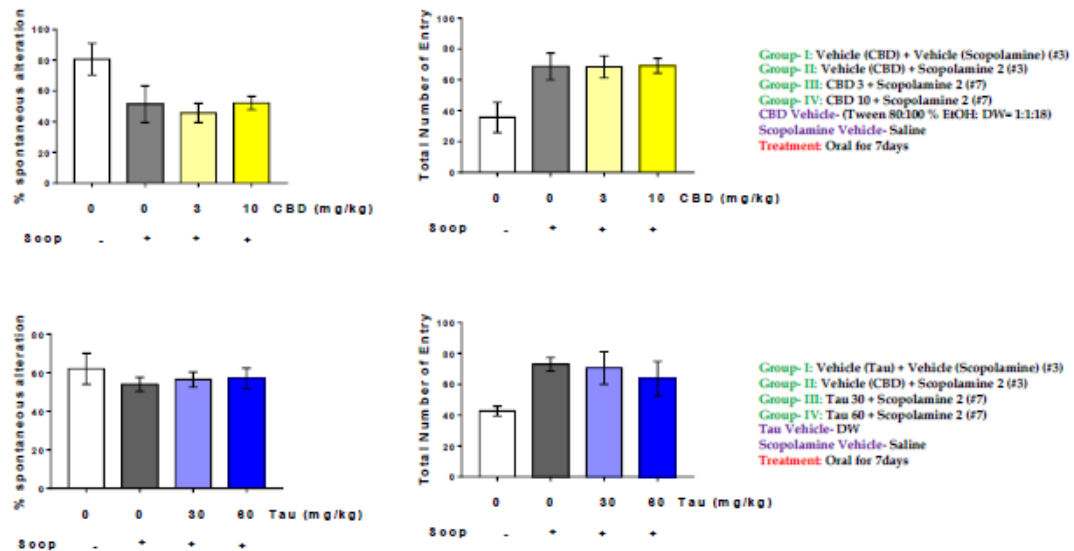


Fig. 11 Effect of Taurine at different doses (30, 60, 100 mg/kg) on the cognitive function of Scopolamine (2 mg/kg) induced Alzheimer's 8-week old ICR male mice after single oral treatment. Each bar represents the mean $\pm$ S.E.M. of the % spontaneous alteration and total number of entry. $n = 5$ (veh) to 10 (tau) animals per group.

Chronic Oral Treatment: for 7days

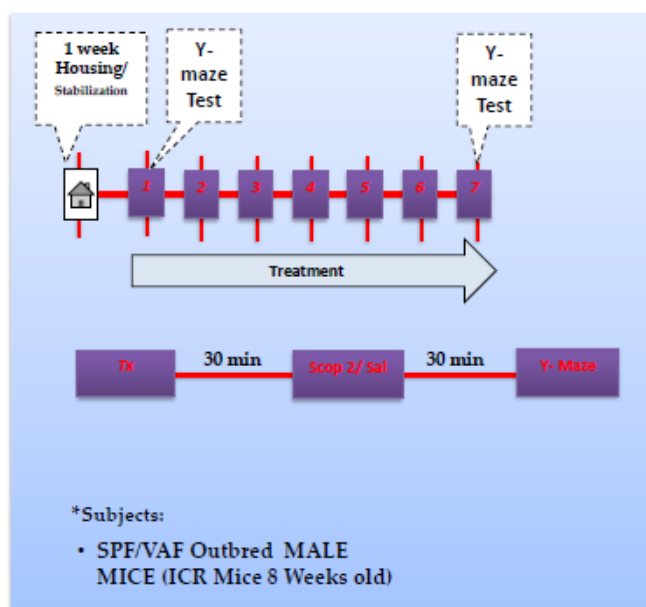


Data Analysis

Assessment the effect of **Combined CBD 10 and Tau 30**
p.o ; on ICR Mice

Y-maze Test after Acute and Chronic oral Treatment

Protocol

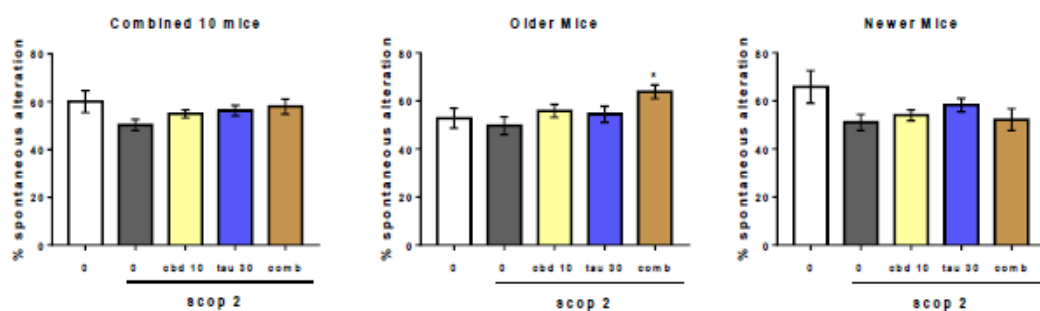


Mice/ Group

Group- I: Vehicle + Saline	#5
Group- II: Vehicle + Scopolamine 2	#5
Group- III: CBD 10+ Scopolamine 2	#7
Group- III: TAU 30+ Scopolamine 2	#7
Group- III: Combined+ Scopolamine 2	#10

CBD Vehicle- (Tween 80:100 % EtOH: DW= 1:1:18)
Scopolamine Vehicle- Saline

Route of Administration: Oral (Tx/ Veh)
IP (Scopolamine 2/ Sal)



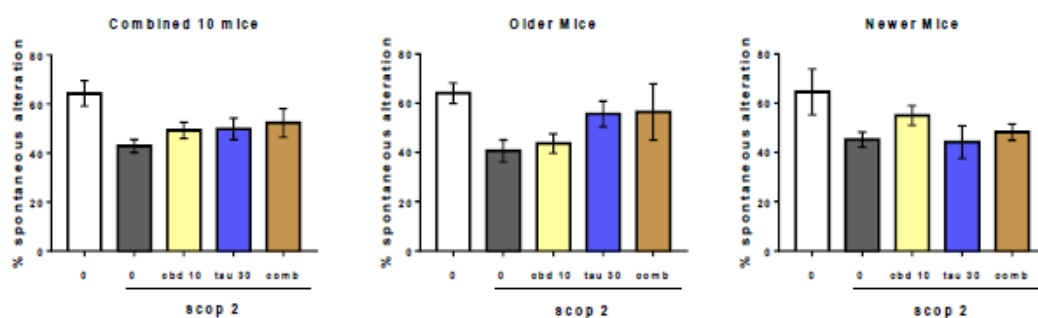
Acute Oral Treatment

Older Mice- 16-17 weeks

Newer Mice- 8 weeks

Vehicle for CBD and Tau- Tween 80: EtOH: DW= 1:1:18

No. of sample- #5 (Except older mice veh group- #4)



Chronic Oral Treatment

Y-Maze- On 7th Day

Older Mice- 17-18 weeks

Newer Mice- 9 weeks

Vehicle for CBD and Tau- Tween 80: EtOH: DW= 1:1:18

No. of sample- #5 (Except older mice veh group- #4)

Summarizes from our in vivo findings

- Scopolamine at dose of 2 mpk causes memory impaired to deficit spatial learning memory.
- CBD (10 mpk) and Taurine (30 mpk) screened dose is effective with consideration of vehicle group but not significantly increased the % spontaneous alterations.
- CBD treatment does not affects spatial learning memory on scopolamine induced Alzheimer's mice model when treat alone.
- Synergistic effects find on older ICR mice in acute treatment. So that why, Aging mice model may be a good candidate for in-vivo model to induce Alzheimer's Disease.

Hypothesis

- Intensity of ER stress (Eustress vs Distress) regulates neuroinflammation in Alzheimer's disease.
- Transmembrane Bax Inhibitor Motif containing 6/ Bax Inhibitor-1 (TMBIM6/BI-1) initially may facilitates ER stress mediated UPR (Unfolded protein response) through interact with IRE-1 for surviving Neuronal cell death.
- Several stressor (sleep disruption mice model/ Alzheimerogenic Chemicals such as LPS, ethanol, Colchicine, A β) may causes gliosis in cortex and hippocampal region.
- Mild ER stress (Eustress/ ER Hormesis) may ameliorate Cognitive impairment via regulation of microglial polarization.
- Chronic stress (Distress) may causes neuronal cell death through RIDD (regulated Ire1 dependent decay) activated pathway
- Aquaporin-4 mediates communication between astrocyte and microglia may be a potential targeting approach for Alzheimer's Diseases by maintaining neuroplasticity.

AD CAUSATIVE HYPOTHESES

HYPOTHESES	POSTULATION	REFERENCES
TAU HYPOTHESIS	Hyper phosphorylation of tau protein leads to the conversion of normal tau into the paired helical filament (PHF-tau) and NFTs (monomer to oligomer)	Mohandas E, Neurobiology of Alzheimer's disease. Indian J Psychiatry. 2009;51(1):55–61. Swerdlow RH. Mitochondria in cybrids containing mtDNA from persons with mitochondrialopathies. J Neurosci Res. 2007;85(15):3416–28. Duan AR, et al. Interactions between tau and different conformations of tubulin: implications for tau function and mechanism. J Mol Biol. 2017;429(9):1424–38. Guerrero-Muñoz MJ, Tau Oligomers: The Toxic Player at Synapses in Alzheimer's Disease. Front Cell Neurosci. 2015;9:464.
AMYLOID CASCADE HYPOTHESIS (widely accepted and is the most well-studied)	Amyloid plaques , made by the accumulation of A β peptides which resulted from the proteolytic separation of APP, are crucial in AD pathology. A β 1-40 (A β 40) and A β 1-42 (A β 42) are the two major occurring isoforms	Golde ET, Biochemical detection of Ab isoforms: implications for pathogenesis, diagnosis and treatment of Alzheimer's disease. Biochim Biophys Acta. 2000;26(1502(1)):172–87. Hardy J, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science. 2002;297(5580):353–6.
CHOLINERGIC HYPOTHESIS (The oldest theory among the AD causative theories)	A decrease in neurotransmitters, known as acetylcholine , in neurons is responsible for AD etiology.	Terry AV, The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug development. J Pharmacol Exp Ther. 2003;306(3):821–7.

AD CAUSATIVE HYPOTHESES

HYPOTHESES	POSTULATION	REFERENCES
INFLAMMATION HYPOTHESIS	Microglia-related pathways were observed as crucial risk factors for AD and its pathogenesis. During the initial phase of AD, microglia and its associated proteins, such as the Triggering Receptor Expressed on Myeloid Cells-2 (TREM2), can influence synaptic reduction. subsequently, amyloid plaques will be surrounded by aberrant microglia and astrocytes, and produce several proinflammatory cytokines.	Bolós M, Alzheimer's disease as an inflammatory disease. Biomol Concepts. 2017;8(1):37–43. Paolicelli RC et al. Synaptic pruning by microglia is necessary for normal brain development. Science. 2011;333(6048):1456–8.
MITOCHONDRIAL CASCADE HYPOTHESIS (Physiologic mechanisms underlying AD and the aging brain are similar)	Mitochondrial abnormality is the main reason for Aβ accumulation, the formation of NFT, and degeneration of synapses in AD. AD brain mitochondrial abnormality propels amyloidosis, phosphorylation of tau, and cell cycle re-entry.	Swerdlow RH, A mitochondrial cascade hypothesis blight for sporadic Alzheimer's disease. Med Hypotheses. 2004;63(1):8–20. Swerdlow RH, Mitochondria in Alzheimer's disease. Int Rev Neurobiol. 2002;53:341–85. PMID: 12512346.
OXIDATIVE STRESS HYPOTHESIS	The brain is known to consume more energy and exert more functions than any other organs during mitochondrial respiration, which also increases the likelihood of reactive oxygen species (ROS) exposure . Furthermore, AD is closely linked to molecular oxidative stress, as well as protein nitration, the rise of protein oxidation, lipid peroxidation, and glycooxidation. Moreover, AD is closely linked to the aggregation of Ab , which has been reported to cause oxidative stress ⁷⁵	Manoharan S, The role of reactive oxygen species in the pathogenesis of Alzheimer's disease, Parkinson's disease, and Huntington's disease: Oxid Med Cell Longev. 2016;2016:8590578. Butterfield DA, Amyloid beta-peptide and amyloid pathology are central to the oxidative stress and inflammatory cascades under which Alzheimer's disease brain exists. J Alzheimers Dis. 2002;4(3):193–201.

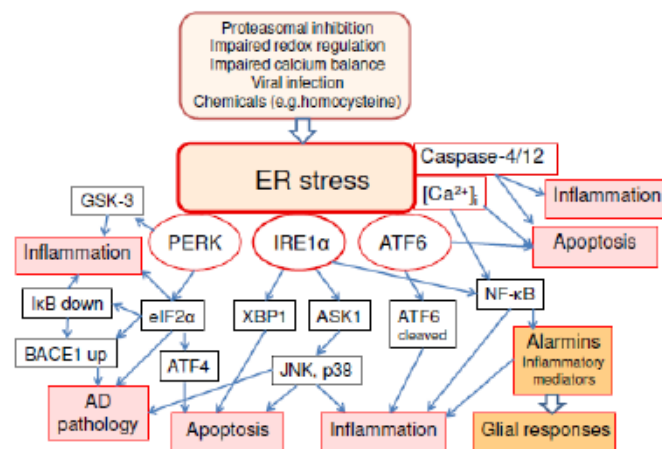


Figure 1

A schematic presentation of the three branches of UPR, their signaling pathways, and pathological responses with respect to inflammation and AD pathology.

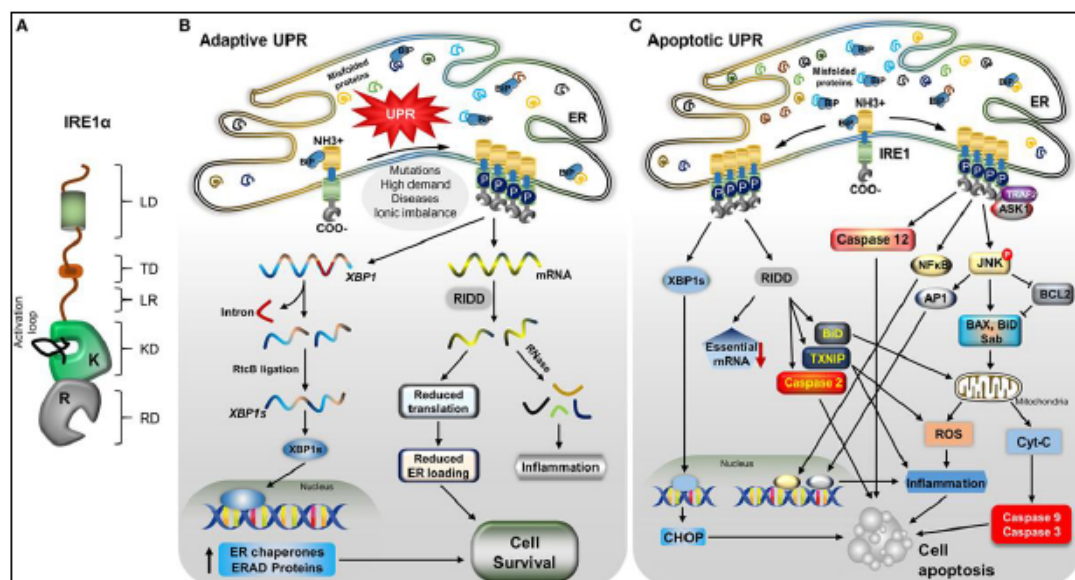


Figure Courtesy: Raghu Patil et al, 2018 Front. Immunol., Sec. Autoimmune and Autoinflammatory Disorders. Volume 9 - 2018



TMBIM6 regulates redox-associated posttranslational modifications of IRE1 α and ER stress response failure in aging mice and humans

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S-nitrosylation

Sulfonation

ER stress response failure

ABSTRACT

Age-associated persistent ER stress is the result of declining chaperone systems of the ER that reduces cellular functions, induces apoptosis, and leads to age-related diseases. This study investigated the previously unknown regulatory mechanism of TMBIM6 during age-associated hepatic abnormalities. Wild-type (WT) and the TMBIM6 knockout (TMBIM6^{-/-}) mice liver, human liver samples from different age groups were used to demonstrate the effect of physiological aging on liver. For TMBIM6 rescue experiments, TMBIM6^{-/-} old mice and stable human hepatic cell lines expressing TMBIM6 were used to study the functional role of TMBIM6 on aging-associated steatosis and its associated mechanisms. In aging humans and mice, we observed declined expression of TMBIM6 and aberrant UPR expression, which were associated with high hepatic lipid accumulation. During aging, TMBIM6-deficient mice had increased senescence than their WT counterparts. We identified redox-mediated posttranslational modifications of IRE1 α such as S-nitrosylation and sulfonation were higher in TMBIM6-deficient aging mice and humans, which impaired the ER stress response signaling. Sulfonation of IRE1 α enhanced regulated IRE1 α -dependent decay (RIDD) activity inducing TMBIM6 decay, whereas S-nitrosylation of IRE1 α inhibited XBP1 splicing enhancing the cell death. Moreover, the degradation of miR-33b-3p by strong IRE1 α cleavage activity enhanced the expression of FTF1B, resulting in diminishing phosphorylation of PERK. The re-expression of TMBIM6 reduced IRE1 α modifications, preserved ER homeostasis, reduced senescence and senescence-associated lipid accumulation in human hepatic cells and TMBIM6-depleted mice. S-nitrosylation or sulfonation of IRE1 α and its controller, the TMBIM6, might be the potential therapeutic targets for maintaining ER homeostasis in aging and aging-associated liver diseases.

Biology 2021, 10, 542

REF: Abate, G.; Uberti, D.; Tambaro, S.
Potential and Limits of Cannabinoids in
Alzheimer's Disease Therapy.
Biology 2021, 10, 542.

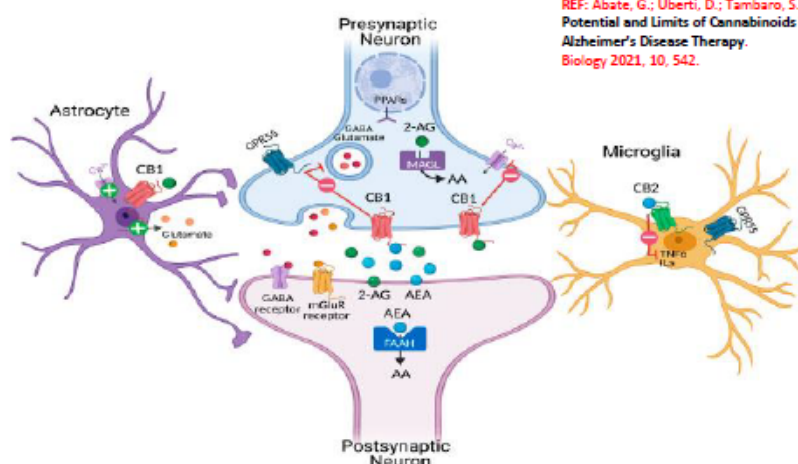
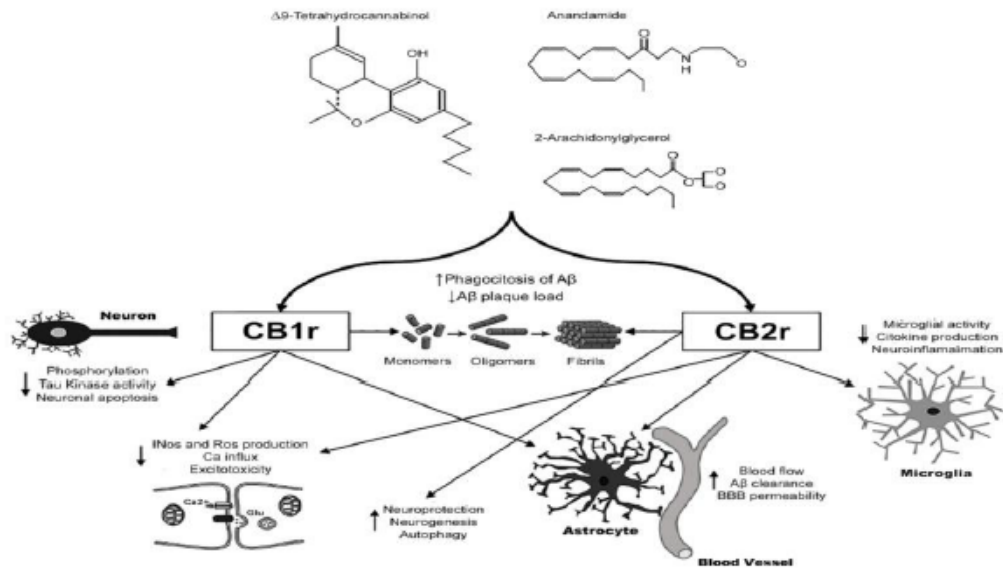
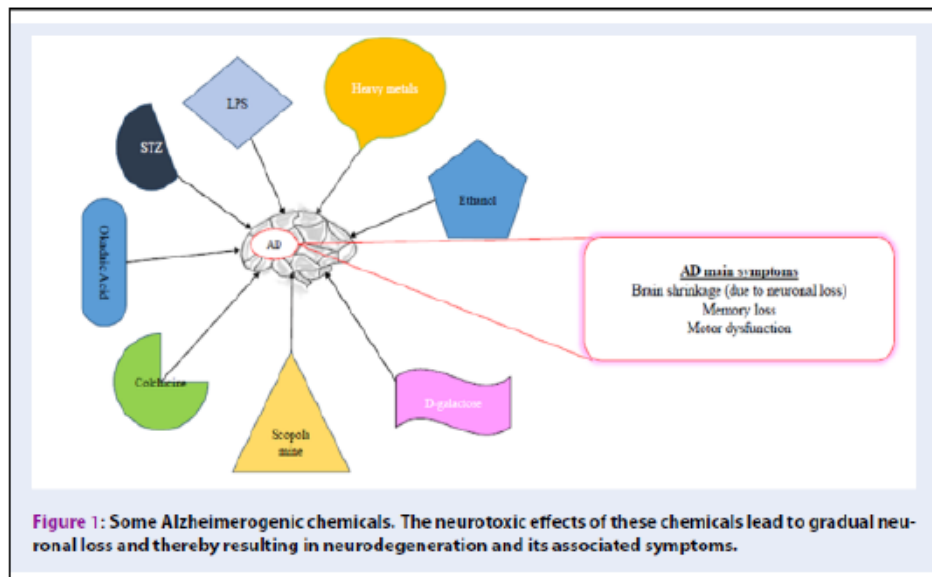


Figure: Schematic representation of the endocannabinoid system in the brain. Putative localization of endocannabinoid receptors in the nervous and glia system. Enzymes involved in endocannabinoid biosynthesis and degradation are reported in both pre- and postsynaptic neurons. 2-AG (green) and AEA (blue) are synthesized from phospholipids on demand. Activation of presynaptic CB1 receptors negatively modulates cell calcium influx and the release of GABA and glutamate neurotransmitters in GABAergic and glutamatergic neurons, respectively. Instead, the stimulation of CB1 in astroglia positively modulates calcium influx and glutamate release. Activation of CB2 in microglia negatively affects the release of TNF and ILs.

AA: arachidonic acid; 2-AG: 2-acylglycerol; AEA: anandamide; PPARs: peroxisome proliferator activated receptors; FAAH: Fatty acid amide hydrolase; MAGL: monoacylglycerol lipase; mGluR metabotropic glutamate receptors; ILs: interleukins; TNF: tumor necrosis factor-.

Commonly Used Chemicals To Induce Ad-like Symptoms (Ad Modelling)



Summary of the beneficial effects of the cannabinoid compounds in Alzheimer's disease pathology. Cannabinoid treatment can modulate multiple disease processes including A β and tau processing, neuroinflammation, microglial activation, mitochondrial dysfunction and excitotoxicity. CB2 receptor agonists reduce the release of pro-inflammatory molecules, facilitate A β clearance by promoting microglia phagocytic phenotype, reduce A β neurotoxicity. Moreover, CB2-mediated activity reduces oxidative stress damage produced by reactive oxidative species (ROS) and tau hyperphosphorylation. CB1 receptor agonists regulate iNOS protein expression and NO production, inhibit hyperphosphorylation of tau protein through the inhibition of tau kinase, reduce Abeta plaque load through the activation of PPAR gamma receptor and the stimulating the expression of LRP1. 337x257mm (96 x 96 DPI).

Future Plan

- Identify the ER stress mediated neuroinflammation and Neuroprotection in Alzheimer's disease
- Design Alzheimer's mice model and investigate the behavioral changes by CBD treatment
- Identify the role of BI-1 in ER stress regulating pathway of Neuroinflammation in AD causative hypothesis.
- Quantify the neuronal cell survival appraise in response to Eustress vs Distress condition in cellular model, preferably; neuronal and Non neuronal cell (Astroglial cell)

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Chemistry & Life Science
Sahmyook University



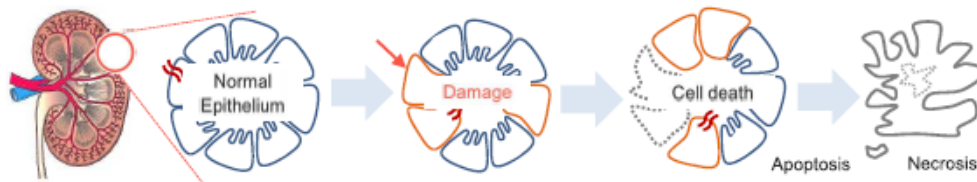
2023.04.05.

우수연구자 초청세미나

성균관대 약대

김형식 교수님

A sensitive urinary biomarker, pyruvate kinase M2, for early detection of diabetic-induced nephropathy



Prof. Hyung Sik Kim, Ph.D.
(hkims@skku.edu)

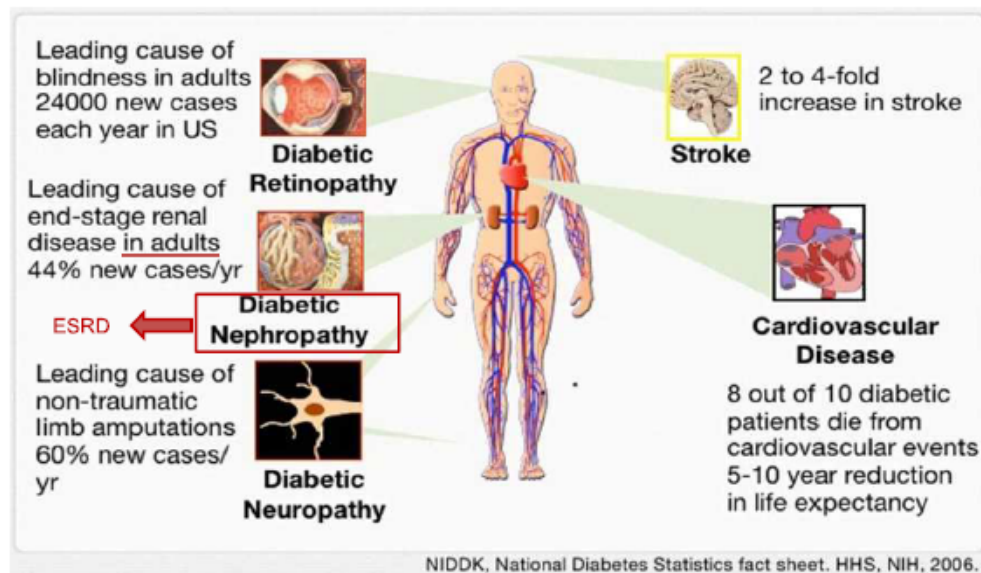
School of Pharmacy
Sungkyunkwan **U**niversity
Suwon, Korea

Disclosures

- what is risk factors of diabetic nephropathy
- why we need new biomarkers to detecting diabetic-induced chronic kidney disease (CKD)
- functional role of PKM2 to understanding cellular mechanism of diabetic-induced renal fibrosis

About diabetes – American Diabetes Association

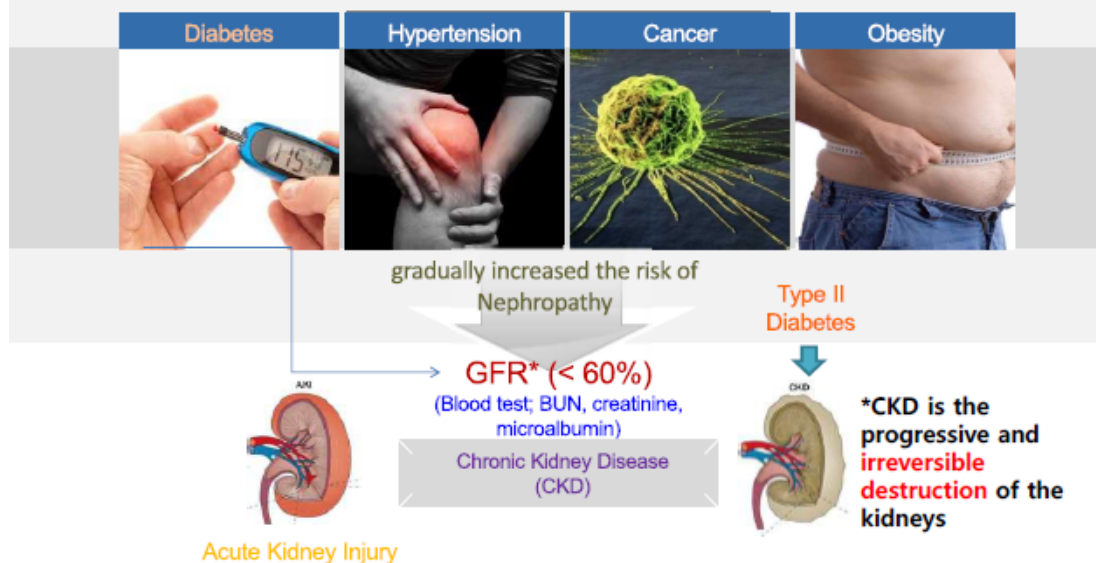
Complications of Diabetes



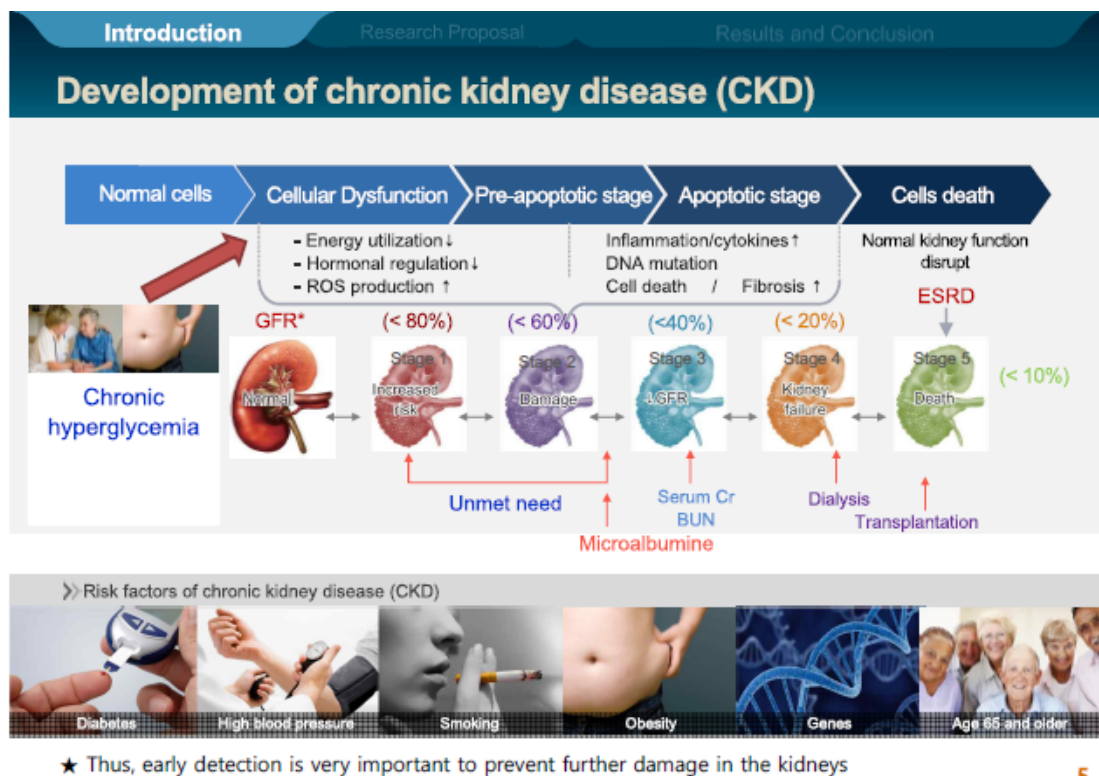
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Risk factors for chronic kidney disease (CKD)

Metabolic Diseases



4



5

Introduction Research Proposal Results and Conclusion

Definition of CKD

- CKD is usually caused by long-term diseases for **≥3 months**, that slowly damages the kidney

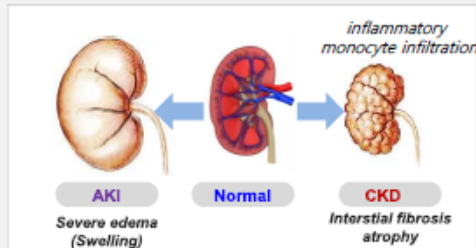
- Kidney damage**, without decreased GFR, as defined by
 - pathologic abnormalities
 - changes of biomarkers for kidney damage, including abnormalities in the composition of the blood or urine
 - abnormalities in imaging tests
- GFR <60%**, with or without kidney damage
- Detect “**microalbumin**” levels in the urine (~30 mg/day) (about **>300 mg/day**)

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Introduction Research Proposal Results and Conclusion

Incidence of chronic kidney disease (CKD)

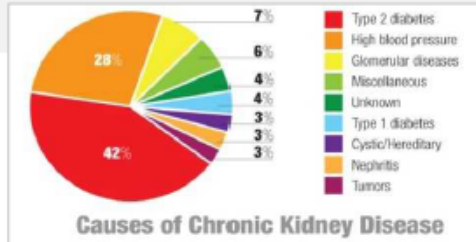
AKI vs. CKD



Incidence of CKD in Korea



Causes of CKD



Dialysis for patients



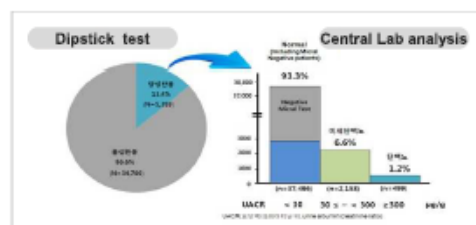
About 10 percent of patients with Type 1 diabetes and 40 percent of those with Type 2 diabetes eventually will suffer from kidney failure (CKD).

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Introduction Research Proposal Results and Conclusion

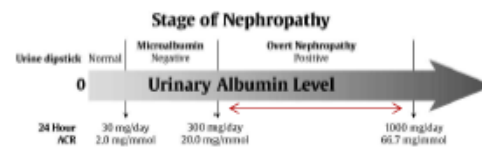
What are the early signs of kidney disease in patients with diabetes?

Urinary microalbumin test

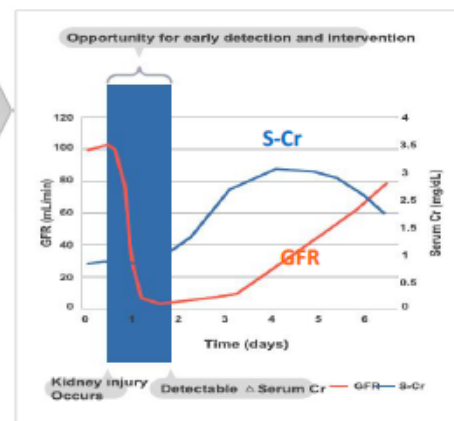
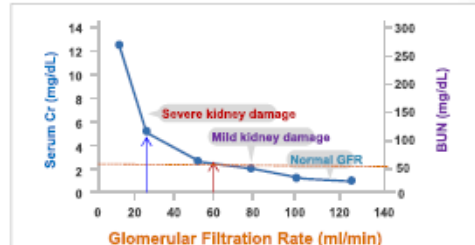


This level of albuminuria is widely accepted as evidence of established nephropathy in both diabetic and nondiabetic renal diseases

Stage-dependent pattern of albumin



Limit of blood BUN or creatinine

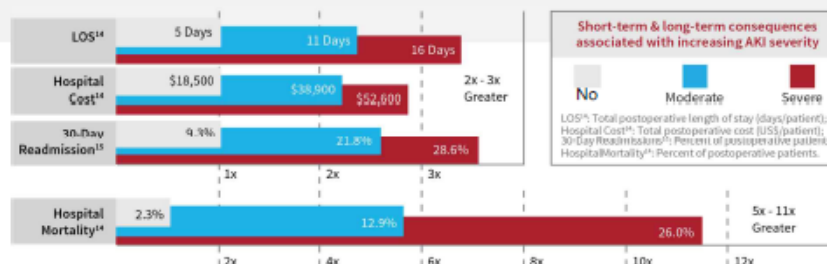


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Introduction Research Proposal Results and Conclusion

Impacts of chronic kidney disease (CKD) induced by diabetic

Mortality and Economic Burden



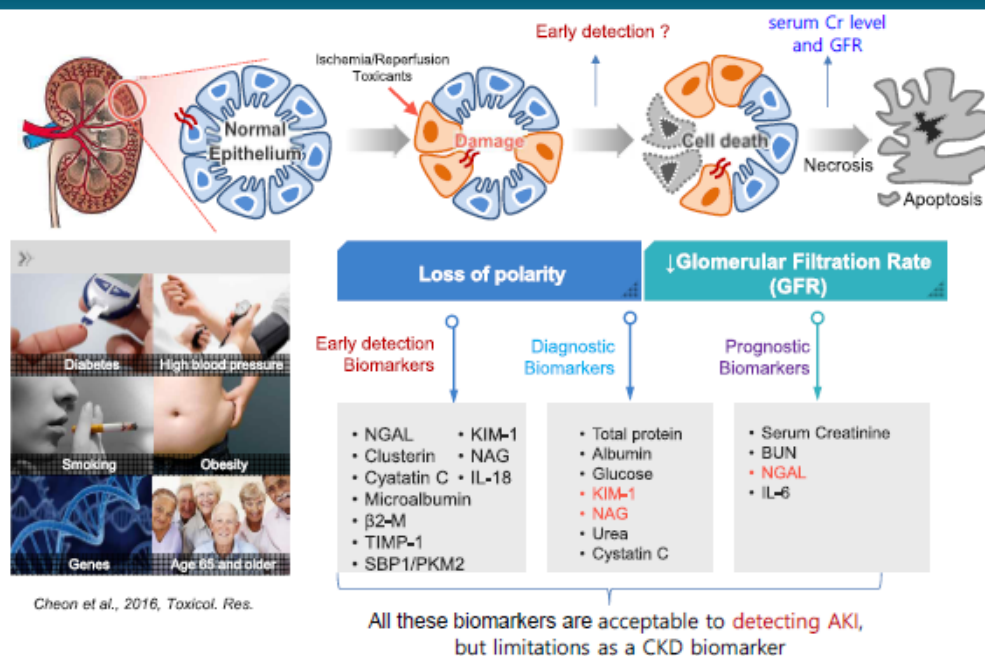
Impacts of CKD

- Hospital cost is increasing for ESRD treatment, **costs the Medicare program are \$52 billion/year (approximately 6.3% of the total annual Health Insurance)**.
- Beginning from **stage 3 CKD**, overall mortality increases to 6 to 13 times higher than that of the general population and, by the onset of **ESRD**, the annual death rate is **>19.2~26%**.
- Therefore, we need sensitive biomarker to early detecting diabetic nephropathy.

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Introduction Research Proposal Results and Conclusion

Importance of new biomarkers to early detecting AKI/CKD



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Our research goals....!



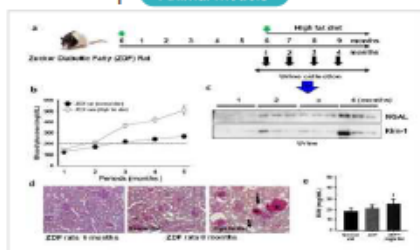
Part I

Identification of New Biomarkers for Early Detection of Diabetic Nephropathy using Proteomic Analysis

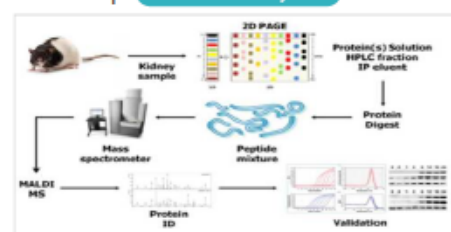
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Experimental design

1st step Animal models



2nd step Proteomics analysis

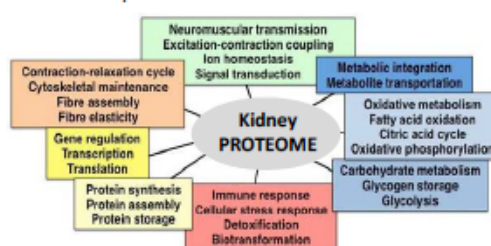


4th step



Clinical study and Validation

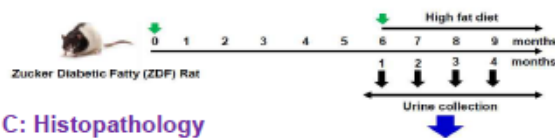
3rd step



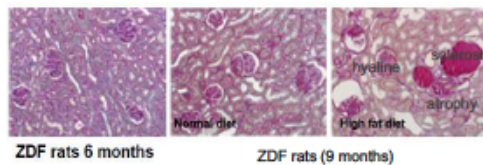
DEPs Identification and its pathway analysis

12

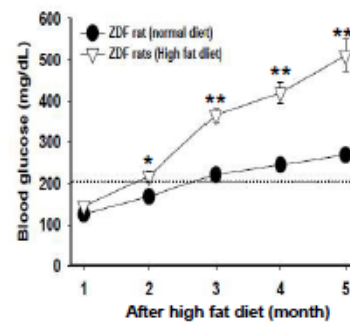
A: Experimental model



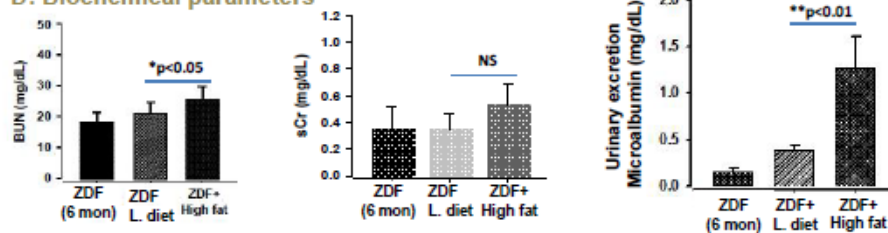
C: Histopathology



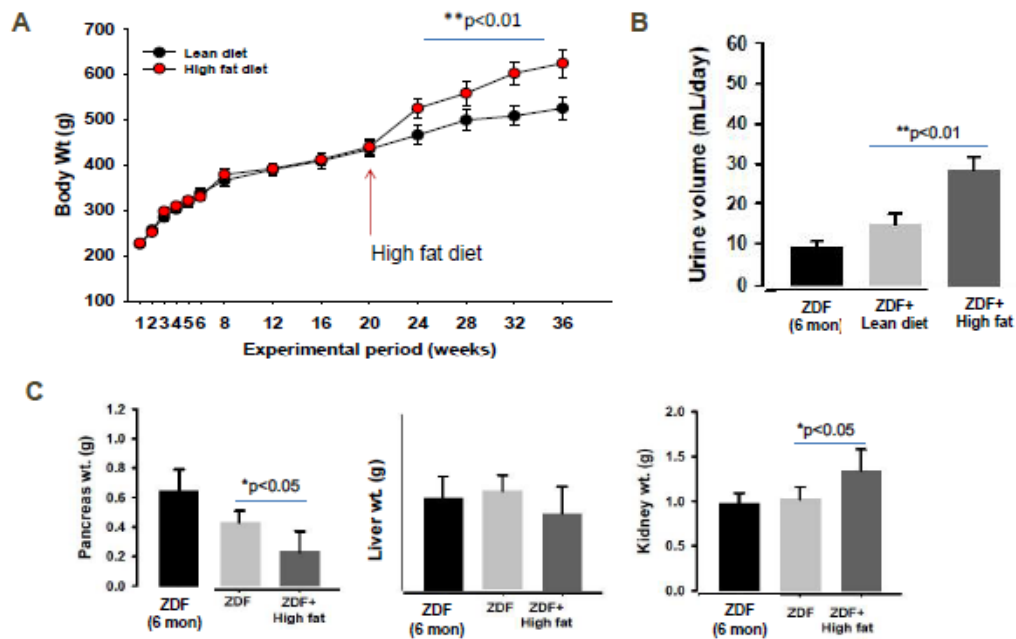
B: Blood glucose



D: Biochemical parameters

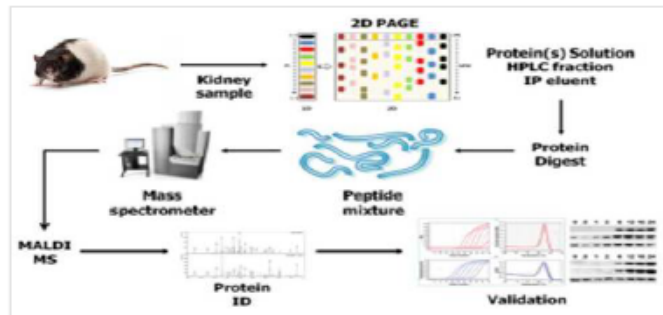


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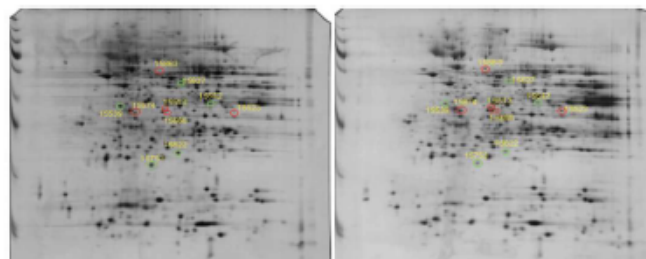
Proteomics analysis in the kidney tissues



Lean diet

vs

High fat diet



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Relative intensities of the positively identified proteins.

prot_hlt_num	protein_description
1	Tubulin alpha-ubiquitous chain (Alpha-tubulin ubiquitous) (Tubulin K-alpha-1)
2	Aldo-keto reductase family 1
3	Pyruvate kinase isozymes M1/M2 (EC 2.7.1.40) (Pyruvate kinase muscle isozyme) (Pyruvate kinase 2/3) → glucose metabolism
4	Keratin, type I cytoskeletal 10 (Cytokeratin-10) (CK-10) (Keratin-10) (K10)
5	Tubulin beta chain (Tubulin beta-5 chain)
6	Actin, cytoplasmic 1 (Beta-actin)
7	Alpha-enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (Non-neural enolase) (NNE) (Enolase 1) (Phosphopyruvate hydratase) (C-myc promoter-binding protein) (MBP-1) (MPB-1) (Plasminogen-binding protein)
8	Elongation factor 1-alpha 1 (EF-1-alpha-1) (Elongation factor 1 A-1) (eEF1A-1) (Elongation factor Tu) (EF-Tu) (Leukocyte receptor cluster member 7)
9	Annexin A2 (Annexin II) (Lipoprotein II) (Calpactin I heavy chain) (Chromobindin-6) (p36) (Protein I) (Placental anticoagulant protein IV) (PAP-IV)
10	Heat shock cognate 71 kDa protein (Heat shock 70 kDa protein 8)
11	Galectin-1 (Lectin galactoside-binding soluble 1) (Beta-galactoside-binding lectin L-14-I) (Lactose-binding lectin 1) (S-Lac lectin 1) (Galaptin) (14 kDa lectin) (HFL) (HBL) (Putative MAPK-activating protein MP12)
12	ATP synthase subunit beta, mitochondrial precursor (EC 3.6.3.14)
13	L-lactate dehydrogenase A chain (EC 1.1.1.27) (LDH-A) (LDH muscle subunit) (LDH-M) (Proliferation-inducing gene 19 protein) (Renal carcinoma antigen NY-REN-59) → glucose metabolism
14	Alpha-actinin-1 (Alpha-actinin cytoskeletal isoform) (Non-muscle alpha-actinin-1) (F-actin cross-linking protein)
15	Beta-enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (Muscle-specific enolase) (MSE) (Skeletal muscle enolase) (Enolase 3)
16	Type I collagen alpha 1 chain → EMT activation
17	Aspartate aminotransferase, mitochondrial precursor (EC 2.6.1.1) (Transaminase A) (Glutamate oxaloacetate transaminase 2)
18	Heterogeneous nuclear ribonucleoprotein U (hnRNP U) (Scaffold attachment factor A) (SAF-A) (p120) (pp120)
19	Importin beta-3 (Karyopherin beta-3) (Ran-binding protein 5) (RanBP5)
20	ATP synthase subunit alpha, mitochondrial precursor (EC 3.6.3.14)
21	Glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) (GAPDH)
22	Heat shock protein HSP 90-beta (HSP 84) (HSP 90)
23	60S ribosomal protein L11 (CLL-associated antigen KW-12)
24	Elongation factor 2 (EF-2)
25	Serum albumin precursor
26	Aconitate hydratase, mitochondrial precursor (EC 4.2.1.3) (Citrate hydro-lyase) (Aconitase)
27	Peroxisome oxidin-1 (EC 1.11.1.15) (Thioredoxin peroxidase 2) (Thioredoxin-dependent peroxide reductase 2) (Proliferation-associated gene protein) (PAG) (Natural killer cell-enhancing factor A) (NKEF-A)
28	Heterogeneous nuclear ribonucleoprotein K (hnRNP K) (Transformation up-regulated nuclear protein) (TUNP)
29	Fructose-bisphosphate aldolase A (EC 4.1.2.13) (Muscle-type aldolase) (Lung cancer antigen NY-LU-1)
32	14-3-3 protein beta/alpha (Protein kinase C inhibitor protein 1) (KCIP-1) (Protein 1054)

Continued

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Relative intensities of the positively identified proteins.

33	Vimentin domain protein -> EMT activation
34	Malate dehydrogenase 1
35	Dihydropyrimidinase-related protein 2 (DRP-2) (Collapsin response mediator protein 2) (CRMP-2) (N2A3)
36	Peptidyl-prolyl cis-trans isomerase A (EC 5.2.1.8) (PP1ase A) (Rotamase A) (Cyclophilin A) (Cyclosporin A-binding protein)
37	Histone H2B type 1-B (H2B.f) (H2B/f) (H2B.1)
38	60S ribosomal protein L14 (CAG-1SL 7)
39	Heat shock protein 75 kDa, mitochondrial precursor (HSP 75) (Tumor necrosis factor type 1 receptor-associated protein) (TRAP-1) (TNFR-associated protein 1)
40	T-complex protein 1 subunit gamma (TCP-1-gamma) (CCT-gamma) (hTRiC5)
41	Coatomer subunit beta (Beta-coat protein) (Beta-COP)
42	Malate dehydrogenase, mitochondrial precursor (EC 1.1.1.37)
43	fibronectin type III domain protein
44	KNG1 kininogen 1
45	GPX1 glutathione peroxidase 1
46	High mobility group protein B1 (High mobility group protein 1) (HMG-1) -> cell cycle
47	Alpha-1-acid glycoprotein
48	Intracellular adhesion molecular 2
49	Protein tyrosine phosphatase D30
50	Protein RCC2 (Telophase disk protein of 60 kDa) (RCC1-like protein TD-60)
51	Probable ATP-dependent RNA helicase DDX5 (EC 3.6.1.-) (DEAD box protein 5) (RNA helicase p68)
52	Retinoblastoma-associated protein (PP110) (P105-RB) (RB)
53	Selenium binding protein 1
54	Orphan nuclear receptor NR1B3 (Constitutive androstane receptor) (CAR) (Constitutive activator of retinoid response) (Constitutive active response) (Orphan nuclear receptor MB67)
55	Uromodulin
56	Serine/threonine-protein kinase tousled-like 2 (EC 2.7.11.1) (Tousled-like kinase 2) (PKU-alpha)
57	60S ribosomal protein L18a
58	Thyroid hormone receptor-associated protein complex 100 kDa component (Trap100) (Thyroid hormone receptor-associated protein 4) (Vitamin D3 receptor-interacting protein complex component DRIP100) (Activator-recruited cofactor 100 kDa component) (ARC100)

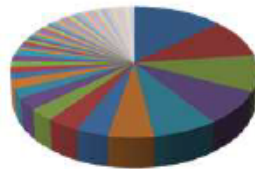
17

Relative intensities of the negatively identified proteins.

prot_hit_num	protein_description
3	Clathrin heavy chain 1 (CLH-17)
30	T-complex protein 1 subunit theta (TCP-1-theta) (CCT-theta) (Renal carcinoma antigen NY-REN-15)
32	T-complex protein 1 subunit delta (TCP-1-delta) (CCT-delta) (Stimulator of TAR RNA-binding)
36	Cullin-associated NEDD8-dissociated protein 1 (Cullin-associated and neddylation-dissociated protein 1) (p120 CAND1) (TBP-interacting protein TIP120A) (TBP-interacting protein of 120 kDa A)
41	Endoplasmic precursor (Heat shock protein 90 kDa beta member 1) (94 kDa glucose-regulated protein) (GRP94) (gp96 homolog) (Tumor rejection antigen 1)
42	78 kDa glucose-regulated protein precursor (GRP 78) (Heat shock 70 kDa protein 5) (Immunoglobulin heavy chain-binding protein) (BIP) (Endoplasmic reticulum luminal Ca(2+)-binding protein grp78)
47	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit precursor (EC 2.4.1.119) (Oligosaccharyl transferase 48 kDa subunit) (DDOST 48 kDa subunit)
51	α2u-Globulin
54	Glutathione S-transferase, pi 2 -> oxidative stress
63	Gamma-enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (Neural enolase) (Neuron-specific enolase) (NSE) (Enolase 2) - Homo sapiens (Human)
5	Regulatory protein SIR2 homolog 1 -> aging and autophagy
19	Cullin-associated NEDD8-dissociated protein 1 (Cullin-associated and neddylation-dissociated protein 1) (p120 CAND1) (TBP-interacting protein TIP120A) (TBP-interacting protein of 120 kDa A) -
21	78 kDa glucose-regulated protein precursor (GRP 78) (Heat shock 70 kDa protein 5) (Immunoglobulin heavy chain-binding protein) (BIP) (Endoplasmic reticulum luminal Ca(2+)-binding protein grp78) -
24	Gamma-enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (Neural enolase) (Neuron-specific enolase) (NSE) (Enolase 2)
34	Peroxiredoxin 1 -> oxidative stress
36	Hemoglobin beta-chain complex
38	T-complex protein 1 subunit theta (TCP-1-theta) (CCT-theta) (Renal carcinoma antigen NY-REN-15)
53	Dolichyl-diphosphooligosaccharide-protein glycosyltransferase 48 kDa subunit precursor (EC 2.4.1.119) (Oligosaccharyl transferase 48 kDa subunit) (DDOST 48 kDa subunit)
56	Clathrin heavy chain 1 (CLH-17)
65	Endoplasmic precursor (Heat shock protein 90 kDa beta member 1) (94 kDa glucose-regulated protein) (GRP94) (gp96 homolog)

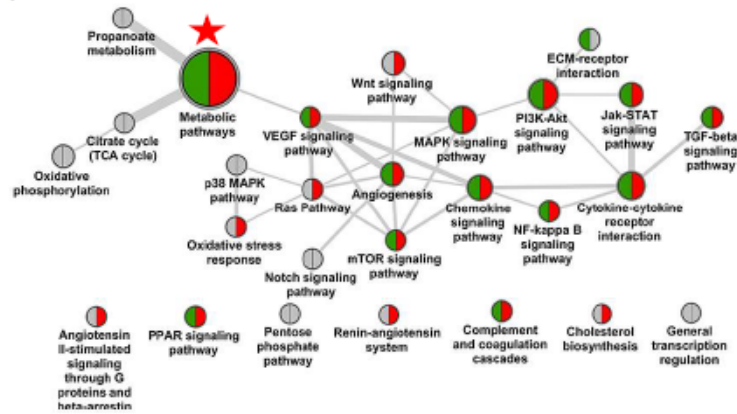
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Differentially expressed proteins (Lean diet vs high fat diet)



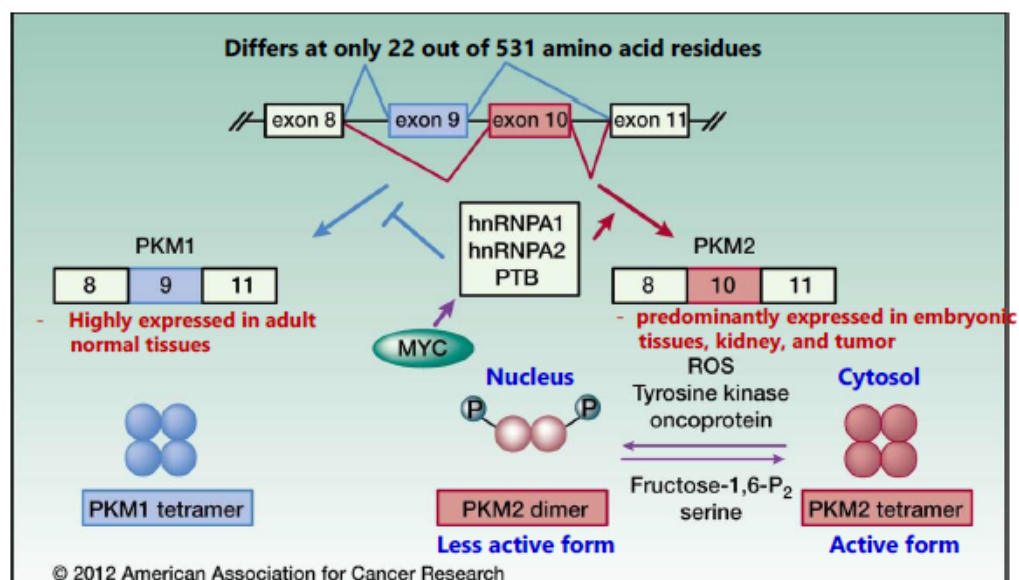
- EMT
- Cell death/cell cycle
- Actin cytoskeleton
- Mitochondrial function
- Inflammation
- Others

Pathway identification



Pathways are marked for holding biomarker candidates (green)

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Introduction Aims of Study **Results and Discussion**

A schematic illustration of the role PKM2 plays in reprogramming cancer metabolism

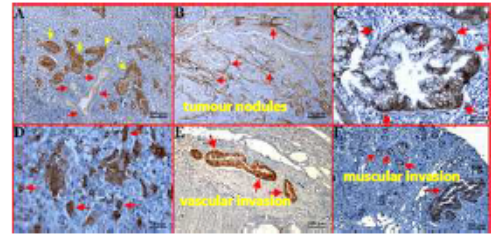
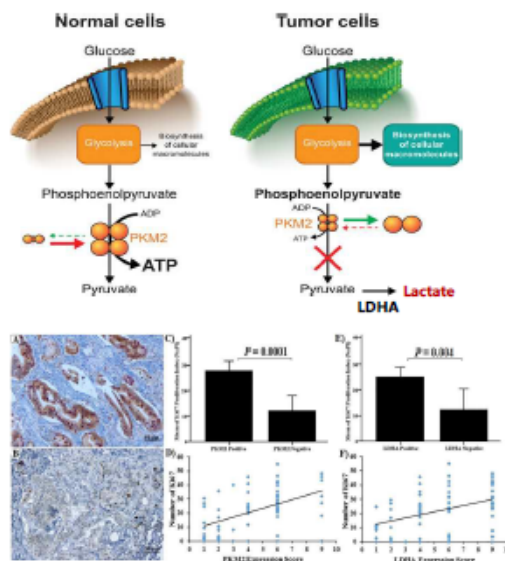


Fig. 2. Immunohistochemical staining of PKM2 expression in representative pancreatic tumor sections. (Yellow, poorly diff.)

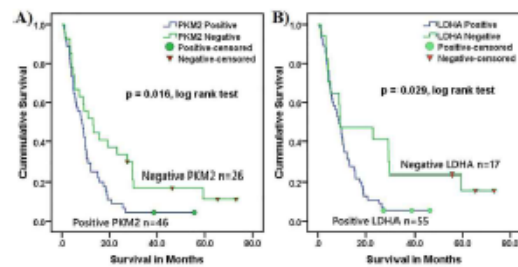
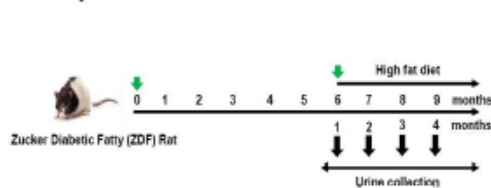


Fig. 4. Survival rates of PKM2 and LDHA expression in the tumor patients

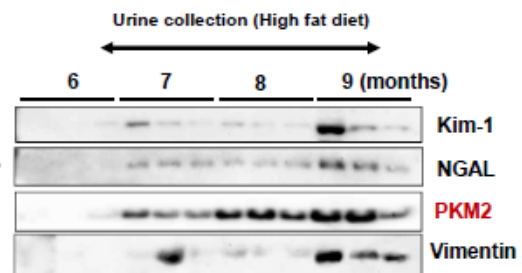
Introduction Research Proposal **Results**

Implications of Results

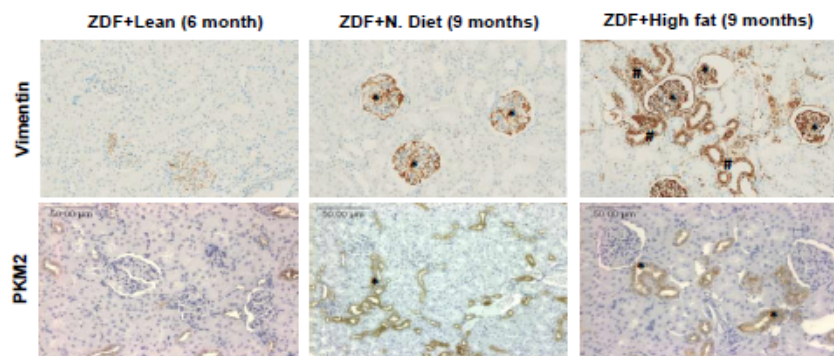
A: Experimental model



B

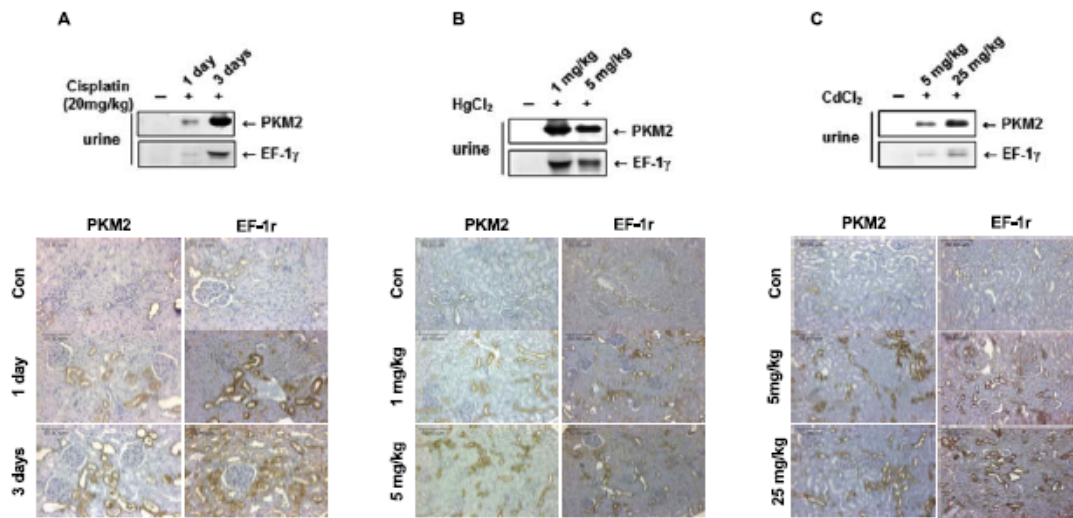


C



Implications of Results

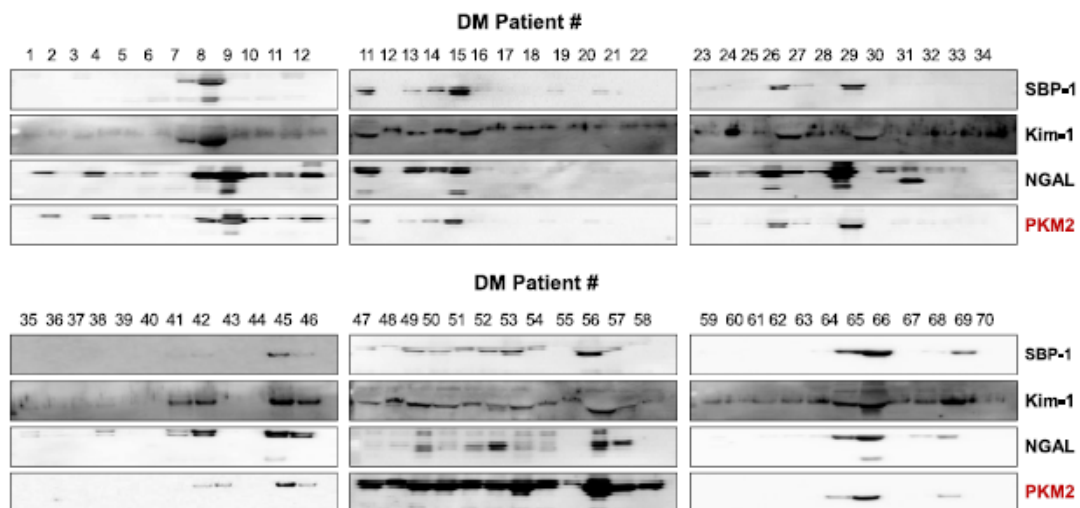
단백체 기법에 의한 신장질환 조기검색을 위한 신규 바이오마커 (PKM2)의 발굴



Kim et al., Toxicological Sci., 2016

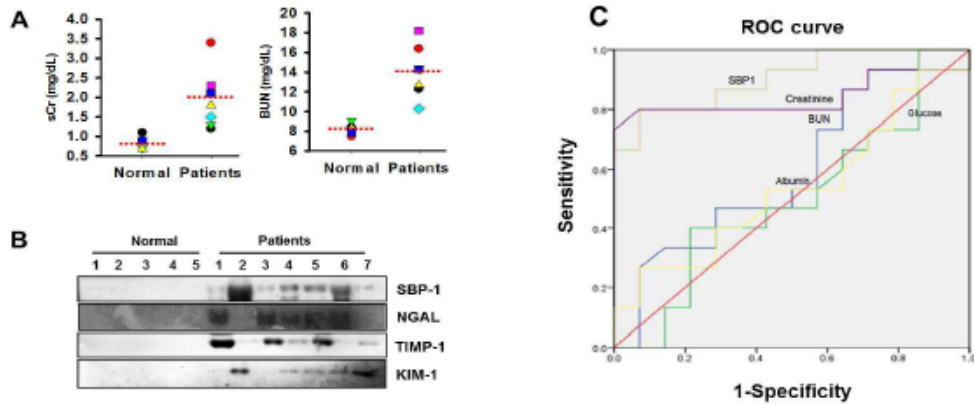
Implications of Results

Validation of new biomarkers PKM2 in the clinical trials



Implications of Results

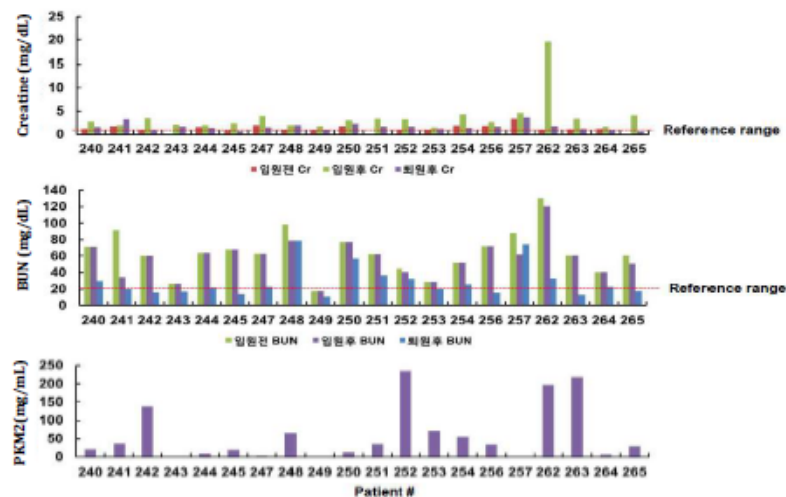
급성신부전증 환자에서 biomarker SBP1에 대한 민감도 및 특이도



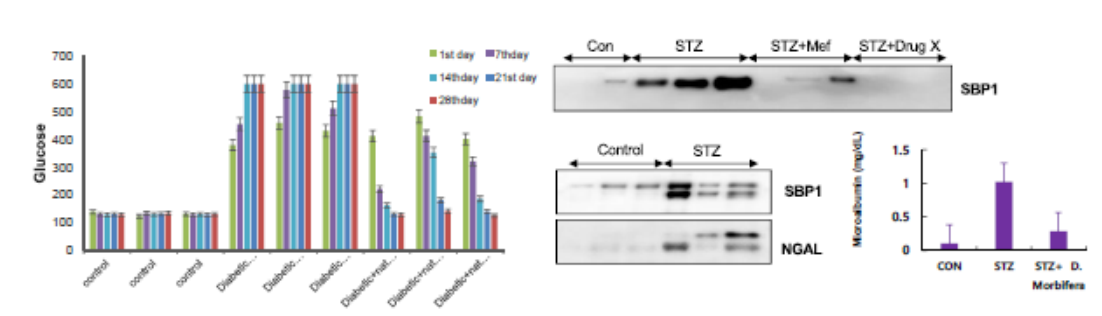
Kim et al., J. Toxicol. Environ Health, 2017

Implications of Results

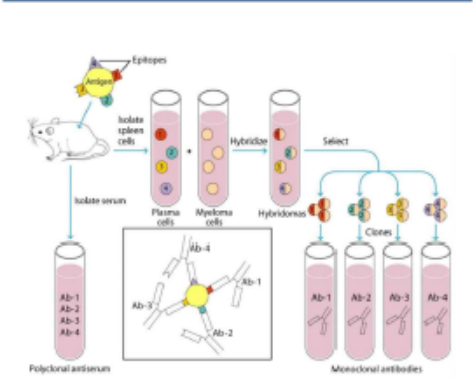
만성 신부전증 환자 중에서 biomarker PKM2에 대한 유용성 평가



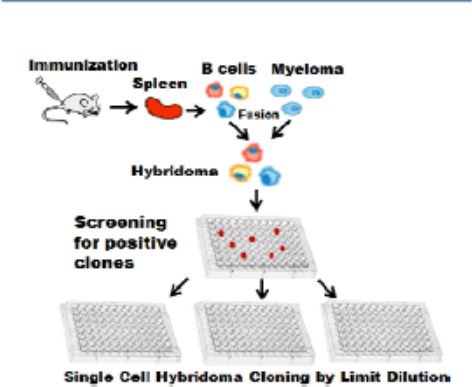
Diabetes-induced animal model에서 biomarker SBP1에 대한 유용성 평가



PKM2 monoclonal antibody 합성

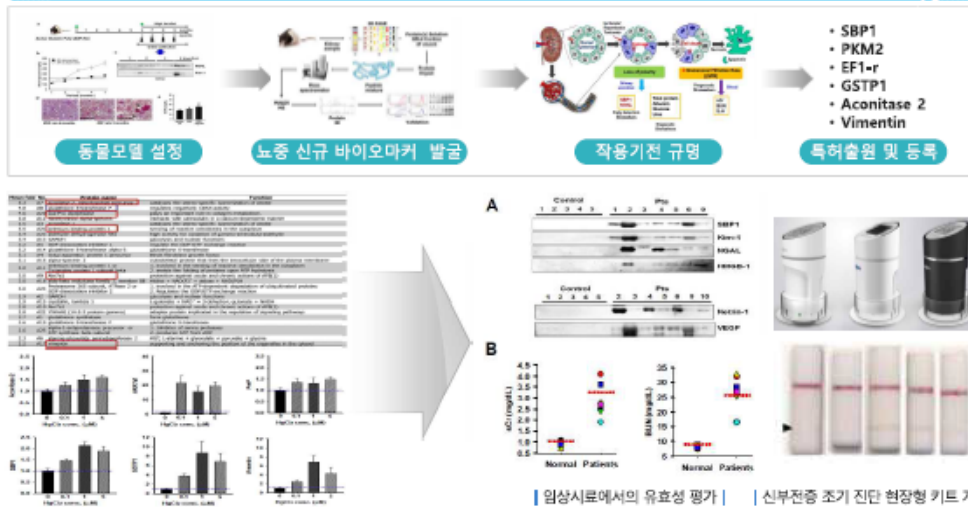


ELISA assay



Implications of Results

만성 신부전증 조기 진단을 위한 뇨 중 바이오마커 발굴 및 특허 확보



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Implications of Results

본 연구진이 기 확보한 신부전증 조기진단 바이오마커 관련 특허 등록 현황

구분	평가항목	검체	특허명칭	
신장질환평가용 바이오마커 SBP1 및 이의 이용	성균관대	신부전증진단	소변	약물로 인한 신장독성의 조기진단 방법
AKR7A1와 6종류의 단백질	성균관대	신장독성 조기진단	신장조직	신장독성 평가용 바이오마커 AKR7A1 및 이를 이용한 신장독성 평가방법
PKM2 (pyruvate kinase isozyme M2)의 당뇨병 신증 조기진단	성균관대	신장독성 조기진단	소변	신장독성 및 부작용 유발 약물 검색용 단백질 바이오마커 및 이를 이용한 당뇨병 신장독성 및 부작용 유발 검색 방법
3-인독실 설페이트(3-indoxyl sulfate) 및 트리코넨린(trigonelline)	성균관대/부산대	신장독성 조기진단	소변 신장조직 및 혈액	약물로 인한 신장독성의 조기진단 방법
아세테이트(acetate) 및 트리메틸아민-엔-옥사이드(trimethylamine-N-oxide, TMAO)	성균관대	신장독성평가	소변	아세테이트 및 트리메틸아민-엔-옥사이드의 농도 변화를 이용한 신장독성 판별방법
아세테이트(acetate) 및 시트레이트(citrate)	성균관대	신장독성평가	소변	아세테이트 및 시트레이트의 농도 변화를 이용한 신장 독성 판별방법
아세테이트(acetate) 및 디메틸글라이신(dimethylglycine)	성균관대	신장독성평가	소변	아세테이트 및 디메틸글라이신의 농도 변화를 이용한 신장독성 판별방법

주관기관 연구내용

MFDS2016-22825

진단기기의 최적화를 위한 설계

진단기기생산공정

- 1) Glass Sub.
- 2) Metal Depo. (Cr/Au=200Å/2000Å)
- 3) PR Patterning
- 4) Metal Etching & PR strip
- 5) Black PR patterning&Curing
- 6) Dicing

PCB 제작



제품 개발



기대효과

신개념 현장형 만성 신부전증 조기진단 기술 개발

만성 신부전증
조기진단 기술 확보

조기진단 기기
개발 및 상용화

특허 출원

바이오마커에 대한
임상시험

바이오마커를 이용한
조기진단 키트 개발

만성 신부전증 조기진단에
의한 막대한 의료비 손실 감소

원격의료진료
서비스 구축

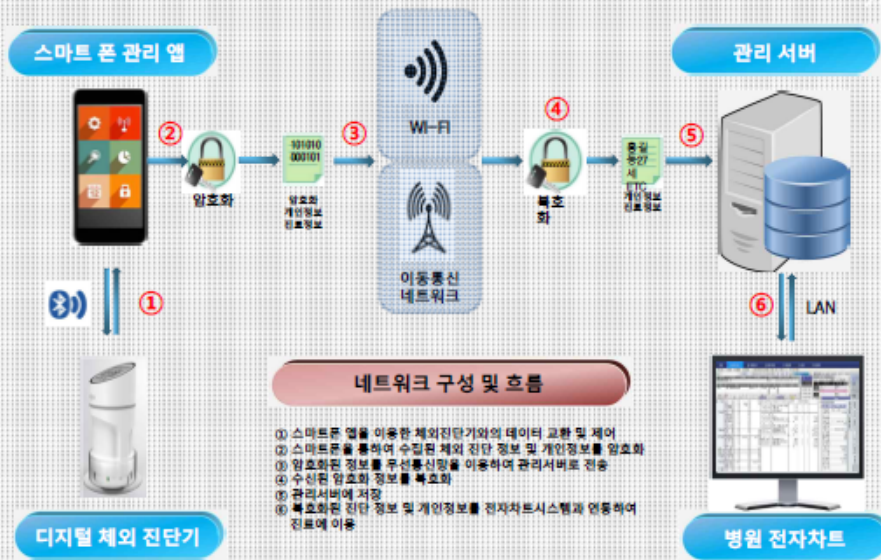
| 기술적 파급효과 |

| 경제·산업적 파급효과 |

활용방안

MFDS2016-22825

진단기기의 병원연계 프로그램 개발



Introduction

Research goals

Results and Conclusion

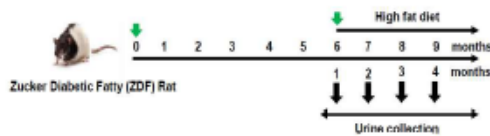
Our research goals....!



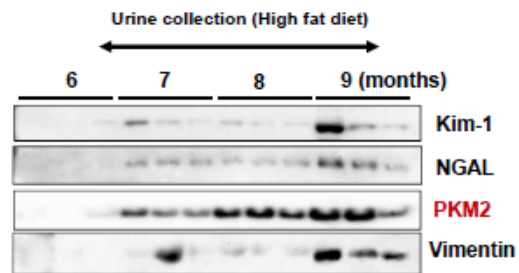
Part II

Identification of New Biomarkers for Early Detection of Diabetic Nephropathy using Proteomic Analysis

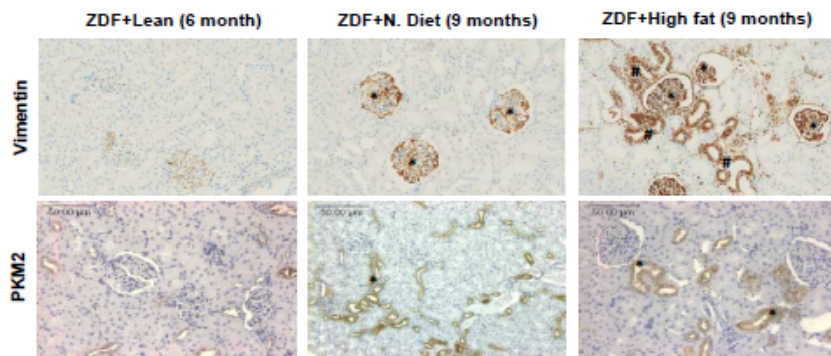
A: Experimental model



B

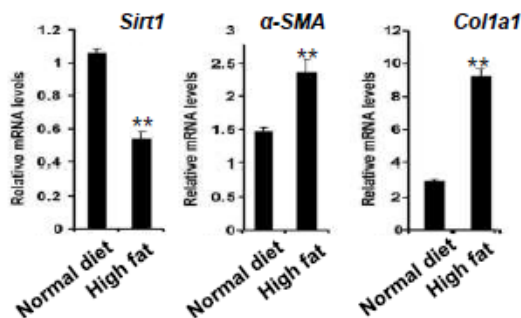


C

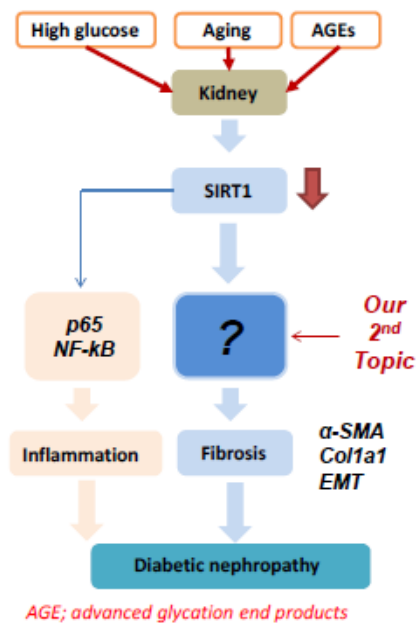
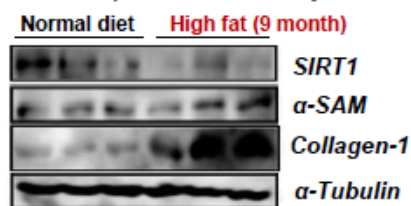


20

A. mRNA levels in kidney



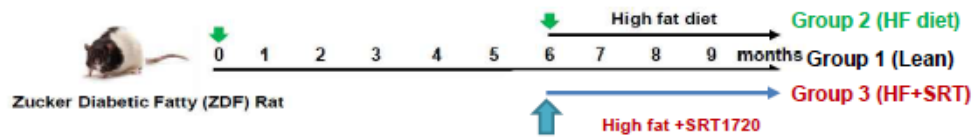
B. Protein expression in kidney



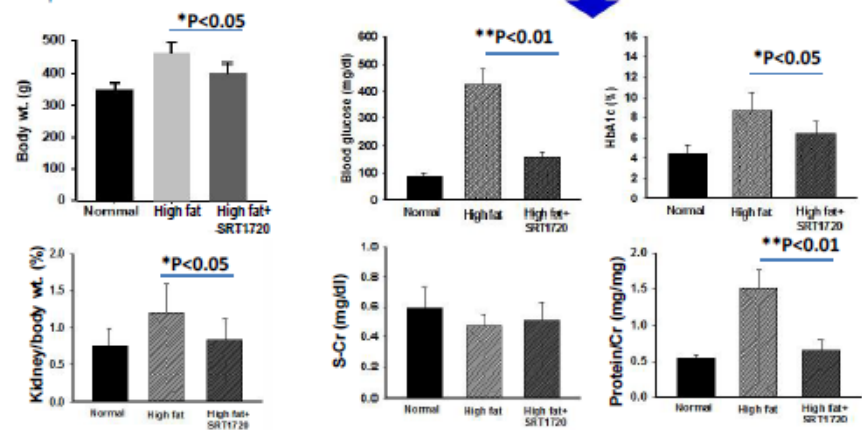
22

Are sirt1 activator prevents diabetic nephropathy?

A: Experimental design

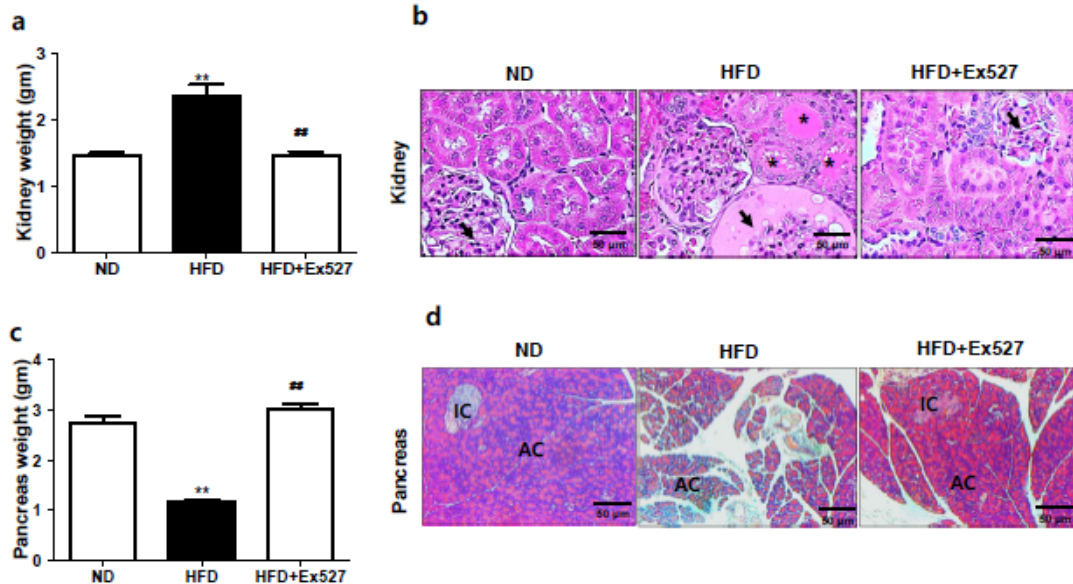


B: Biochemical parameters

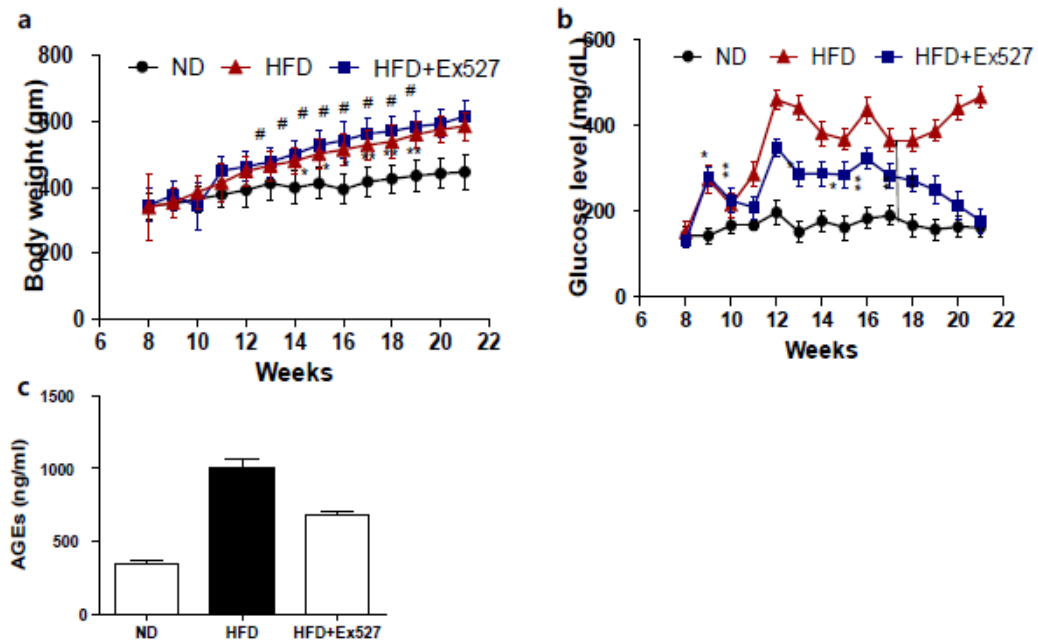


25

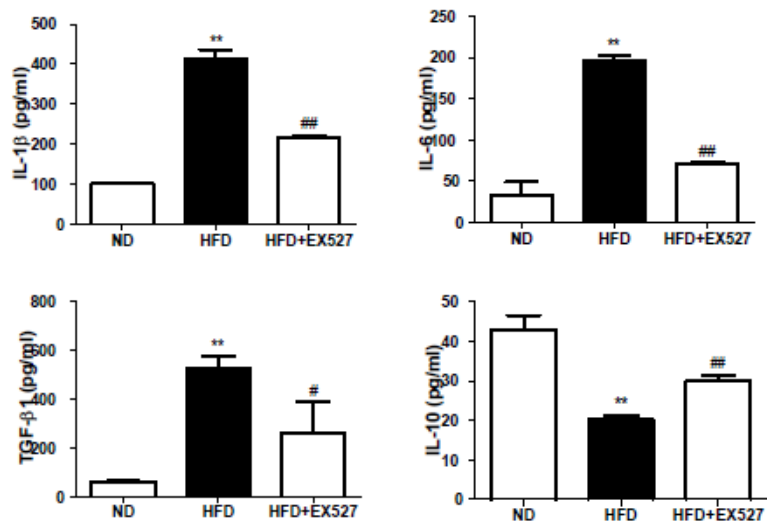
Implications of Results

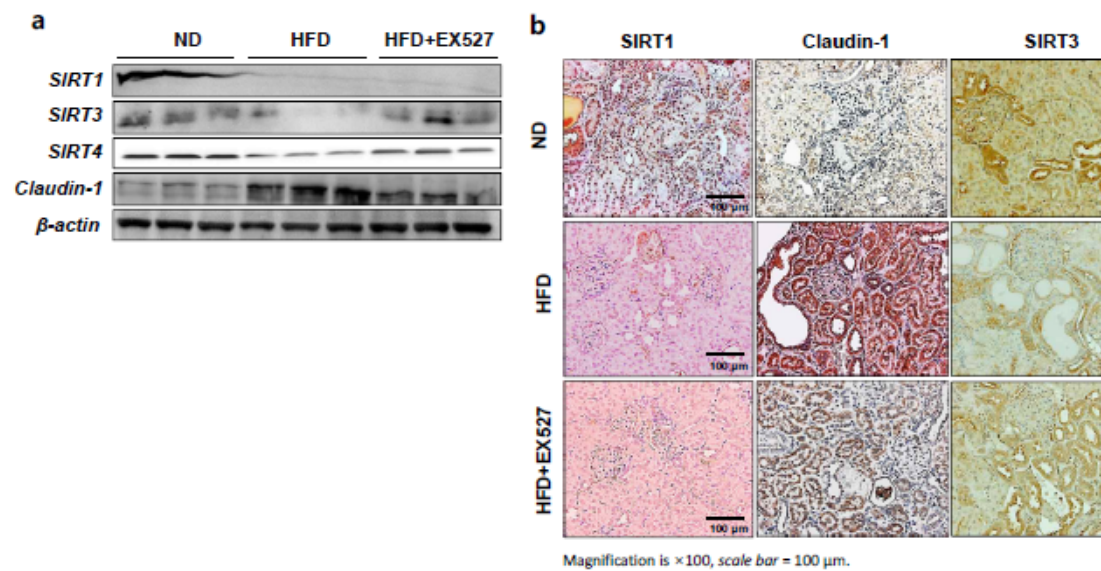
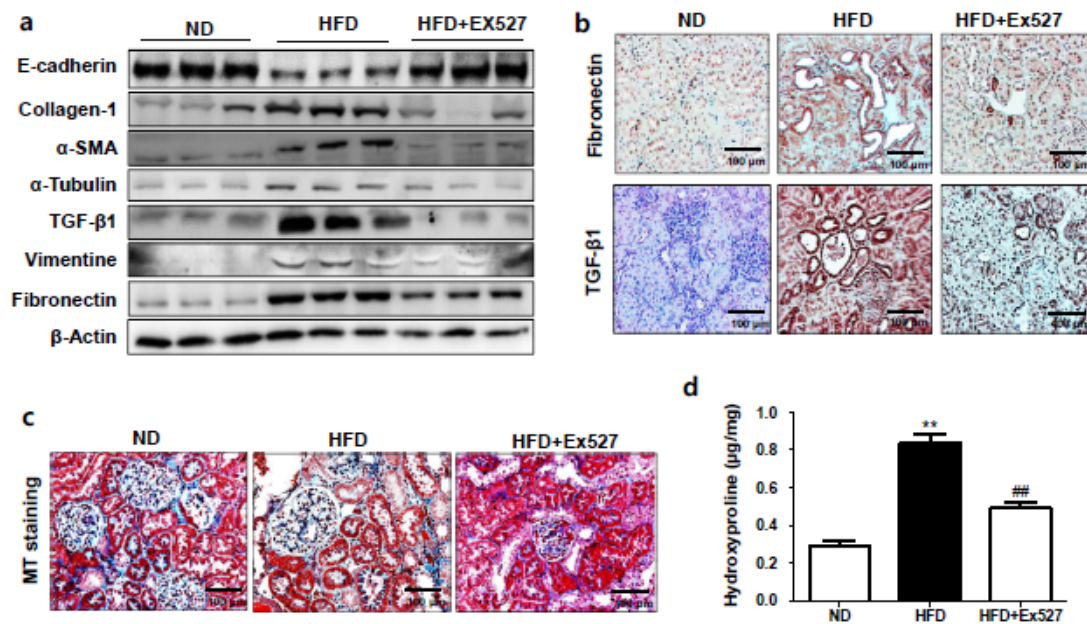


Implications of Results

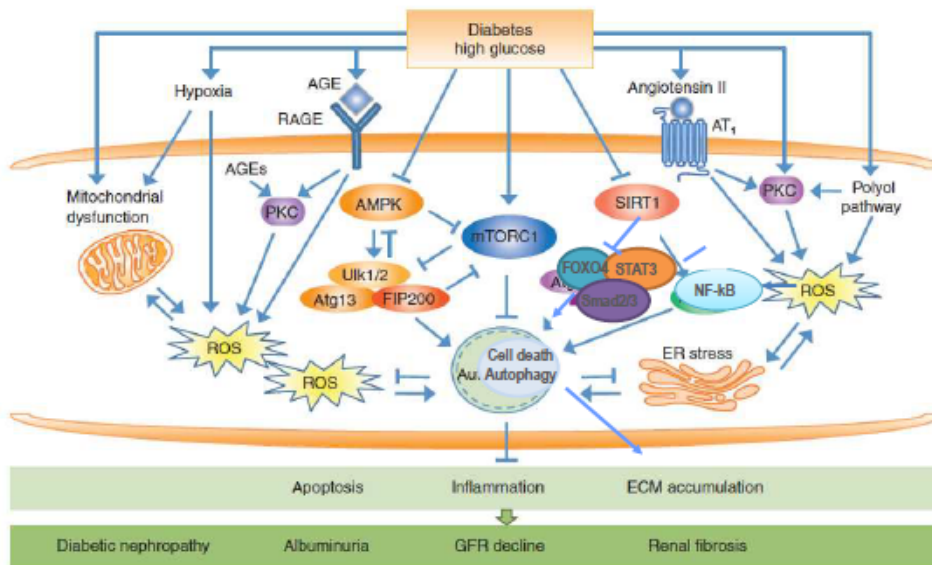


Implications of Results





Conclusions



AGE; advanced glycation end products

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Science is Orchestra....!



Thank you for your time and attention..!

Acknowledgements:

- ▶ Hanyang University
Ok-Nam BAE, Ph.D.
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Amit Kundu
Richa Sancha
Ji Yeon Son
In Hwan Tae
Hoon Young Yang
Ji Soo Kim
Yu Jin Park
Ji Young Kim
Hae Ree Kim
Yu Jung Park
Jae Hyun Park
Yu Jung Park

This work was supported by grants from the BRL (2016-2021)

Thank you for listening

2023.04.12

**병태생리학 전공
대학원생 발표 세미나**

양소혜 학생

Protective effect of cannabidiol against anaphylaxis

Xiaohui Yang

(directed by professor Eun Ju Bae)

School of Pharmacy,
Jeonbuk National University,
Jeonju, Republic of Korea

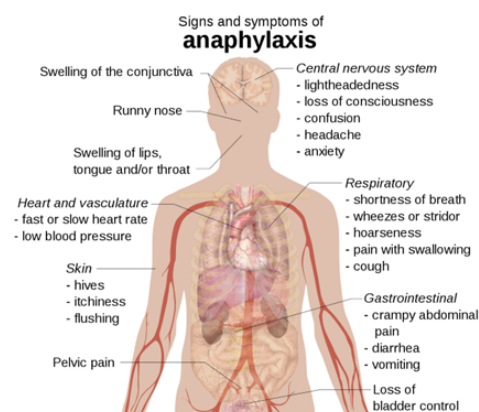
1

Introduction

Anaphylaxis is a life-threatening allergic reaction that can be triggered by exposure to many different substances. (By Daniel More, MD, January 27, 2023)



- Foods
- Insect bites or stings
- Medications
- Latex
- Dusts and pollens
- Exercise-Induced Anaphylaxis
- Cold-Induced Urticaria/Anaphylaxis

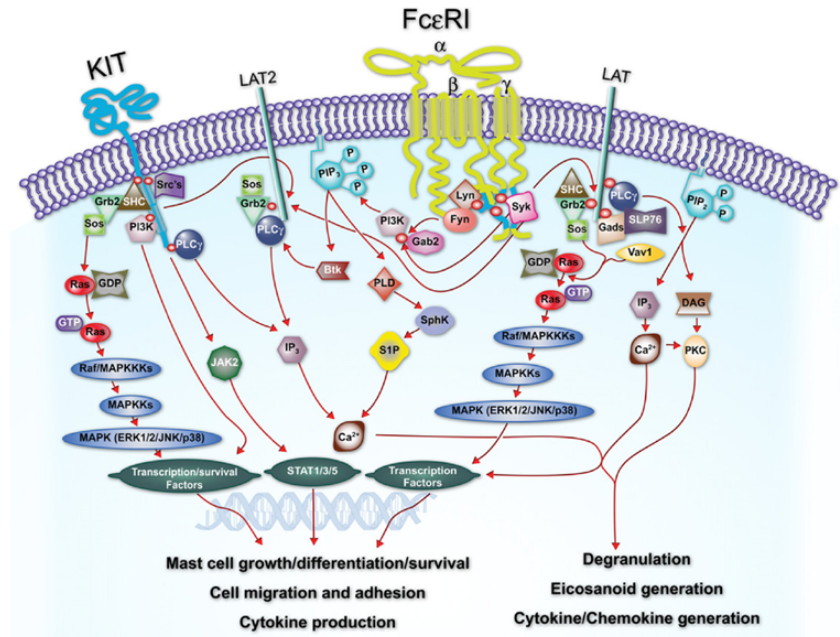


2



Introduction

Mast cells are the primary effector cells in anaphylactic reactions.



3

Introduction

- Cannabidiol (CBD), the most abundant non-psychoactive constituent in the plant Cannabis Sativa
- CBD is known to possess immune modulatory properties.



4

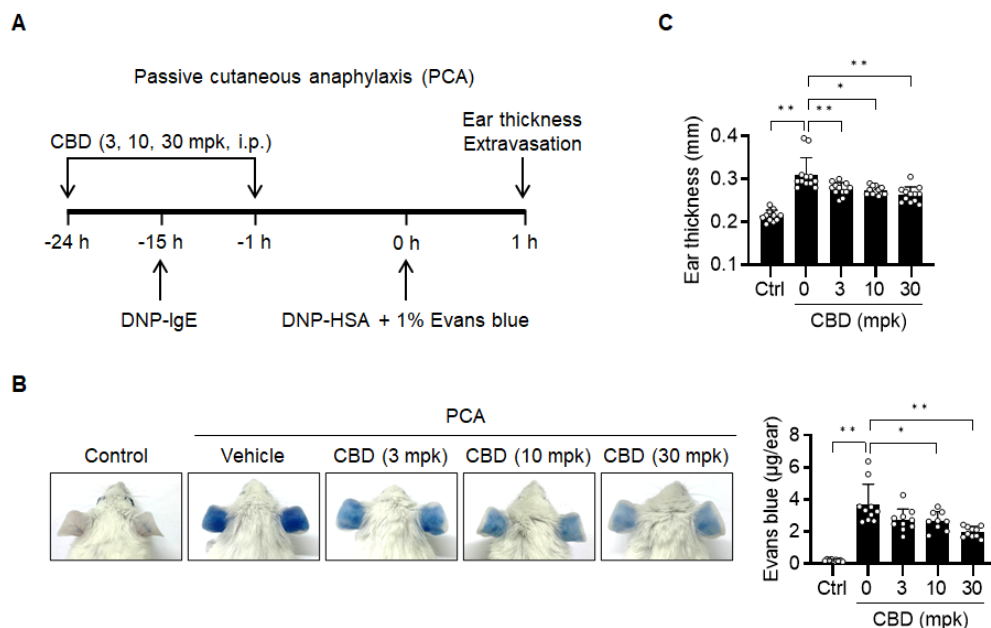
Objective

To evaluate

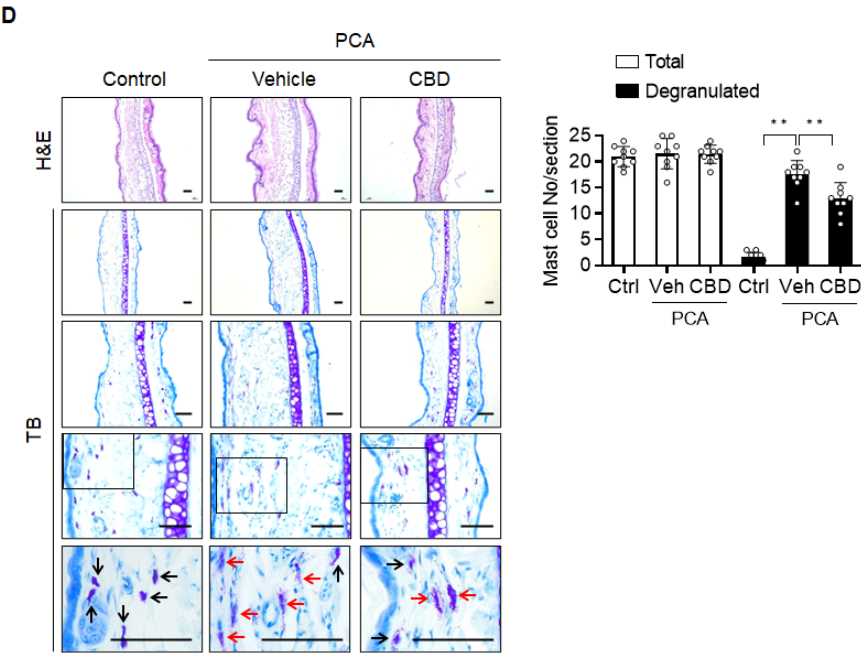
- the effects of CBD on murine and human mast cells degranulation and passive cutaneous anaphylaxis in mice.



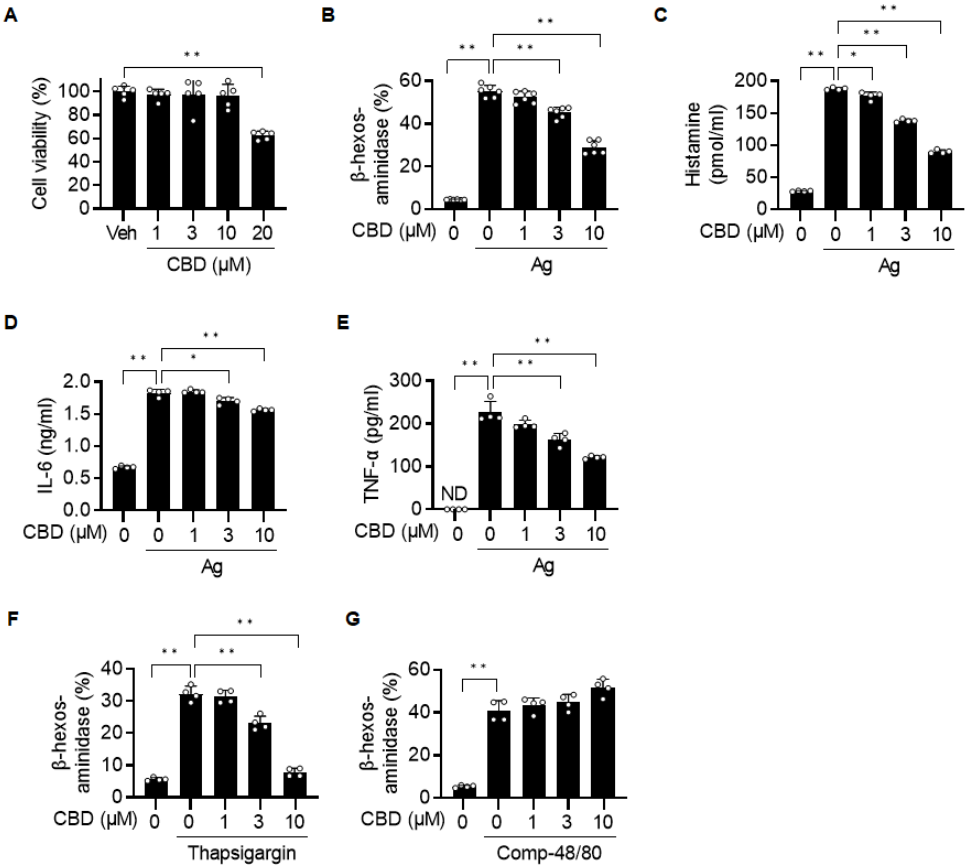
CBD inhibits the Ag-mediated passive cutaneous anaphylactic response



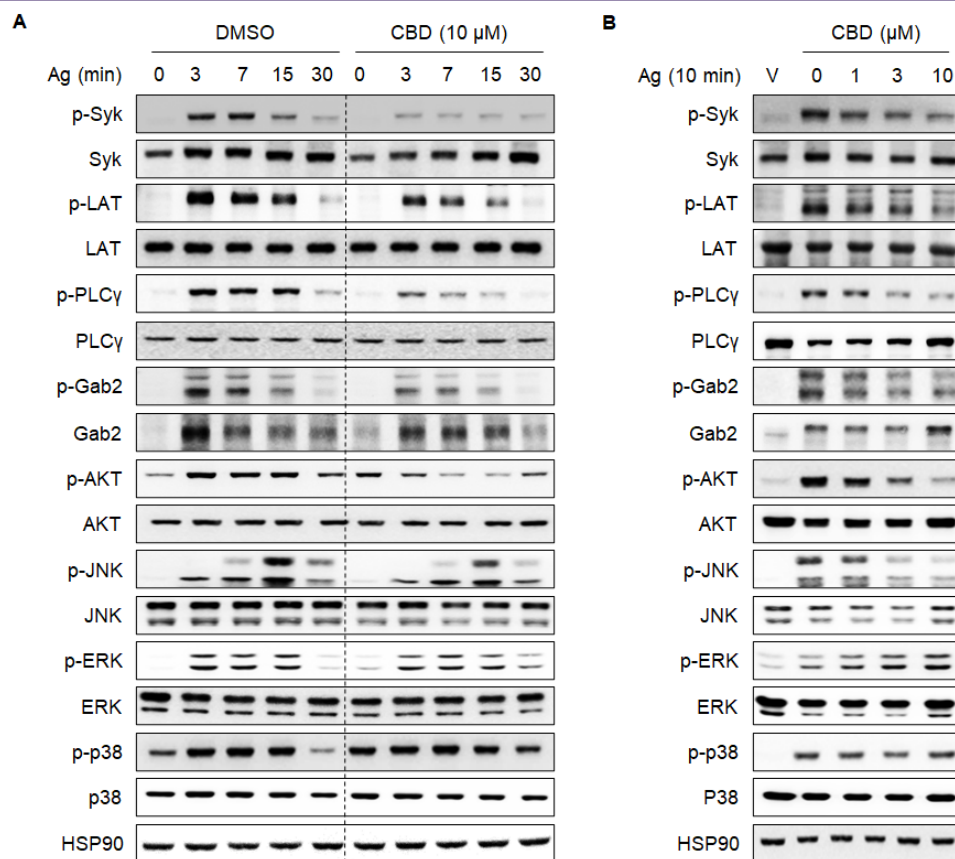
CBD inhibits the Ag-mediated passive cutaneous anaphylactic response



CBD inhibits Ag- and thapsigargin-stimulated mast cell activation



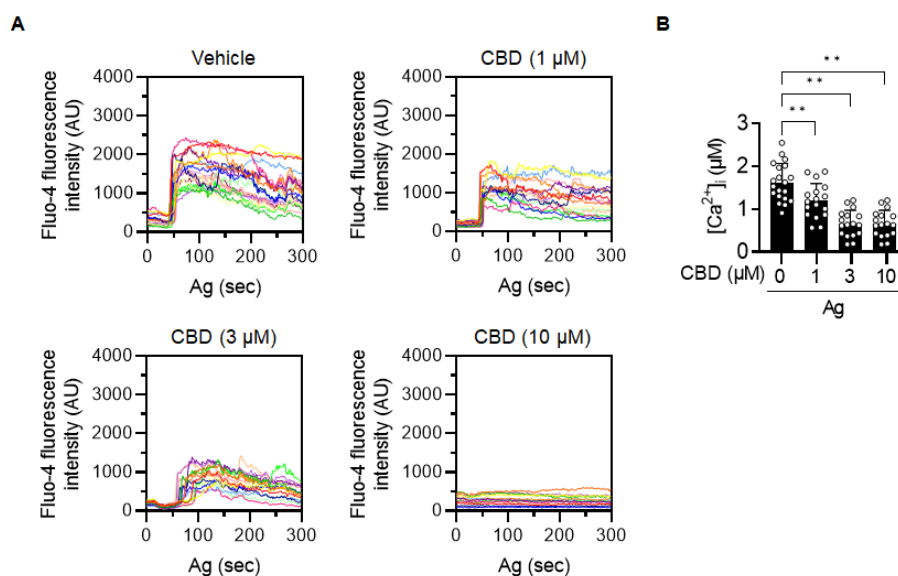
CBD inhibits Ag-stimulated intracellular signaling in mast cells



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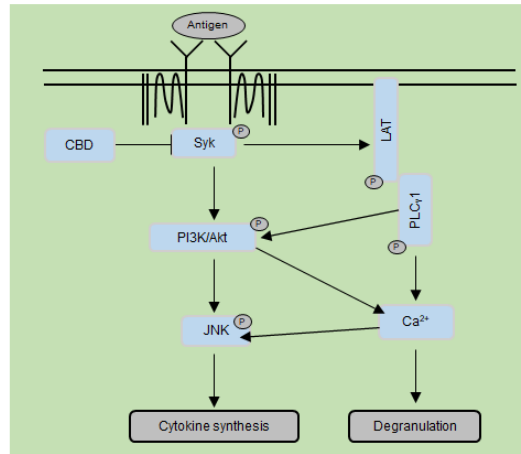


Fluorescence emission spectral scans of intracellular calcium influx



Summary I

- CBD inhibits FcεRI-mediated mast cell signaling and intracellular calcium mobilization, thereby suppressing mast cell degranulation.
- Figure out the mechanism of CBD action on mast cell degranulation.

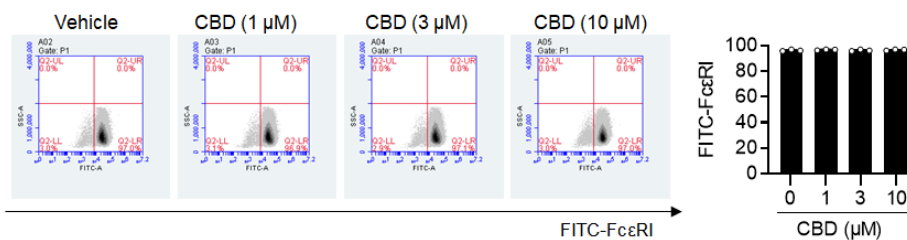


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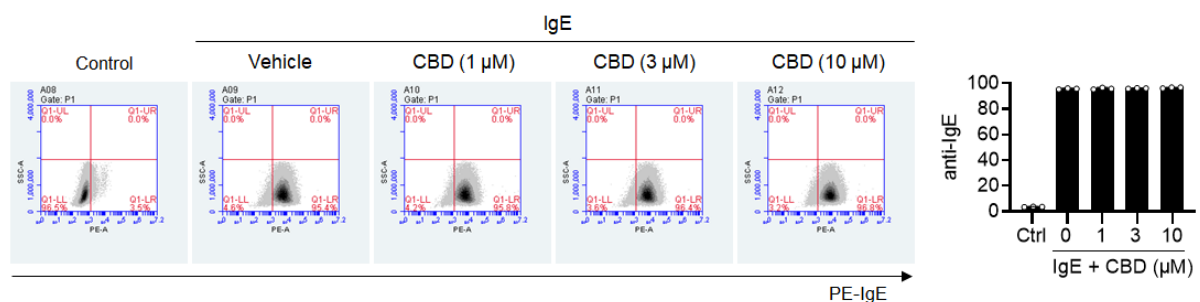


Effects of CBD on FcεRI expression and IgE binding to FcεRI in BMDCs

A



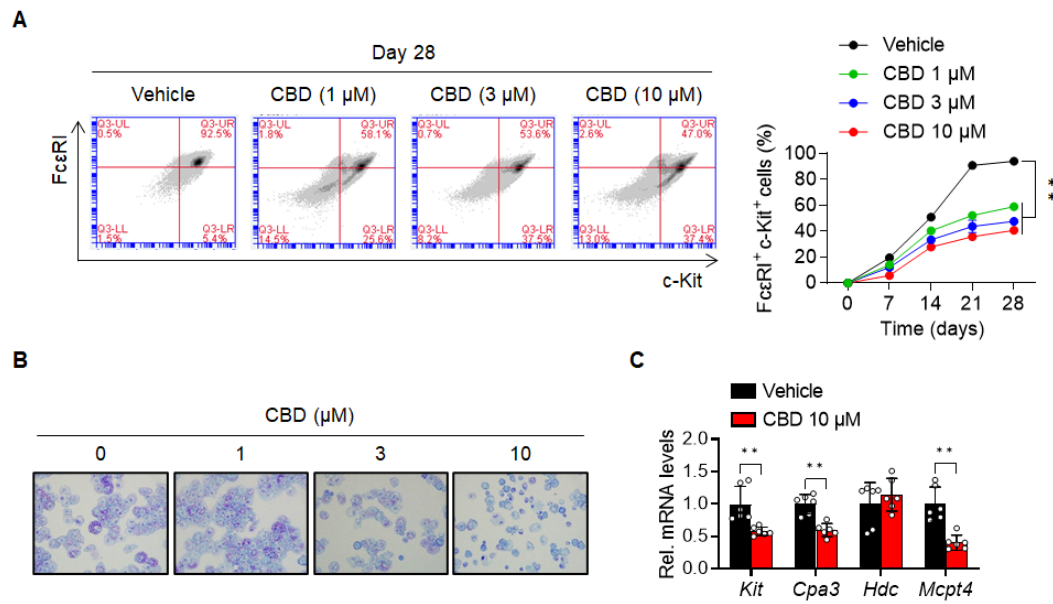
B



12



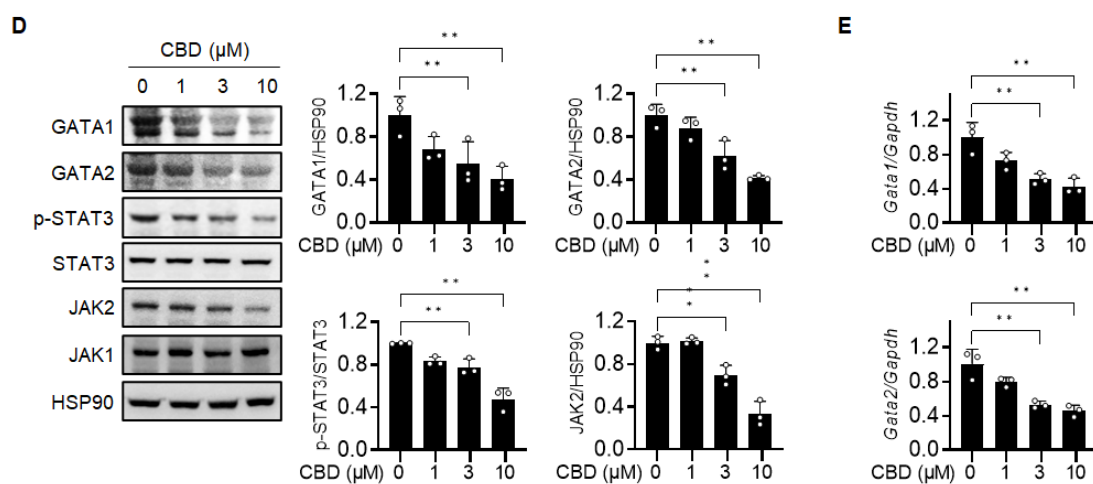
CBD suppresses SCF-induced mast cell differentiation of bone marrow cells



13



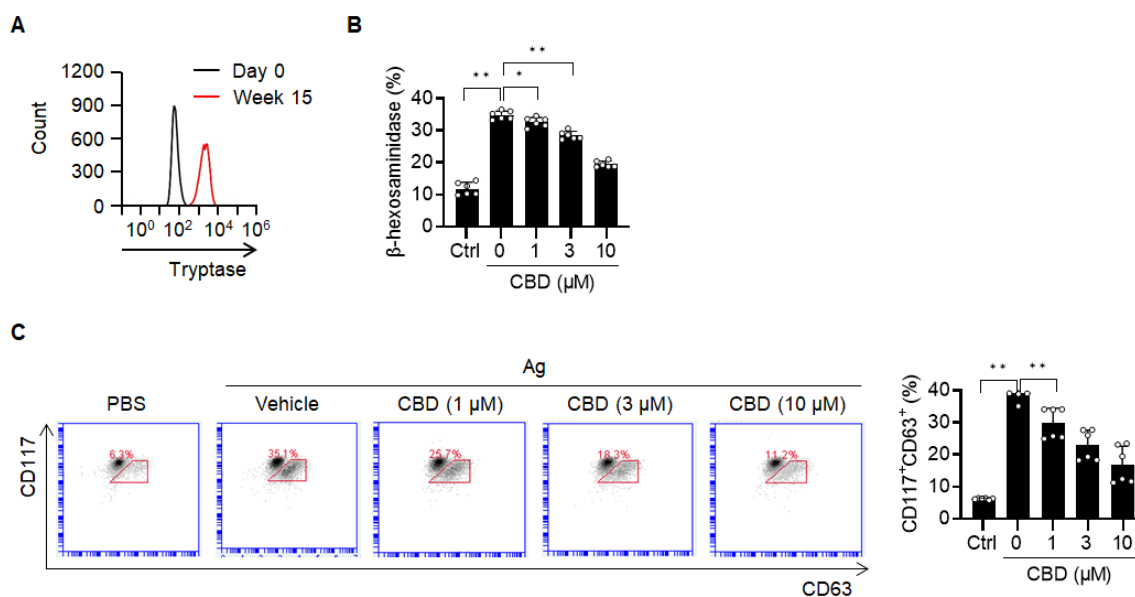
CBD suppresses SCF-induced mast cell differentiation of bone marrow cells



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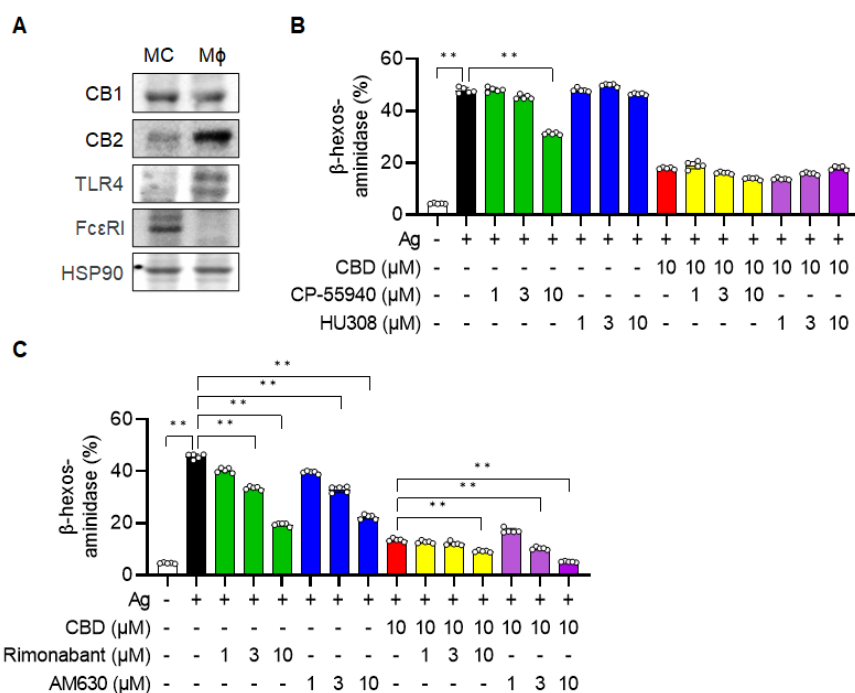
CBD suppresses Ag-stimulated degranulation of human cord blood-derived mast cells (hCBMCs)



15



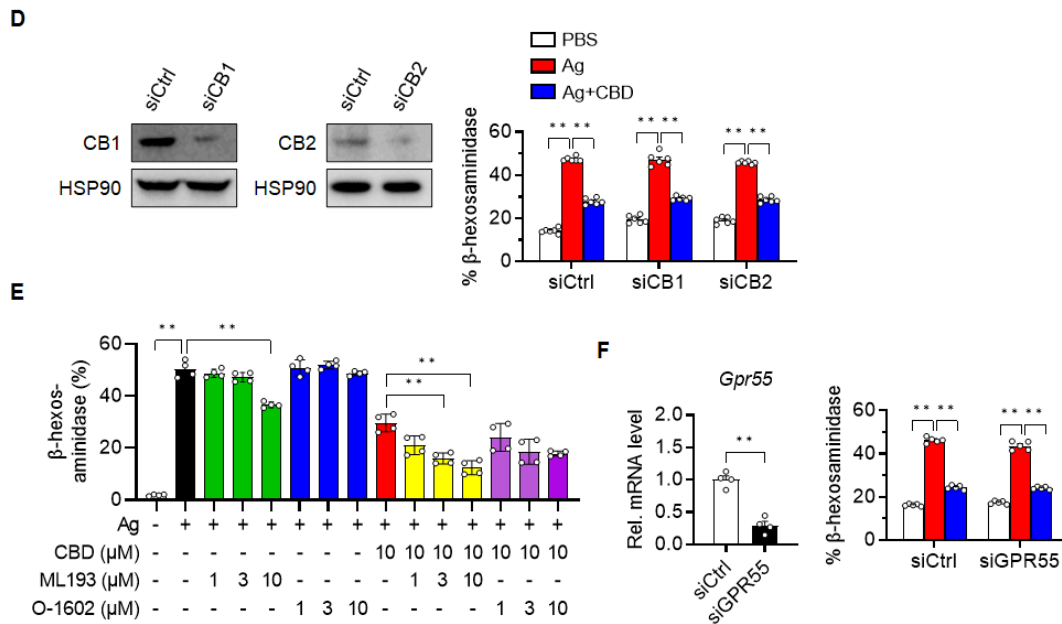
CBD inhibits Ag-stimulated mast cell degranulation independent of CB1, CB2 or GPR55 receptors



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CBD inhibits Ag-stimulated mast cell degranulation independent of CB1, CB2 or GPR55 receptors



Summary II

- CBD inhibits antigen-stimulated mast cells activation independent of CB1, CB2 or GPR55 receptors.
- CBD was also observed to exert a profound inhibitory effect on the development of mast cells in a long-term culture.
- These findings identify that administration of CBD is a new therapeutic intervention in mast cell-associated anaphylactic diseases.



Thank you

Q&A

2023.04.12

**약품분석학 전공
대학원생 발표 세미나**

김애림 학생

약학세미나

Profiling Cannabinoid Contents and Expression Levels of Corresponding Biosynthetic Genes in Commercial Cannabis (*Cannabis sativa L.*) Cultivars

약학대학원 약품분석학 통합과정 김애림

Part1. 연구 목적



CBDAS knockout

THC 40% Marijuana



THCAS knockout

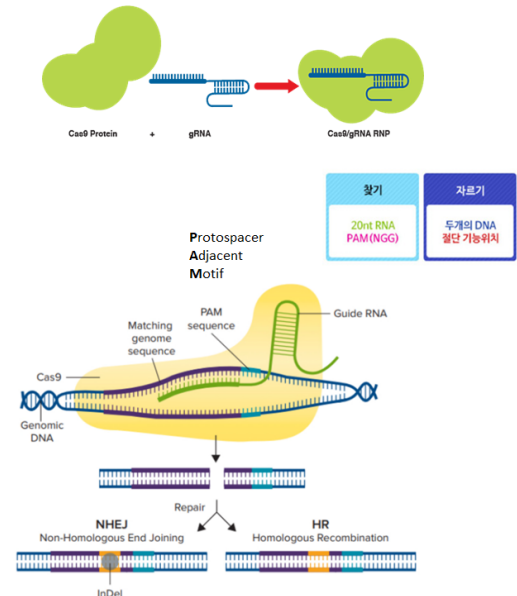
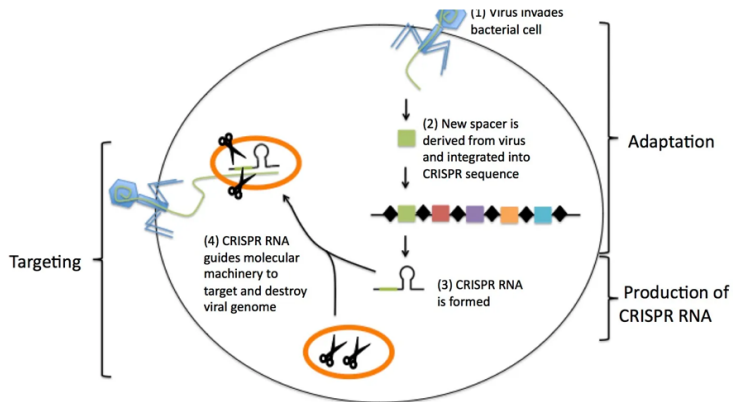
CBD 40% hemp

Part2. Method _ CRISPR/Cas9

CRISPR

Clusters of Regularly Inter-Spaced Palindromic Repeats

세균의 DNA에 보편적으로 존재하는 규칙적, 반복적인 염기서열을 일컫는 용어
세균 세포가 파아지라고 불리는 세균 특이적 바이러스에 감염되면 대부분 세균은 죽지만, 일부는 살아 남아 바이러스의 DNA 일부를 잘라 자신의 유전체에 차곡차곡 삽입한다. CRISPR 영역은 RNA로 전사되고 잘게 쪼개진 후 세포 내 Cas9 단백질과 결합해 RNA-단백질 복합체를 형성한다. 같은 바이러스가 다시 그 세균에 침입하면, CRISPR-Cas9 복합체가 바이러스 염기서열을 인식해 결합한 후 절단해 세포를 보호한다.

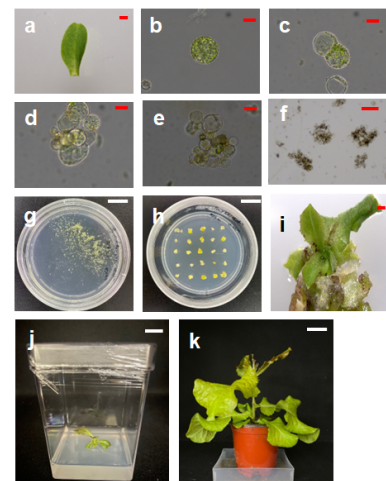


Part2. Method_조직배양

THCAS	TGGATCAATGAGAAAGATGAGAATCTTAGTTTCTG6TG	sgRNA1	540
CBDAS	TGGGTTAATGAGAAAAAGAGAGTCTTAGTTTGGCTGCTGGGATTTGCCCTACTGTTTGC		540
CBCAS	TGGATCAATGAGATGAATGAGAATTTTAGTTTCTG6TG		540

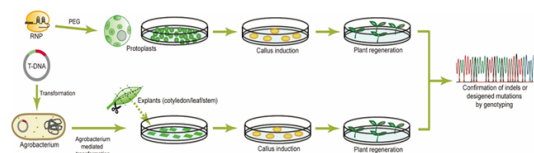
THCAS	TCCATGGGAGAAGATCTGTTT	sgRNA2	720
CBDAS	TCTATGGGGAGAAGATCTCTTTTGGGCTTTACGTGGTGGTGGAGCAGAAAGCTTCGGAATC		720
CBCAS	TCCATGGGAGAAGATCTATTT		720

THCAS	AATCATGGG	sgRNA3	960
CBDAS	AATCAAGGGAGAAATAGACAGCAATACACACTTACTTCTTCAGTTTTCTTGGTGGA		957
CBCAS	AATCATGGG		960



CBCAS, CBDAS, THCAS, CBGAS

Target 유전자 탐색
유전자 염기서열 확보 및
sgRNA 설계 및 제작
sgRNA 효능 확인



(2021, Young-Cheon Kim)

Part3. 논문1 대마 원형질체를 이용한 THCAS, CBCAS, CBDAS 단백질의 Subcellular localization(세포 내 위치) 확인

Plant Biotechnology Reports
https://doi.org/10.1007/s11816-022-00786-6

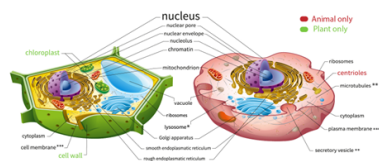
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SHORT COMMUNICATION

Establishment of efficient *Cannabis* (*Cannabis sativa* L.) protoplast isolation and transient expression condition

Ae Lim Kim^{1,4} · Young Jae Yun¹ · Hyong Woo Choi² · Chang-Hee Hong³ · Hyun Joo Shim⁴ · Jeong Hwan Lee¹ · Young-Cheon Kim¹

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Subcellular localization?

세포 기능 및 단백질을 이해하기 위한 중요한 정보. 단백질은 각각의 위치에서 서로 상호작용하기 때문에 세포 소기관에서 단백질 분포가 어떠한지 파악하는 것이 중요하다. 단백질의 상호작용에 대한 정보를 얻으로써 단백질의 기능을 예측할 수 있으며, 외부 자극으로 인한 단백질 변화를 예측할 수 있다.

Protoplast (원형질체)

Protoplasts are spherical bare plant cells produced by the elimination of the cell wall by means of digestive enzymes; plant protoplasts can be grown in defined environments and will form a new cell wall, divide and, in many cases, regenerate whole plants. Protoplasts are a unique experimental system for studying the structure and function of plant cells.

1. Gene function analysis
2. Genome editing

Part3. 논문1_protoplast isolation



Fig. 1 Two-month-old *Cannabis* plant and explant used for protoplast isolation

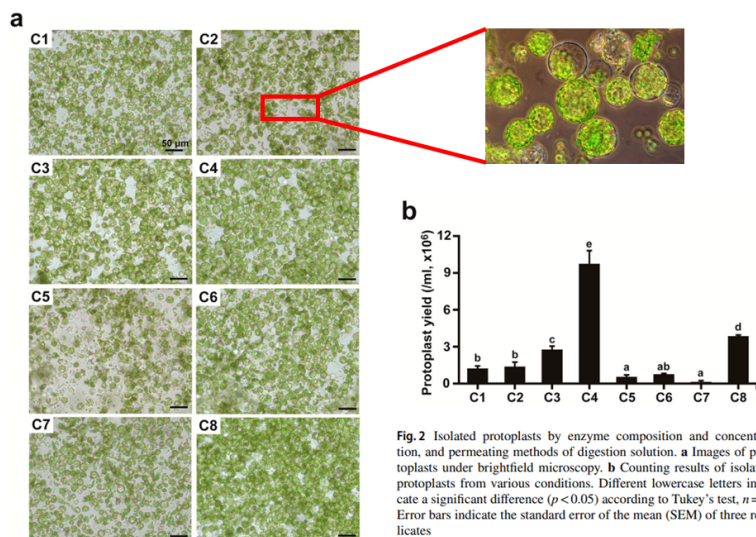


Fig. 2 Isolated protoplasts by enzyme composition and concentration, and permeating methods of digestion solution. **a** Images of protoplasts under brightfield microscopy. **b** Counting results of isolated protoplasts from various conditions. Different lowercase letters indicate a significant difference ($p < 0.05$) according to Tukey's test, $n = 3$. Error bars indicate the standard error of the mean (SEM) of three replicates

Part3. 논문1_subcellular localization

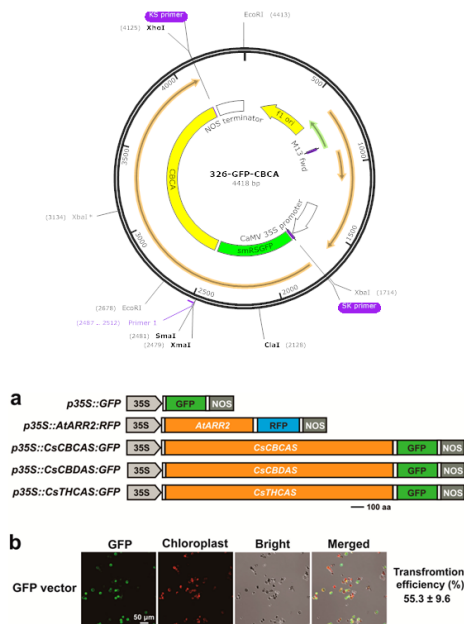


Fig. 3 Subcellular localization of CxCBCAS, CxCBDAS, and CxTHCAS proteins. **a** Schematic diagram of the constructs used for this analysis. **b** Efficiency of protoplast transformation was assessed by manually counting the GFP protein signals in protoplasts under a confocal laser scanning microscope. Mean $\pm$ SEM values are shown ($n=3$). **c** Respective CxCBCAS::GFP, CxCBDAS::GFP, and CxTHCAS::GFP localization. GFP and chloroplast are indicated in fluorescence images. AtARR2::GFP was used as a nuclear marker protein. "Bright" indicates light microscopy images. "Merged" indicates the overlapped image of the GFP, AtARR2::RFP, chloroplast, and brightfield images

Part4. 논문 2



Article Profiling Cannabinoid Contents and Expression Levels of Corresponding Biosynthetic Genes in Commercial *Cannabis (Cannabis sativa L.)* Cultivars

Ae Lim Kim ^{1,2,†}, Young Jae Yun ^{1,†}, Hyong Woo Choi ³, Chang-Hee Hong ⁴, Hyun Joo Shim ^{2,*}, Jeong Hwan Lee ^{1,*} and Young-Cheon Kim ^{1,*}

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[†] These authors contributed equally to this work.

1. RT-qPCR 활용 대마품종/종자별 THCAS, CBCAS, CBDAS 유전자 발현양상 분석
2. HPLC 기기분석을 통한 대마품종/종자별 THC 및 CBD 함량 profiling
3. HPLC 결과를 토대로 향후 가용 종자 line 고정 (3-5종의 종자 line 압축)

Part4. 논문2_Biosynthesis pathway

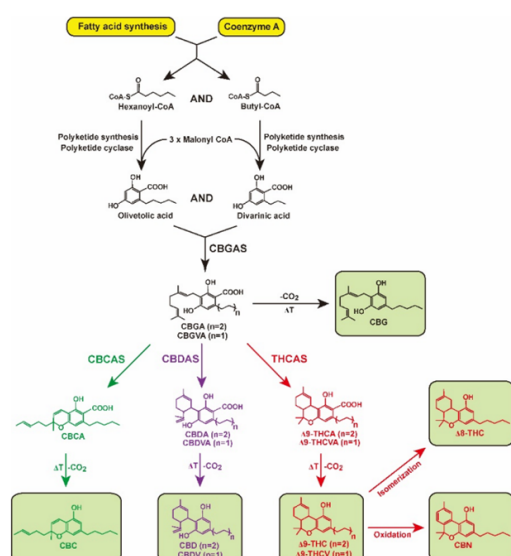
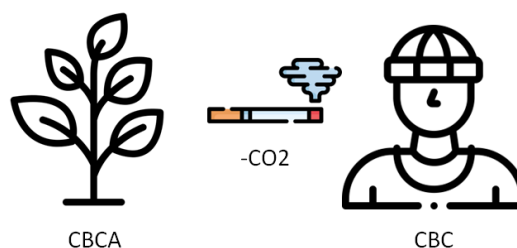


Figure 1. Biosynthesis pathway of cannabinoids. Hexanoyl-CoA and butyryl-CoA are used as substrates to generate olivetolic and divarilic acid. Through the action of cannabigerolic acid synthase (CBGAS), olivetolic and divarilic acids are converted into cannabigerolic acid (CBGA) and cannabigerovarinic acid (CBGVA), the central precursor for cannabichromenic acid (CBCA), cannabidiolic acid (CBDA), cannabidivarinic acid (CBDVA), Δ⁹-tetrahydrocannabinolic acid (Δ⁹-THCA), and Δ⁹-tetrahydrocannabivarinic acid (Δ⁹-THCVA). After exposure to heat (ΔT), CBGA, CBCA, CBDA, CBDVA, Δ⁹-THCA, and Δ⁹-THCVA decarboxylate (-CO₂) are converted into cannabigerol (CBG), cannabichromene (CBC), cannabidiol (CBD), cannabidivarin (CBDV), Δ⁹-tetrahydrocannabinol (Δ⁹-THC), and Δ⁹-tetrahydrocannabivarin (Δ⁹-THCV), respectively. Cannabinoids Δ⁸-tetrahydrocannabinol (Δ⁸-THC) and cannabinol (CBN) are formed by isomerization and oxidation, respectively, of Δ⁹-THC.



Part4. 논문2_HPLC chromatograms of standard

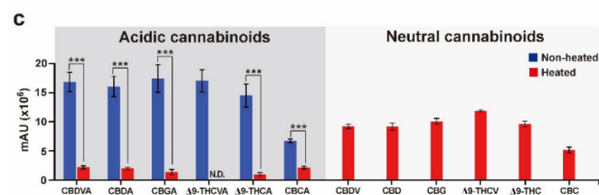
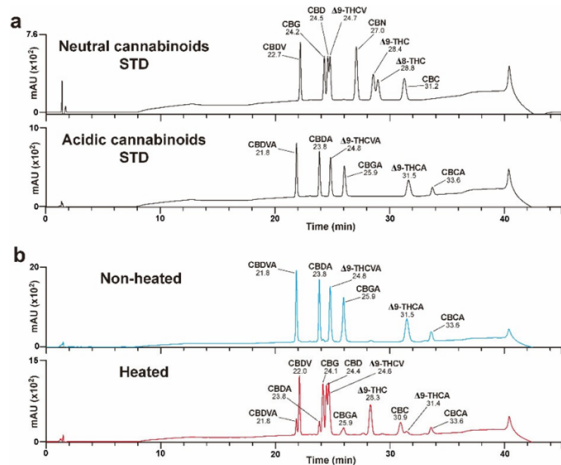


Figure 2. Decarboxylation of the acidic cannabinoid mixture. (a) High-performance liquid chromatography (HPLC) chromatograms of standard (STD) neutral and acidic cannabinoid mixtures. (b) Resulting HPLC chromatograms after heat and acid treatment of acidic cannabinoid mixtures. (c) Results for the decarboxylation of acidic cannabinoids. CBDVA, CBDA, CBGA, Δ⁹-THCVA, Δ⁹-THCA, and CBCA signals were decreased, and their corresponding neutral cannabinoid signals were newly detected after heat treatment. The Δ⁹-THCVA signal was non-detectable (nd) owing to the overlap in retention time between Δ⁹-THCVA and Δ⁹-THCV after heat treatment. Error bars indicate the SEM of three replicates (Student's *t*-test, *** *p* < 0.001).

Part4. 논문2_HPLC chromatograms of plant extract

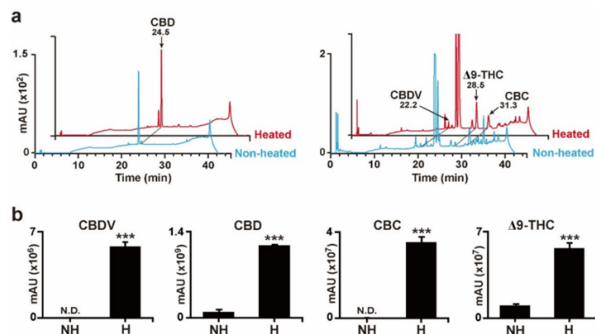


Figure 3. Decarboxylation results of the *Cannabis* plant extract. (a) HPLC chromatograms of enriched neutral cannabinoids, CBD, CBDV, Δ9-THC, and CBC after heat treatment. (b) Results for enriched CBD, CBDV, Δ9-THC, and CBC signals after heat treatment. nd indicates non-detectable. Error bars indicate the SEM of three replicates (Student's *t*-test, *** *p* < 0.001).

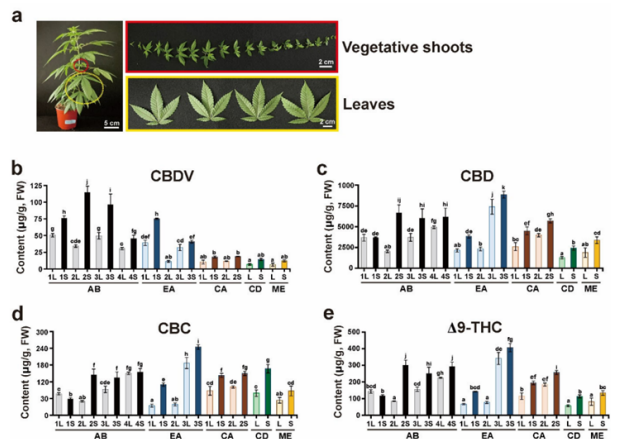


Figure 4. Quantitative analysis of neutral cannabinoids. (a) Vegetative shoots (S) and true leaves (L) of 2-month-old *Cannabis* plants were used for cannabinoid analysis. (b) CBDV, (c) CBD, (d) CBC, and (e) Δ9-THC levels were quantified from S and L extracts of three individuals each of abacus 2.0 (AB) and early abacus (EA), two individuals each of cherry abacus (CA), and a single plant each of cherry diesel (CD) and merlot (ME). The Y axis represents the contents of cannabinoids in fresh weight (FW). Different lowercase letters indicate significant difference (*p* < 0.05) according to Tukey's test.

Part4. 논문2_RT-qPCR assay

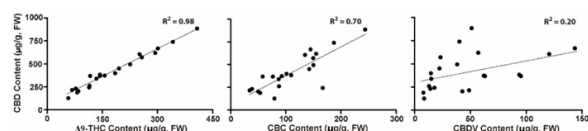


Figure 5. Paired comparison of cannabinoid levels. Comparison of CBD content (Y axis) with the contents of Δ9-THC, CBD, and CBDV (X axis) detected in vegetative shoots and leaves of independent individuals of the *Cannabis* cultivars AB, EA, CA, CD, and ME. Cannabinoid values in each sample are plotted.

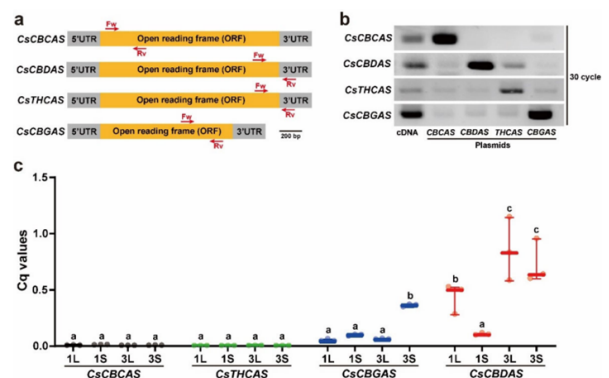


Figure 6. Specificity of primers for cannabinoid biosynthesis genes and RT-qPCR assay results. (a) Diagram of the primer sets designed for RT-qPCR assay of *CsCBCAS*, *CsCBDAS*, *CsTHCAS*, and *CsCBGAS*. Locations of gene-specific primers used for the RT-qPCR experiment are indicated. (b) Specificity of the designed primer sets for *CsCBCAS*, *CsCBDAS*, *CsTHCAS*, and *CsCBGAS*. The expected amplicon bands of *CsCBCAS*, *CsCBDAS*, *CsTHCAS*, and *CsCBGAS* were mainly obtained from cDNA and plasmids containing the corresponding open reading frames (ORFs) or 3'-untranslated regions (UTRs). (c) Transcriptional levels of *CsCBCAS*, *CsCBDAS*, *CsTHCAS*, and *CsCBGAS* in leaves (L) and vegetative shoots (S) of EA1 and EA3 plants. Different lowercase letters indicate significant difference (*p* < 0.05) according to Tukey's test.

감사합니다.

2023.04.19.

우수연구자 초청세미나

경상대 약대

김정환 교수님

Lysosomal SLC46A3 modulates hepatic cytosolic copper homeostasis, mitochondrial function, and triglyceride accumulation

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4/19/2023



Dept. Pharmacology, College of Medicine
Gyeongsang National University, Republic of Korea



Background

During Vietnam War (1955~1975)



A U.S. Air Force crew member stands by a defoliant mixing machine inside one of three C-123 aircraft used to clear jungle growth in South Vietnam; August 1963.

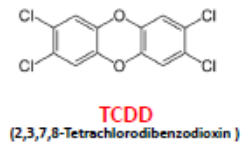
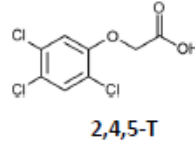
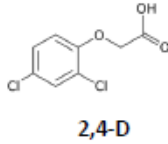


Before



After

Agent Orange



ScienceDirect

Dioxin

Dioxin is formed as an unintentional by-product of many industrial processes that involve chlorine, such as waste incineration, chemical and pesticide manufacturing, and pulp and paper bleaching.

Human case: the highest concentration of TCDD: 144,000 pg/g-fat tissue

AGENT ORANGE: Painful legacy

Agent Orange, a combination of herbicides and defoliants, was used by the U.S. military, particularly during the Vietnam War. The substance caused extensive medical problems, including cancers and birth defects, among people exposed to it.

In 1991, the **Agent Orange Act** made veterans exposed to the chemical eligible for compensation and medical care. The U.S. Department of Veterans Affairs recognizes a "presumptive" link between Agent Orange and a range of illnesses:

- Type II diabetes
- Hodgkin's disease
- Soft-tissue sarcoma
- Peripheral neuropathy
- Spina bifida in children of veterans
- Various other forms of cancer, including prostate and respiratory cases

Added to the list in August 2010:

- B-cell leukemias
- Parkinson's disease
- Ischemic heart disease

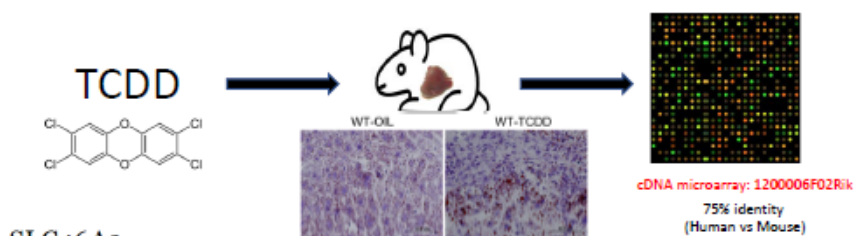
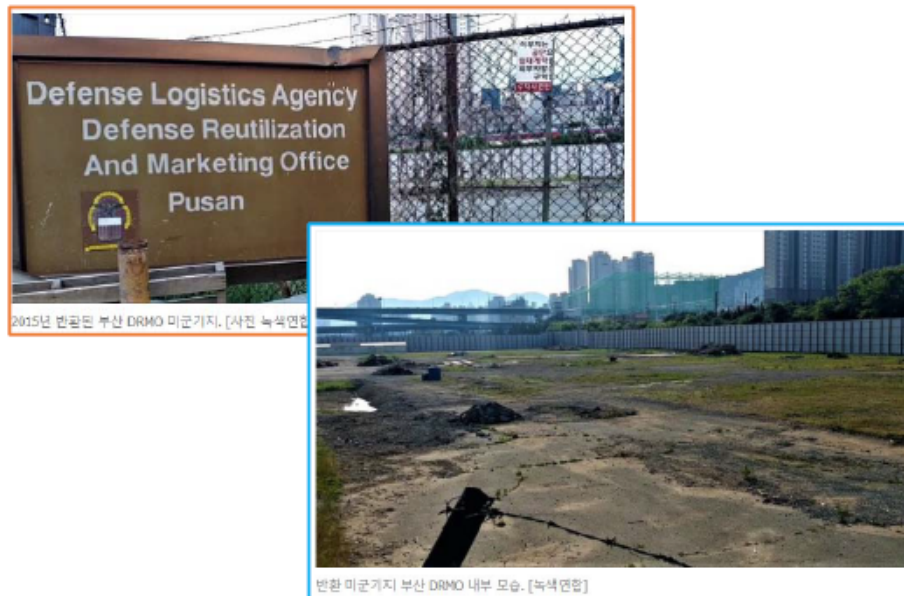


Viktor Yushchenko



National Academies Press

Risk of Dioxin Exposure is still on in South Korea



SLC46A3

From Wikipedia, the free encyclopedia

Solute carrier family 46, member 3 is a [protein](#) that in humans is encoded by the [SLC46A3 gene](#).^[5]

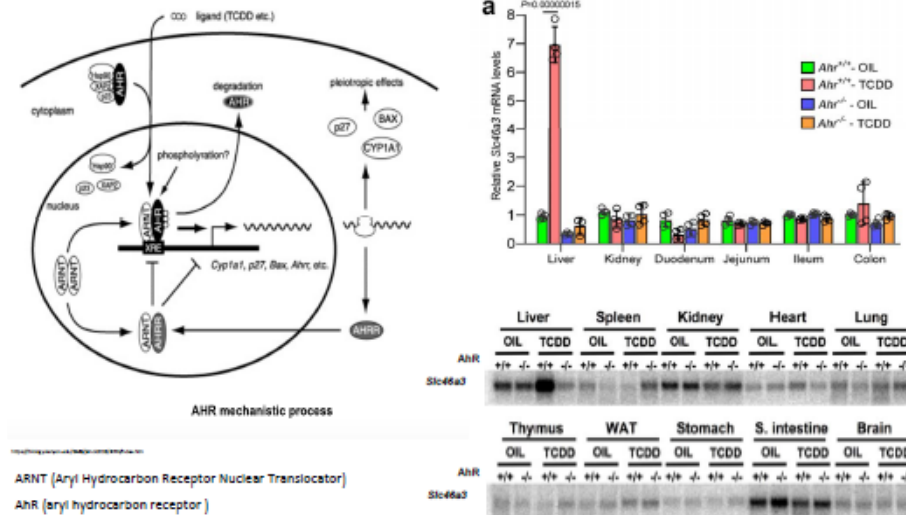
References [[edit](#)]

- ↑ *GRCh38*. Ensembl release 89: ENSG00000139508 [[?](#)]. Ensembl, May 2017.
- ↑ *GRCh38*. Ensembl release 89: ENSMUSG00000029650 [[?](#)]. Ensembl, May 2017.
- ↑ "Human PubMed Reference:" [[?](#)]. National Center for Biotechnology Information, U.S. National Library of Medicine.
- ↑ "Mouse PubMed Reference:" [[?](#)]. National Center for Biotechnology Information, U.S. National Library of Medicine.
- ↑ "Entrez Gene: Solute carrier family 46, member 3" [[?](#)].

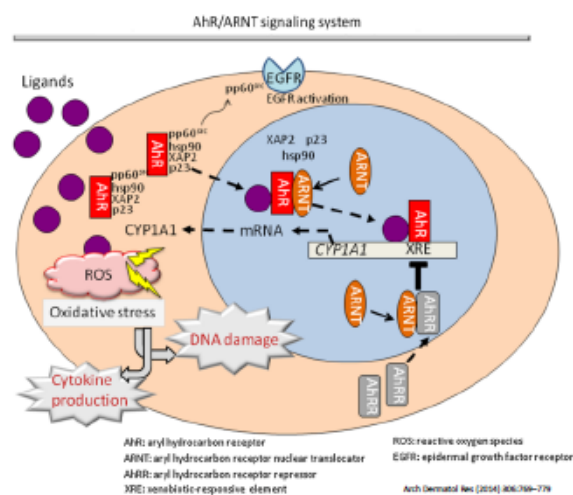
2000 → 2020

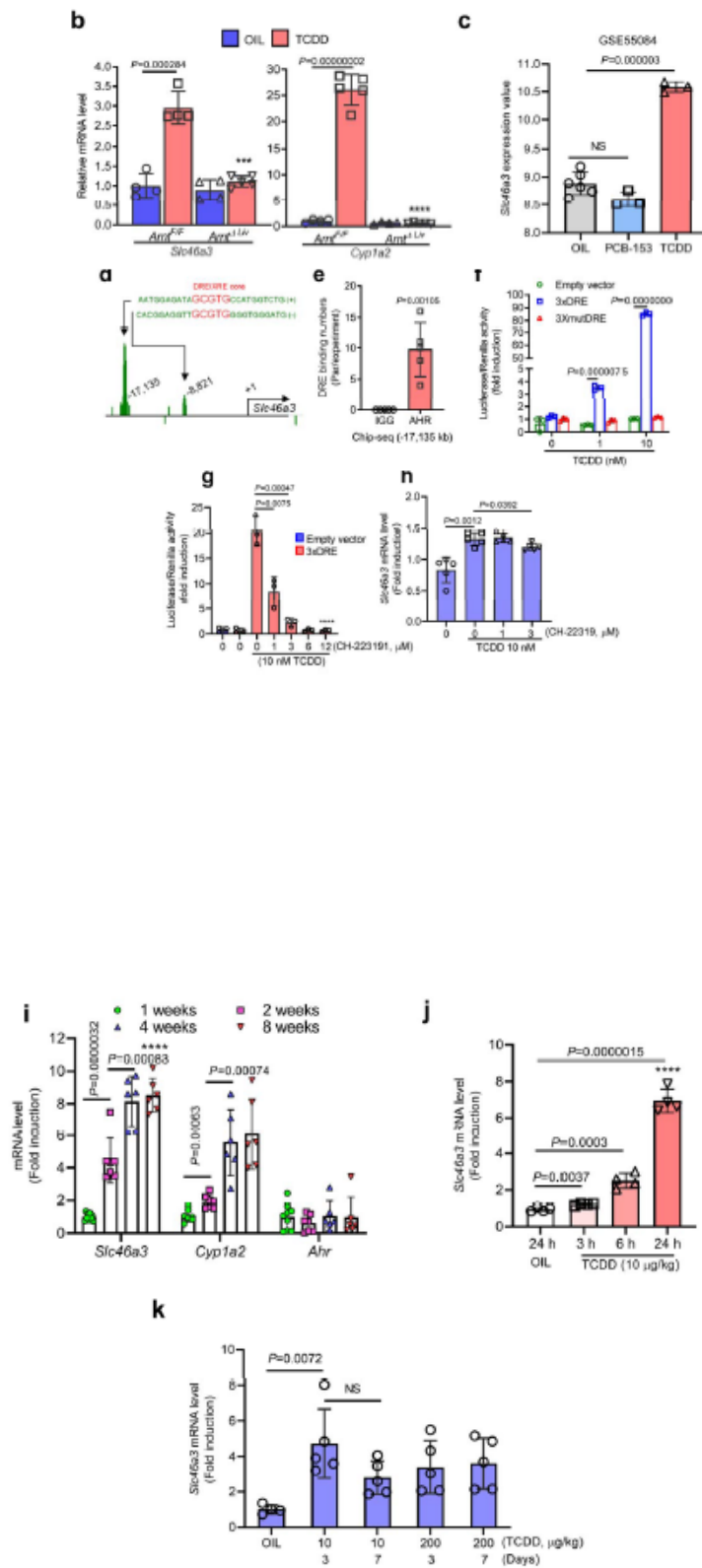
Slc46a3 KO Ahr KO Arnt cKO Hepatocytes Hepa1c1c7

Regulation of *Slc46a3*

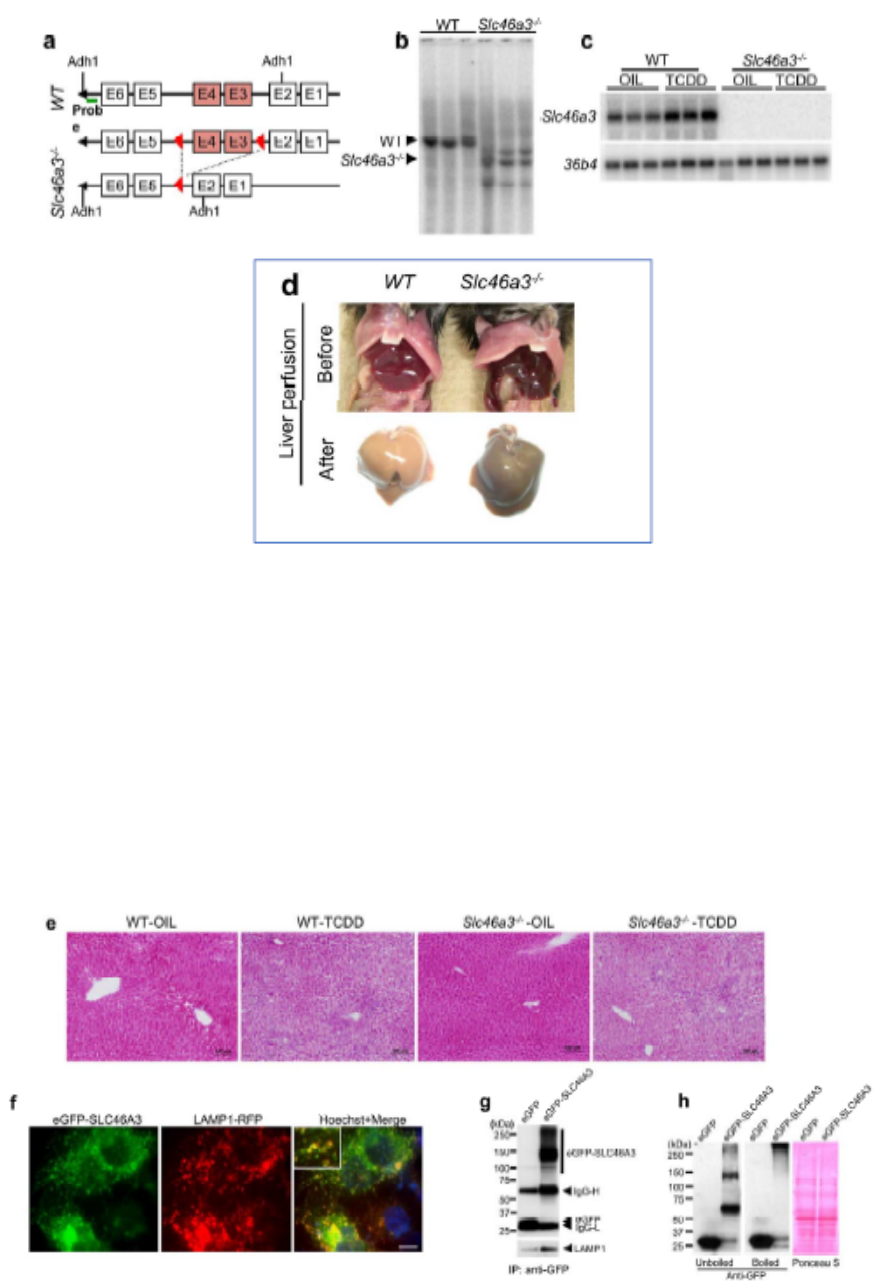


AhR/ARNT and oxidative stress

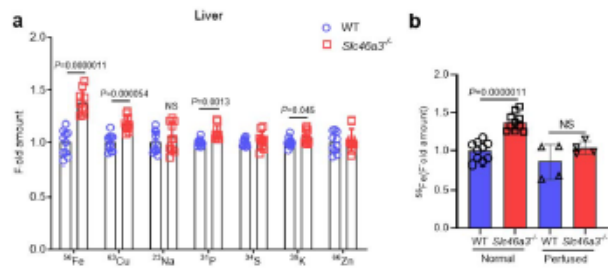


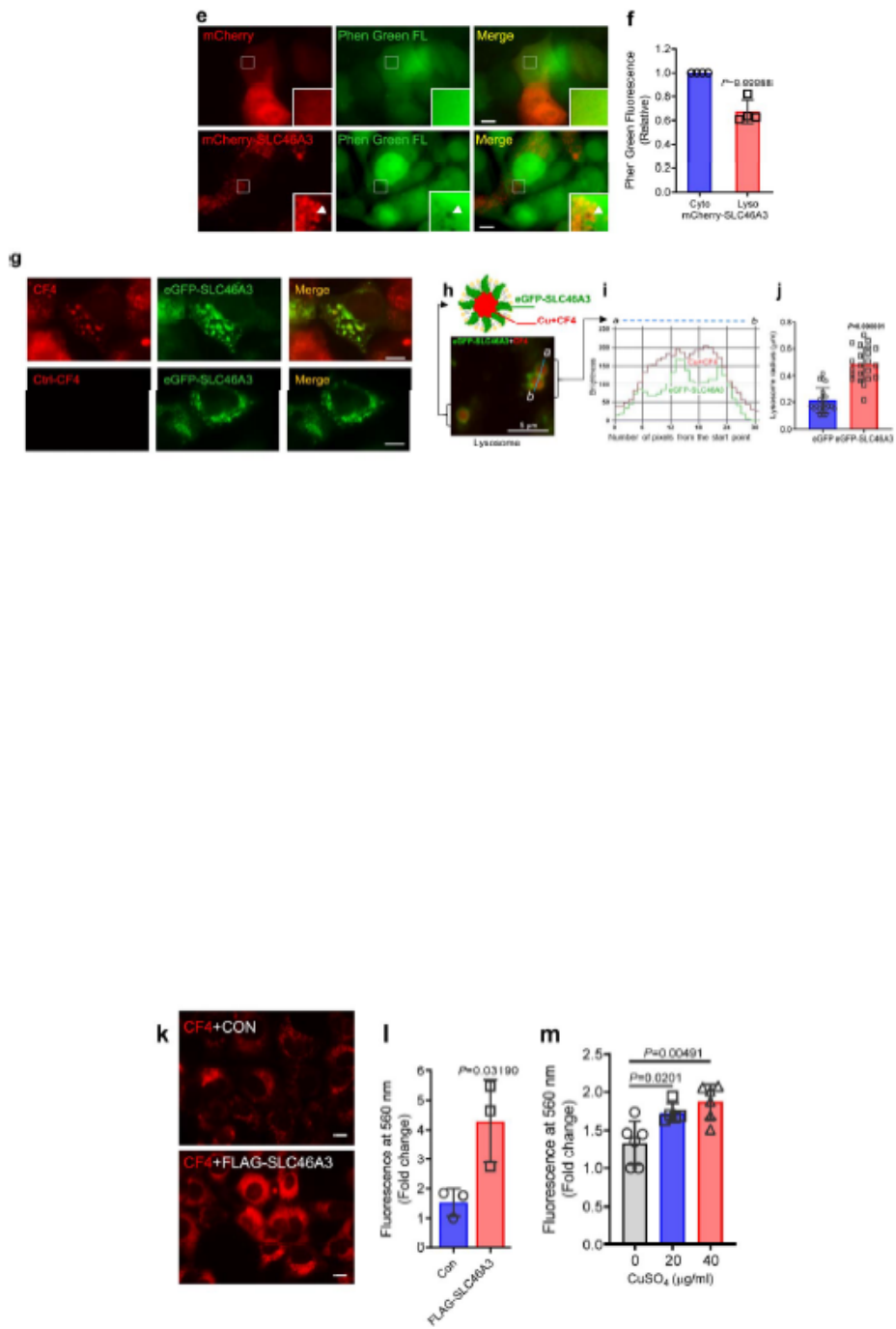


Generation and characterization of *Slc46a3*^{-/-} mice



ICP (Inductively coupled plasma) spectroscopy



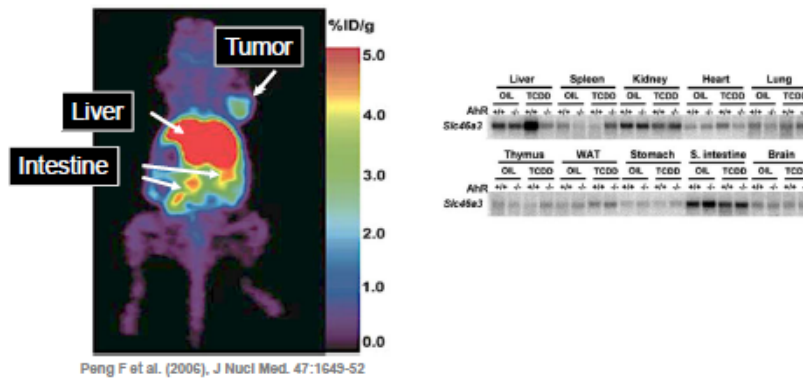


[illegible]

The diagram illustrates the cellular copper homeostasis and its roles in various organelles. It is divided into several sections:

- Cu⁺ transporters:** Cu⁺ enters the cell via CTR1 and is transported into the mitochondria by ATP7A/B.
- Cu binding molecules:** Cu⁺ is bound by CCS, ATOX1, SCO1, SCO2, COX17, and CCO.
- Enzymes acquiring Cu at the TGN:** Ceruloplasmin (Cp; Fe oxidase), Hephastin (HEPH; Fe oxidase), Superoxide dismutase 3 (SOD3), Dopamine β hydroxylase (DBH), and Peptidyl-glycine α-amidating monooxygenase (PAM).
- Enzyme in the melanosome:** TYR (Tyrosinase).
- Enzymes in the cytosol:** SOD1 (Superoxide dismutase 1) and CCS (Copper chaperone for SOD1).
- Enzymes in the mitochondria:** ATOX1 (Copper chaperone for ATOX1), ATP7A/B (Copper-transporting ATPase), and MT1,2 (Metallothionein).
- Transcription regulators affected by Cu levels:** MT1, p53, Hif1a, and others.

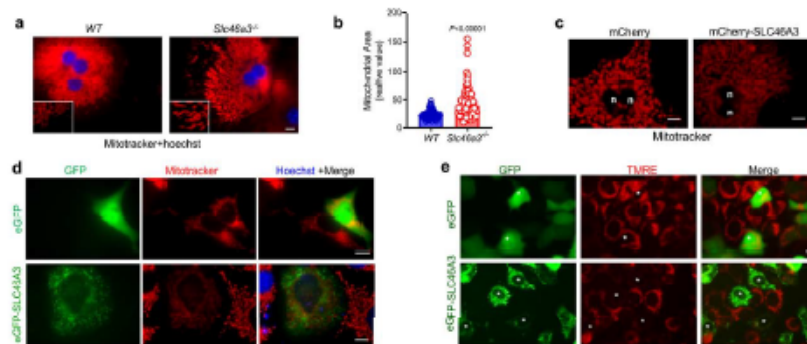
High Copper Levels in the Liver, Intestine, and Tumor

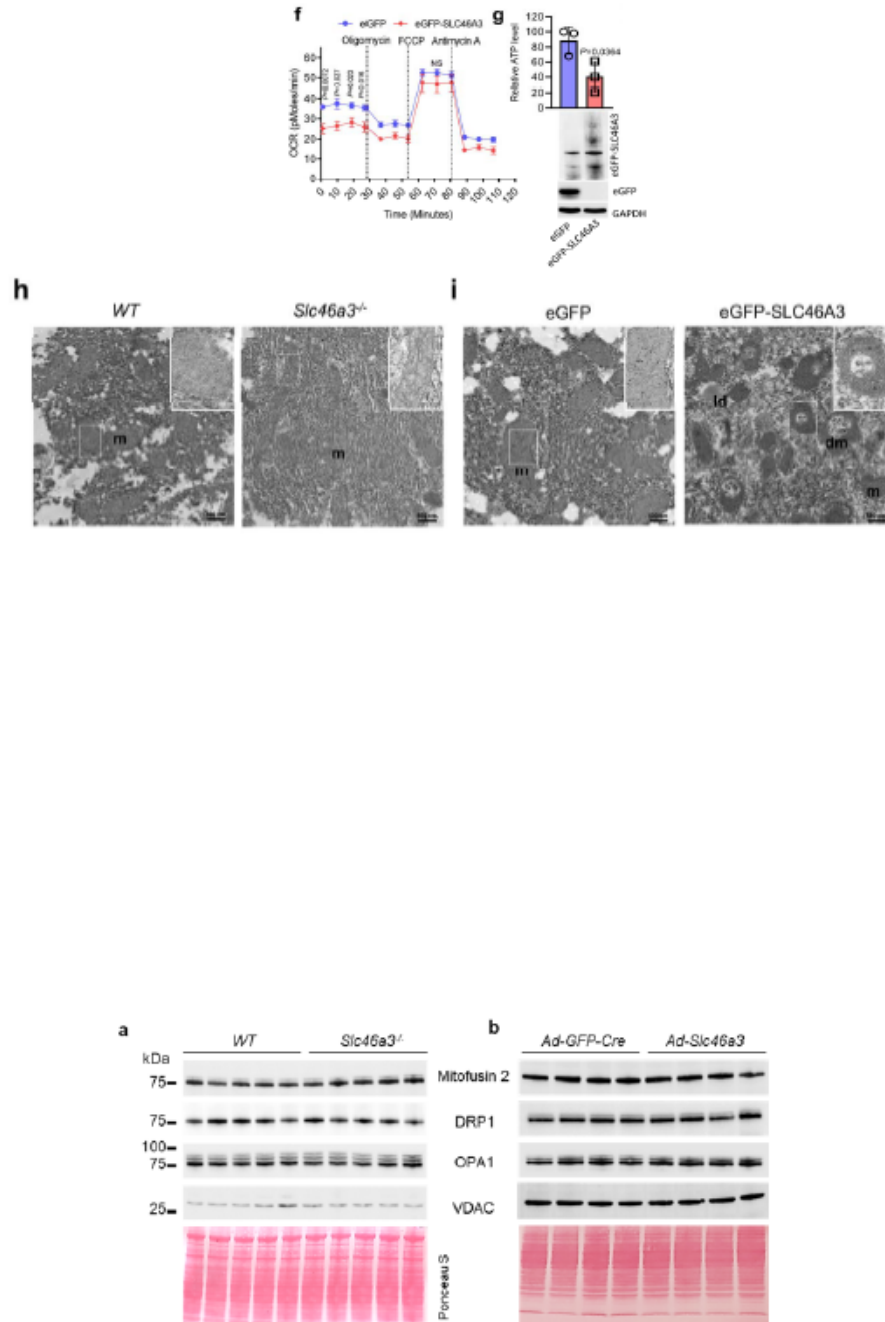


The athymic mice bearing human prostate cancer xenografts were received $^{64}\text{CuCl}_2$ tracer via tail vein.

Positron emission tomography (PET) visualized ^{64}Cu distribution.

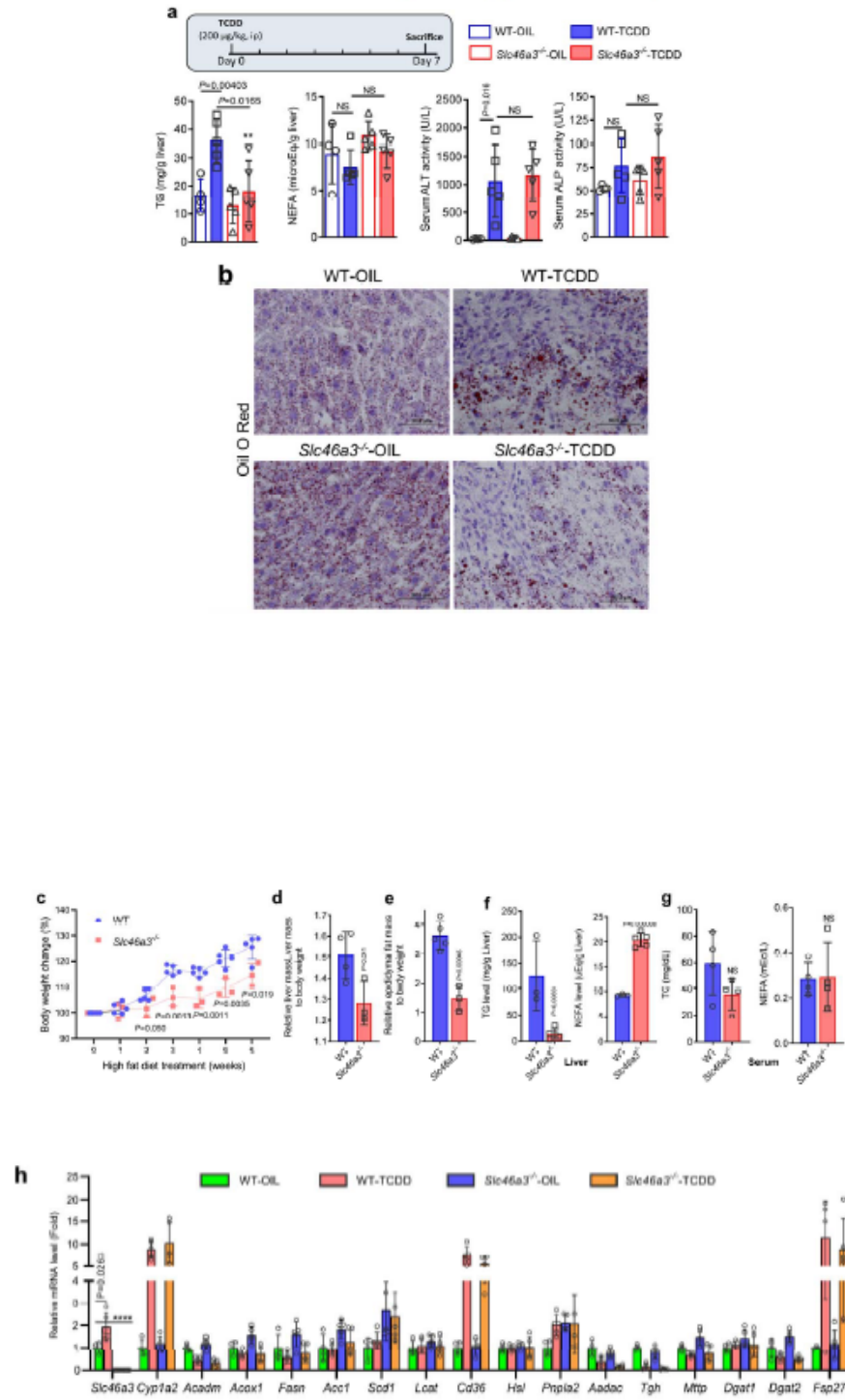
Slc46a3 affects mitochondrial potential and morphology



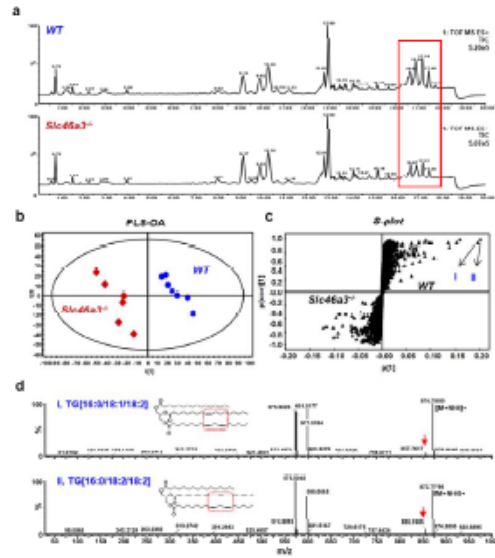


Supplementary Figure 9. Mitochondrial fission or fusion is not affected by SLC46A3. Liver samples from *Slc46a3*^{-/-} mice (*n*=5 biologically independent liver samples) (a) and adenoviral SLC46A3 expressing mice (*n*=4 biologically independent liver samples) (b) were subjected to western blotting. Antibodies against mitochondrial fission or fusion were used.

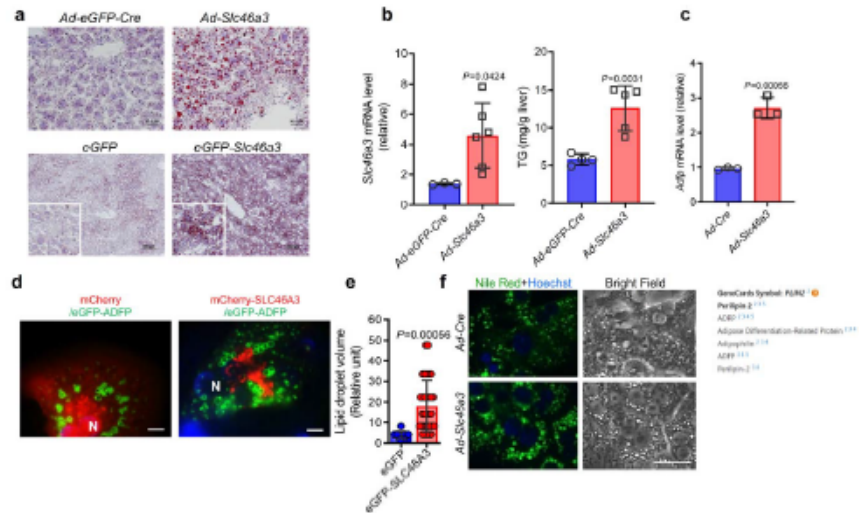
TCDD induces accumulation of hepatic triglyceride through SLC46A3.



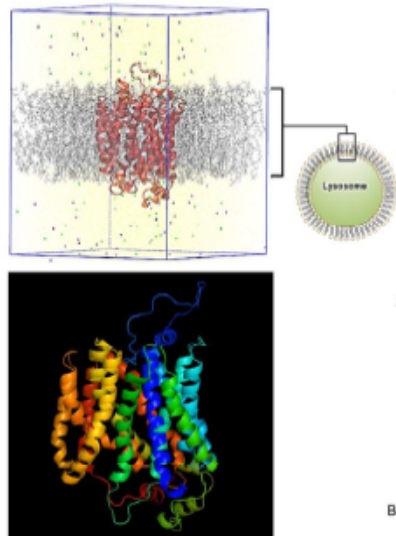
Ablation of the *Slc46a3* gene decreases hepatic triglyceride levels.
(Lipidomics)



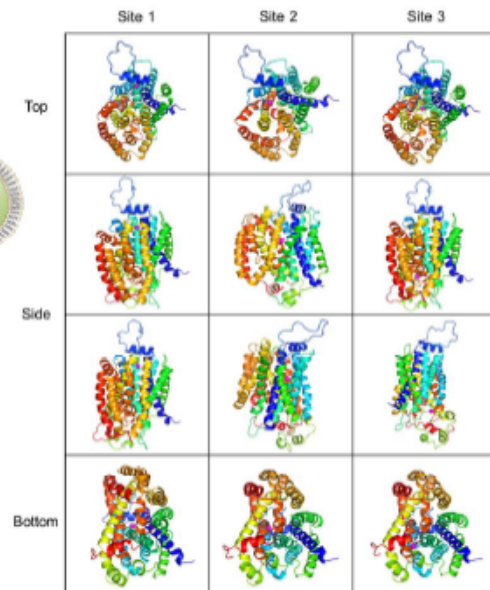
SLC46A3 overexpression triggers TG accumulation in the liver.



SLC46A3 modeling

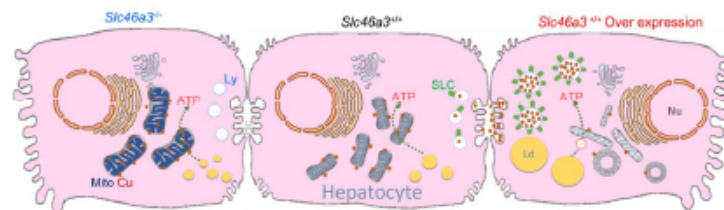


Copper-SLC46A3 docking



Conclusion

Scheme showing a possible role of SLC46A3 in hepatic lipid accumulation in mice



ARTICLE



<https://doi.org/10.1038/s41467-020-20461-0>

OPEN

Lysosomal SLC46A3 modulates hepatic cytosolic copper homeostasis

Jung-Hwan Kim^{1,2}, Tsutomu Matsubara^{2,7}, Jaekwon Lee³, Cristina Fenollar-Ferrer⁴, Kyungreem Han⁵, Donghwan Kim², Shang Jia⁶, Christopher J. Chang⁶, Heejung Yang^{2,8}, Tomokazu Nagano^{2,9}, Kristopher W. Krausz², Sun-Hee Yim^{2,10} & Frank J. Gonzalez²

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Heejung Yang
Tomokazu Nagano
Sun-Hee Yim
Dong-Hwan Kim
Kristopher W. Krausz
Linda Byrd
Frank J. Gonzalez

Kyungreem Han (NHLBI/NIH)
Jaekwon Lee (Nebraska Univ.)
Cristina Fenollar-Ferrer (NIMH/NIH)

Christopher Chang (UC Berkeley)-future collab.
Si Chen (Argonne National Laboratory) future collab.



감사합니다

2023.05.03.

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서울대 약대

강건욱 교수님

TYRO3-targeting strategy to overcome metastasis and immune evasion of cancer cells

Miso Park & Keon Wook Kang

College of Pharmacy, Seoul National University

1

Contents

PART I.

Circulating Small Extracellular Vesicles Activate TYRO3 to Drive Cancer Metastasis and Chemoresistance

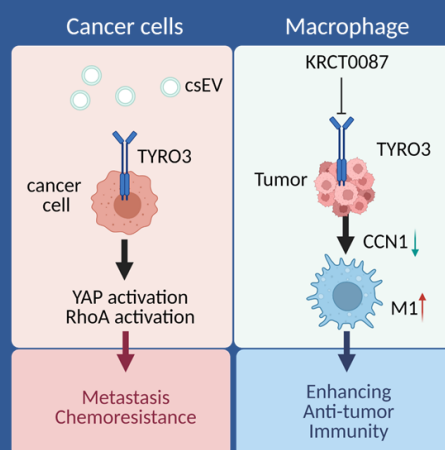
(Park M, et al. *Cancer Res* **2021**)

PART II.

Immuno-oncological efficacy of KRCT0087, a novel TYRO3 small-molecule kinase inhibitor

(Park M, et al. *J Immunother Cancer* **2023**)

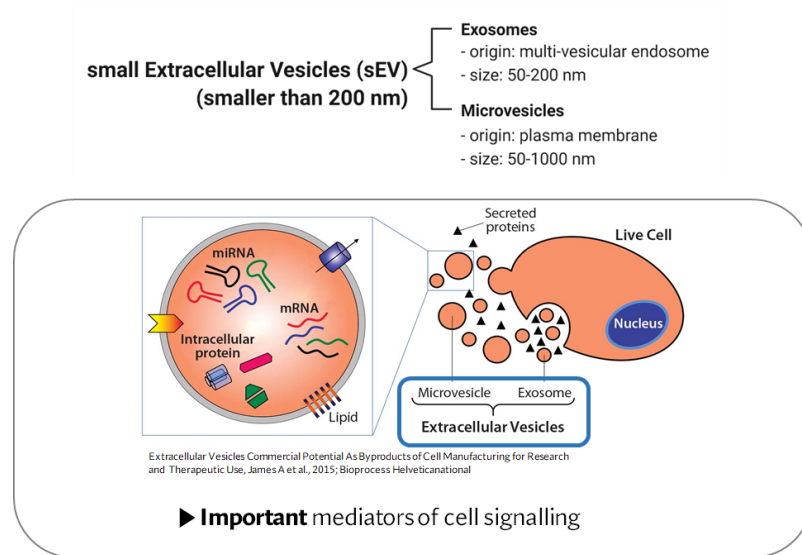
► Circulating Small Extracellular Vesicles (csEV)



2

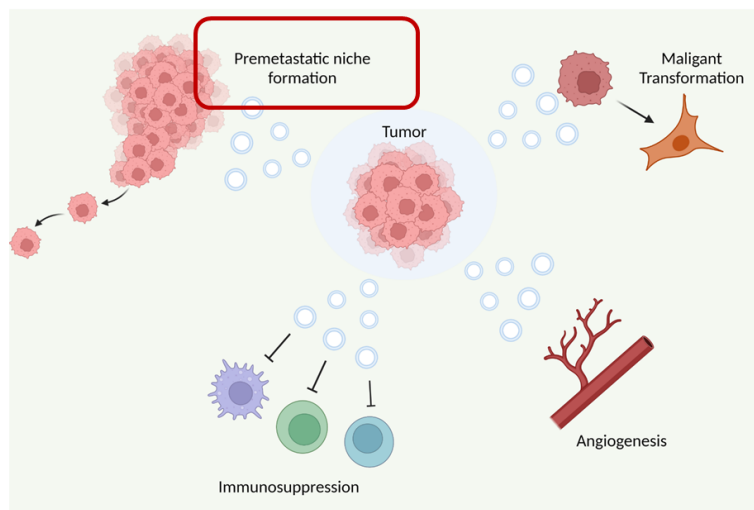
Small Extracellular vesicles (sEV)

3



Tumor derived extracellular vesicles (TdEV)

4

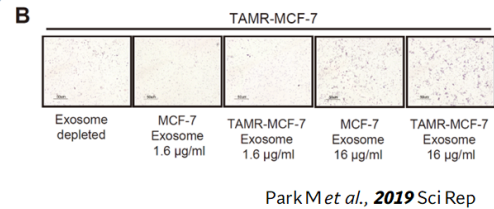


▪ **Our focus has moved on...**

Tumor derived extracellular vesicles (TdEV)

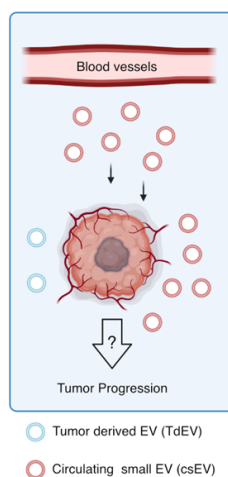


Circulating small extracellular vesicles (csEV)



▪ **The vascular system**

- ① Critical for maintaining **cancer cell survival**
- ② Inevitable space for circulating tumor cells (CTC) to begin **metastasizing**
- ③ The levels of circulating angiogenic factors correlate with **the aggressiveness of cancer**



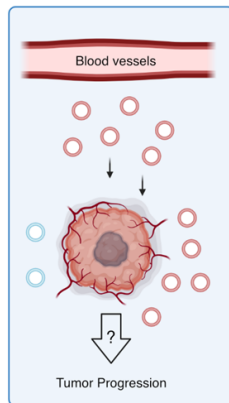
- The number in blood of patients with metastatic cancer:
Circulating tumor cells << platelets and erythrocytes (10^8 fold)
→ **most csEVs do not originate from tumors**

- Most have focused on the clinical potential of csEVs **as a biomarker**



Little information about their role in cancer progression.

Circulating small extracellular vesicles (csEV)

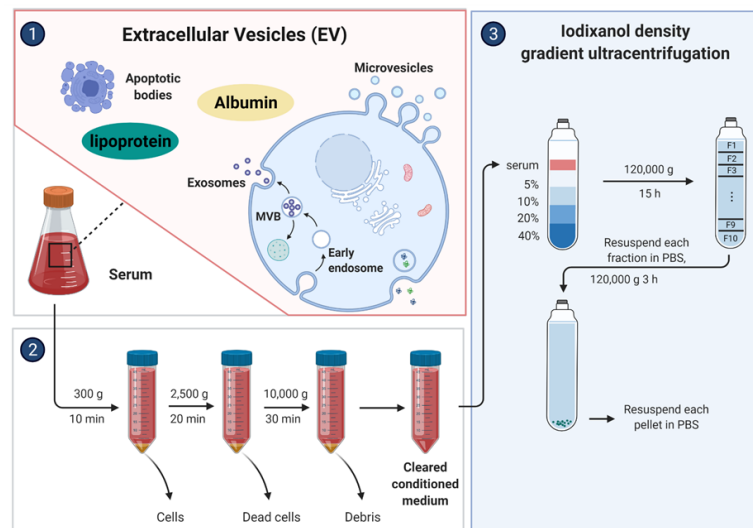


- Tumor derived EV (TdEV)
- Circulating small EV (csEV)

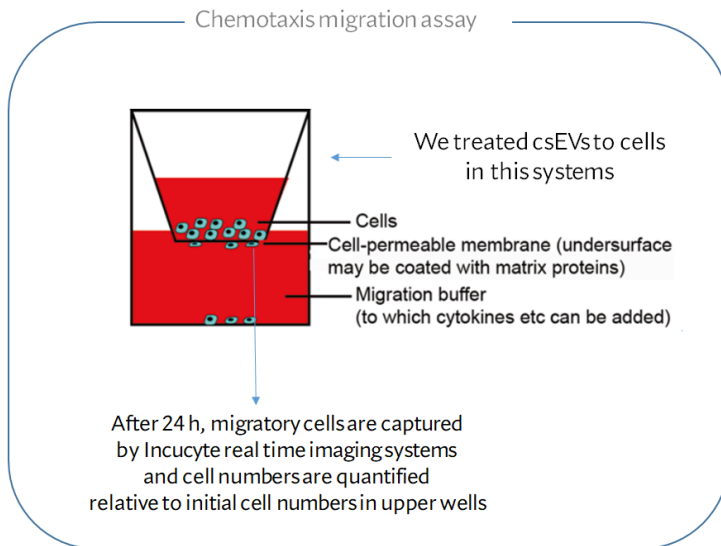
- csEVs are more abundant than TdEVs in the blood
(median value of plasma EV particle number = $2 \times 10^{10}/\text{mL}$)

Hypothesis 1:
csEVs play an crucial mediator in cancer progression

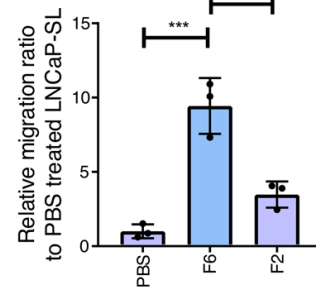
Isolation of csEV by serial centrifugation



edited from biorender templates

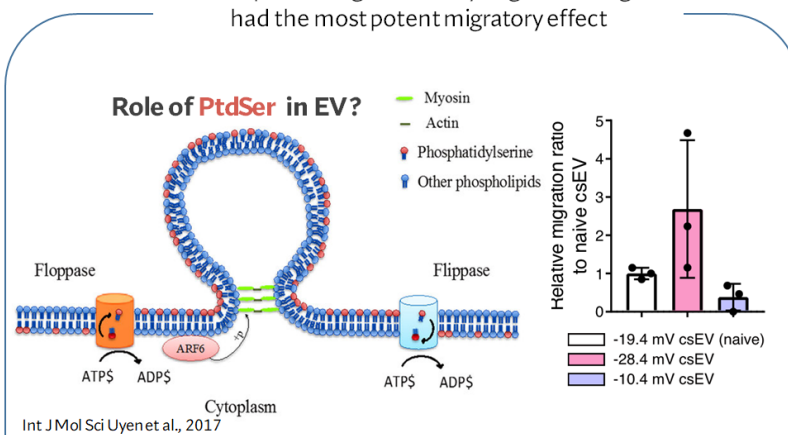


Chemotaxis migration assay

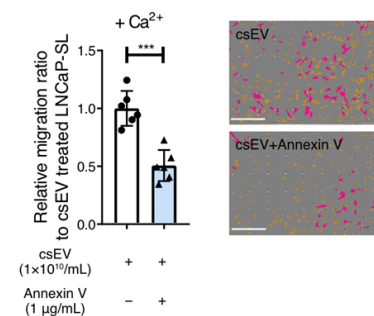


Cell line: LNCaP-SL
(a subline of LNCaP cells, a very aggressive prostate cancer cell line)

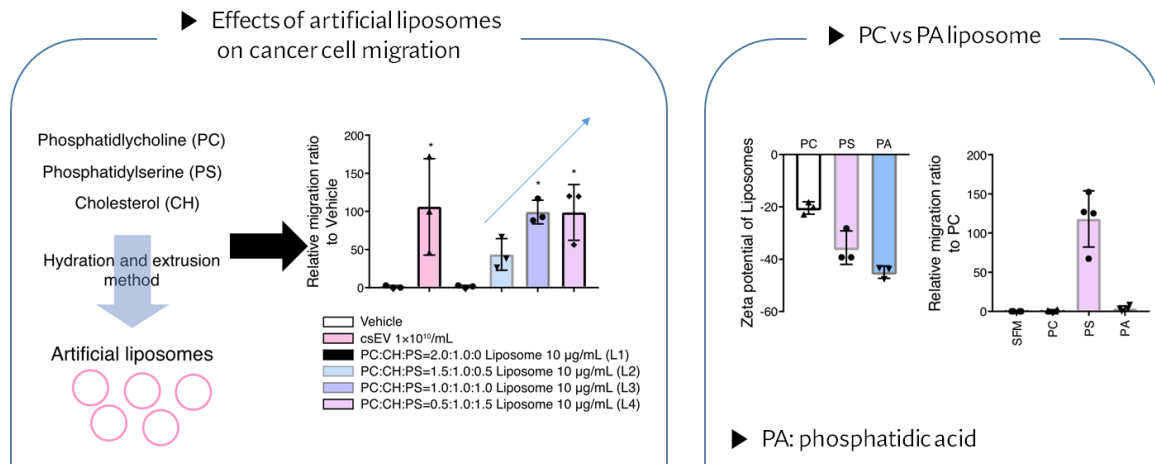
csEVs possessing a relatively negative charge had the most potent migratory effect



csEV-induced cell migration were significantly inhibited by Annexin V

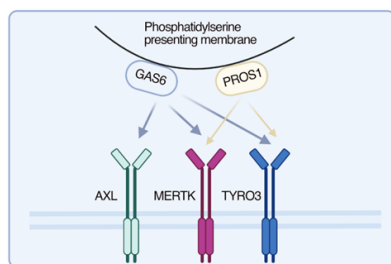


- PtdSer: **fourth most abundant** and **most negatively charged** lipid of small EVs
- Annexin V: a PS-binding protein



Artificial liposomes were provided by Prof. Jinki Kim (Hanyang Univ., South Korea)

Phosphatidylserine receptor: TAM RECEPTOR family



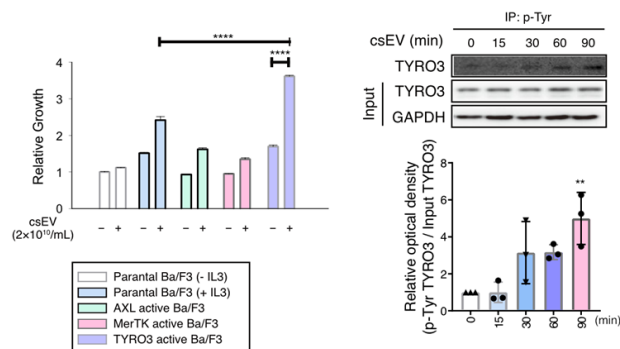
- They require both a **bridging protein ligand** (e.g., **Gas6** and **PROS1**) and **phosphatidylserine lipid moiety** for **maximizing the effector coupling of TAM receptors**

- Role of TAM receptors in cancer**

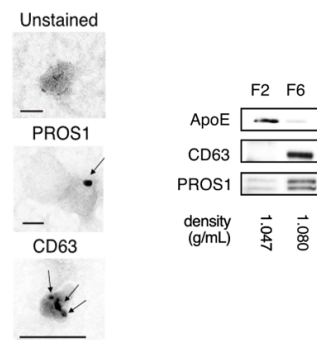
- Abnormal expression in cancer cells
- Proliferation and invasiveness of cancer cells

Investigating whether csEV could activate TAM receptors

► csEV-driven TYRO3 activation



► Existence of **PROS1** in csEV?



PROS1 is a ligand for TYRO3 and exists in plasma at 346 nmol/L

2023.05.10.

우수연구자 초청 세미나

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SYNTHETIC DRUG DEVELOPMENT PROCESS

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목차

- 01. Merck 소개
- 02. 합성의약품의 종류
- 03. 의약품 개발과정
- 04. 부서별 소개와 역할
- 05. Preformulation
- 06. Q&A

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연구자가 잠재 약물 발굴



실험실

연구자가 정확한 실험, 테스트 및 진단을 통해 의약품의 식별하고 개발할 수 있도록 지원



약물 재형화

연구자가 정제, 주사액, 액상 등 약물 제형 결정



약물 생산

대규모에서 소규모까지 다양한 규모의 약물 대량 생산



규제 및 임상 시험

모든 규칙을 준수하여 프로세스가 진행되는지 확인



현지 실험실 및 진료실

정확한 진단을 받아 최종 의약품을 수령할 수 있도록 보장



백신 및 의약품

안전하게 제조된 약물 받을 수 있도록 검사 시행



식용료

구매하는 식용료의 안전 보장



이동

안전하고 건강한 대기 환경을 위한 대기질 모니터링 수행

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PROCESS
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products

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새로운 제품의 수

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등록된 고객수

500,000~

한국 시스템에
등록된 제품수

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매년 등록되는
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7

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사용 분석 기기 및 시험

Medi-Chem analysis (U)HPLC-(UV, MS, MS/MS, ToF, etc.)
PK analysis GC-(FID, ECD, MS, etc.)
조제물 분석 Titration
제제(완제)분석 Karl Fischer
원료분석 Thermogravimetric analysis (TGA)
공정분석 Differential scanning calorimetry (DSC)
Nuclear magnetic resonance (NMR)
X-ray Powder Diffraction (XRD)
Dynamic vapor sorption (DVS)
Melting Point (MP)
Etc.

**Sangseol
Jeon**

2015.03 ~ 2021.12



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분석연구팀



합성공정팀 - 분석

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유한양행 - 참여 과제

레이저티닙(YH25448, GNS-1480): EGFR 변이 폐암 3세대 표적치료제

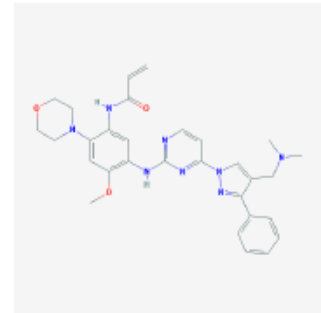
YH12852: 과민성 장증후군(irritable bowel syndrome, IBS)

YH33619: 비알콜성 지방간염 (Non-alcoholic Fatty Liver Disease, NASH)



출처: 유한양행 홈페이지

Amivantamab (JNJ-61186372) 병용투여 연구 중



janssen

유한양행(주)
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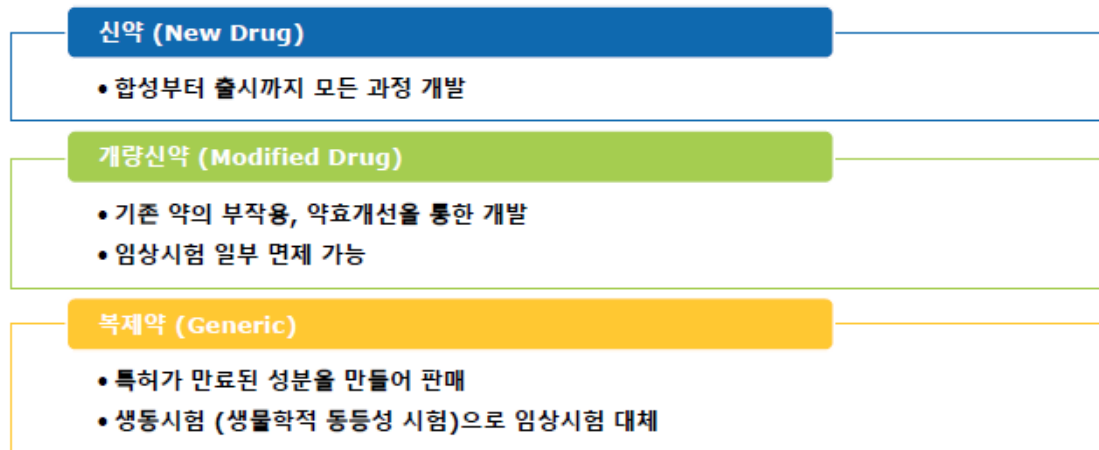


02

합성의약품의 종류

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합성의약품의 종류



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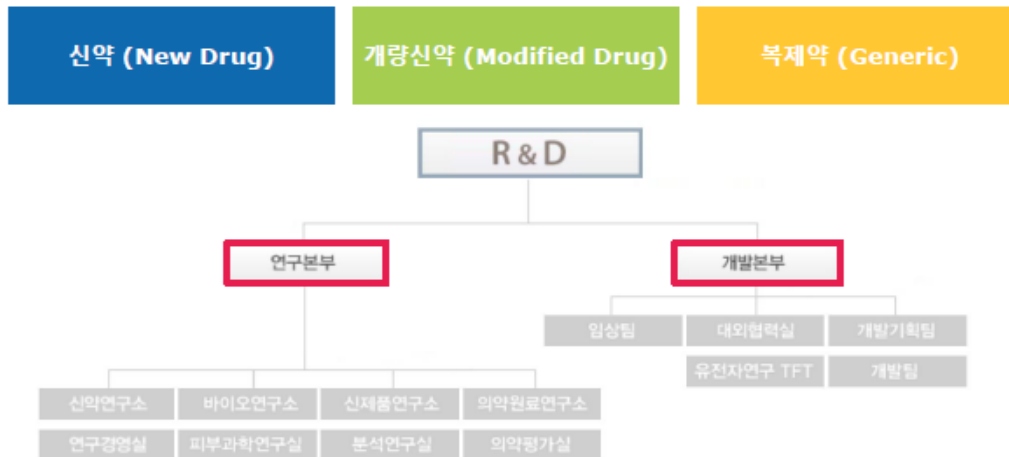
합성의약품의 종류 – 연구와 개발



12 SYNTHETIC DRUG DEVELOPMENT PROCESS

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합성의약품의 종류 - 제약회사의 부서



13 SYNTHETIC DRUG DEVELOPMENT PROCESS

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연구소 부서 별 역할

제제연구 (제제기술)

- 의약품 제형 연구 (전문의약품, 일반의약품, 개량신약, 신약 등) 및 신제품 개발 등
- 신제품 현상화 및 scale-up 연구, 기하가 품목의 허가유지 및 품질 개선 연구 등
- DDS첨가기술 개발 및 개량신약 재용화 연구 등

합성연구

- 신규합성법 연구, 생산공정 최적화 연구, 신규물질 합성 연구 등

의약화학

- 합합, 신물질, 신물질개발 등 전임상 후보물질 도출 등

약리연구

- 신약 후보물질의 약리 연구/약효평가 및 임상 개발물질의 약리연구 및 임상성능 분석 등

비임상 연구

- 비임상 이상의 개발단계 후보물질의 비임상과 임상의 비교 연구 및 신약 후보물질의 안전성 평가 및 약동학 특성 연구 등

공정개발

- 전임상 단계 신약의 신규 합성공정 개발 및 전임상/임상 연구용, 실험 제제연구용 DS 생산 등

임상개발

- 임상과제 Operation, 임상 타당성 검토, 임상자료 관리 및 통계

분석

- 각 Stage에서 필요한 다양한 분석업무 수행

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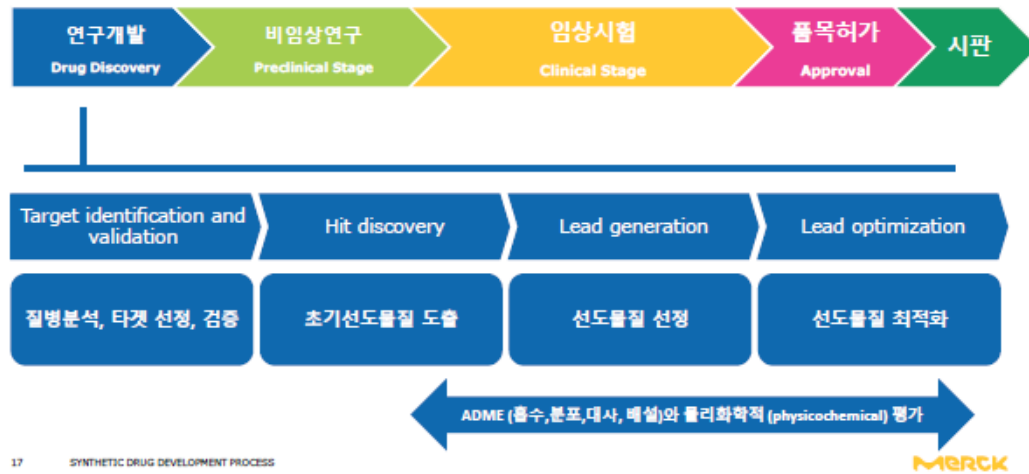
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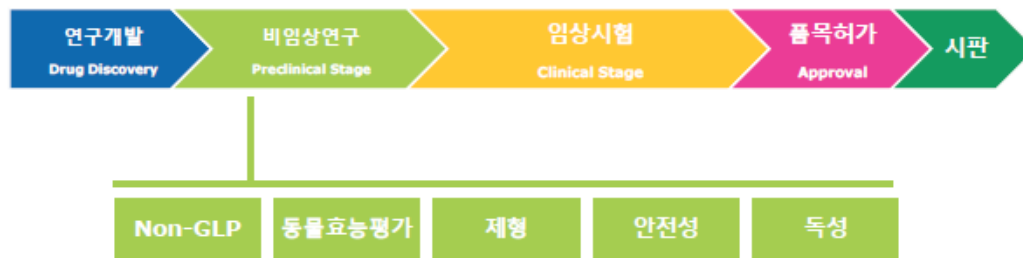


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의약품 개발 과정



의약품 개발 과정



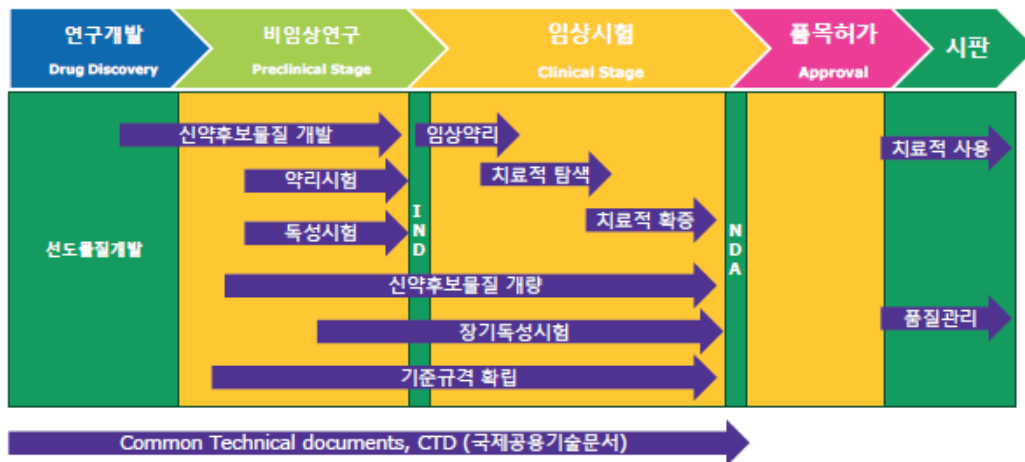
의약품 개발 과정



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의약품 개발 과정



20 Title of Presentation | DD.MM.YYYY

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의약품 개발 과정

Common Technical documents, CTD (국제공용기술문서)

Module 1: 각 지역별 행정요구 정고

Module 2: 목차 및 서론

Module 3: 품질평가보고서

Module 4: 비임상시험결과 보고서

Module 5: 임상시험결과 보고서



의약품 개발 과정

Common Technical documents, CTD (국제공용기술문서)

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	3.2.5.2.4.42. 유효성평가(Validation of Specification)	34	3.2.5.2.4.42. 유효성평가(Validation of Specification)	34
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	3.2.5.2.4.44. 유효성평가(Validation of Specification)	34	3.2.5.2.4.44. 유효성평가(Validation of Specification)	34
	3.2.5.2.4.45. 유효성평가(Validation of Specification)	34	3.2.5.2.4.45. 유효성평가(Validation of Specification)	34
	3.2.5.2.4.46. 유효성평가(Validation of Specification)	34	3.2.5.2.4.46. 유효성평가(Validation of Specification)	34
	3.2.5.2.4.47. 유효성평가(Validation of Specification)	34	3.2.5.2.4.47. 유효성평가(Validation of Specification)	34
	3.2.5.2.4.48. 유효성평가(Validation of Specification)	34	3.2.5.2.4.48. 유효성평가(Validation of Specification)	34
	3.2.5.2.4.49. 유효성평가(Validation of Specification)	34	3.2.5.2.4.49. 유효성평가(Validation of Specification)	34
	3.2.5.2.4.50. 유효성평가(Validation of Specification)	34	3.2.5.2.4.50. 유효성평가(Validation of Specification)	34

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04

부서별 소개와 역할

MERCK

Research/Pharma
Functions

Supelco.



합성



제제



비임상



분석

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Research/Pharma Functions



합성 • Scale에 따른 분류

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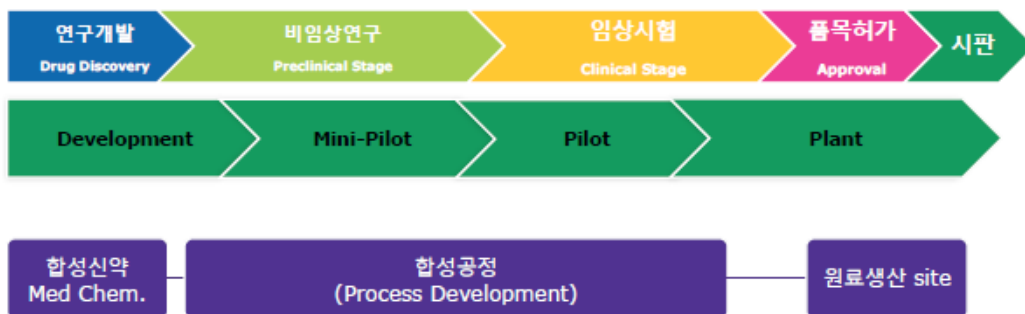


25

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Research/Pharma Functions

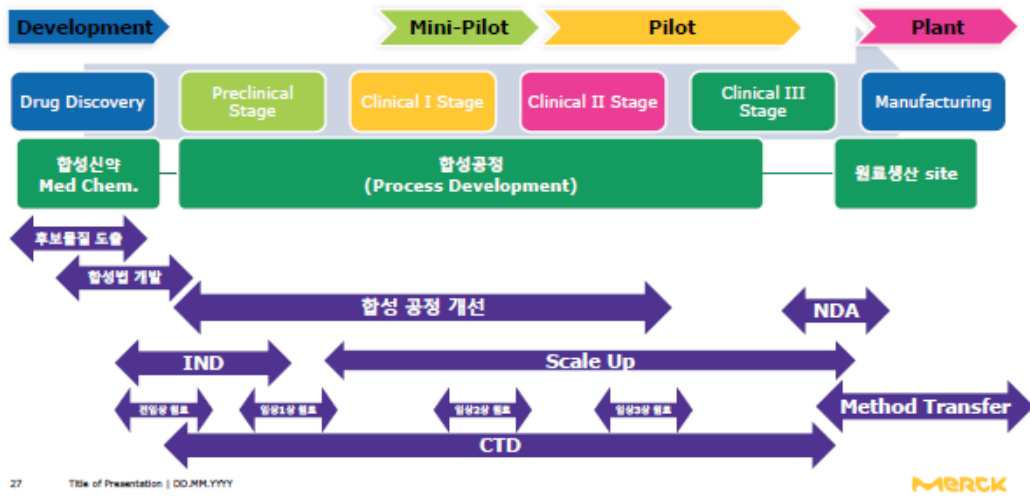
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26

MERCK

Drug Development Stages – 합성



Research/Pharma Functions



합성



제제



비임상



분석



제제 (Formulation)

DDS 관련 제형, platform 기술 개발 (신약, 개량신약 및 OTC 일반의약품 등)

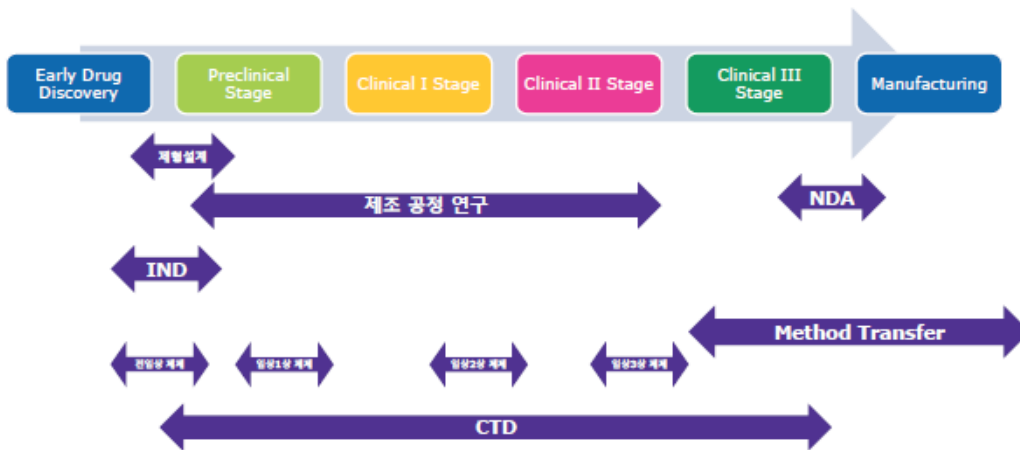


- **합성 신약**
 - 제형설계, 조성 연구, 제조공정 연구, 임상시험용의약품 제조, CTD 작성 등
- **개량 신약**
 - 제형설계, 조성 연구, 제조공정 연구, 임상시험용의약품 제조, CTD 작성 등
- **Generic 제품**
 - 생동성 시험, CTD 작성 등

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Drug Development Stages – 제제

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30

Title of Presentation | DD.MM.YYYY

MERCK



합성



제제



비임상



분석



비임상
(전임상, 독성평가)



세포실험 (in vitro)

동물실험 (in vivo)

- 약효평가
 - 세포실험
- 독성평가
 - 세포 및 동물실험
- Pharmacokinetics (PK)
 - 동물실험



비임상 (전임상, 독성평가)

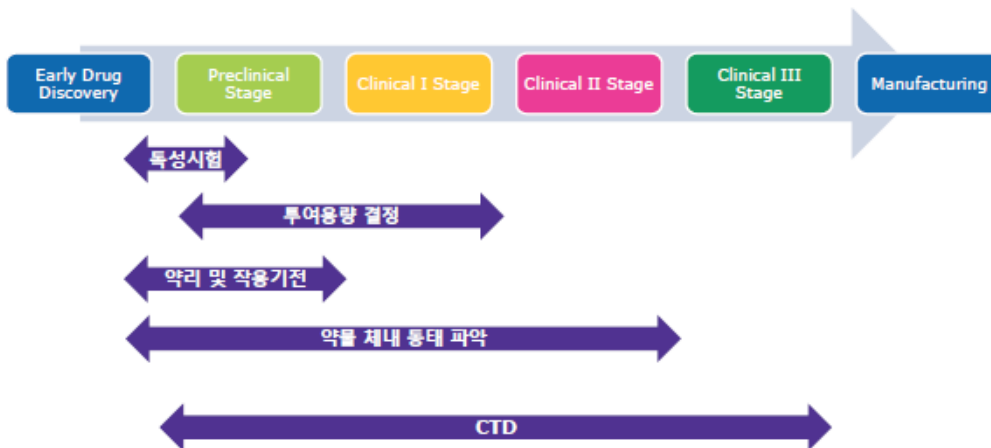


- **약효평가**
 - 물질의 작용기전 연구, 효능과 물성 측정 → 의약품 개발 가능성 검증 (ADME, DMPK)
- **독성평가**
 - 비임상 안전성 평가 및 연구
- **Pharmacokinetics (PK)**
 - 동물실험

ADME: Absorption (흡수), Distribuion (분포), Metabolism (대사), Extretion (배설)
DMPK: Drug Metabolism and Pharmacokinetics (약물동력학)



Drug Development Stages – 비임상



Research/Pharma
Functions

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합성



제제



비임상



분석

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Research/Pharma
Analytical methods

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분석

**확인시험 (Identification) and
정량시험 (Quantification)**
of the active pharmaceutical ingredient

안정성시험 (Stability Testing)
of drugs

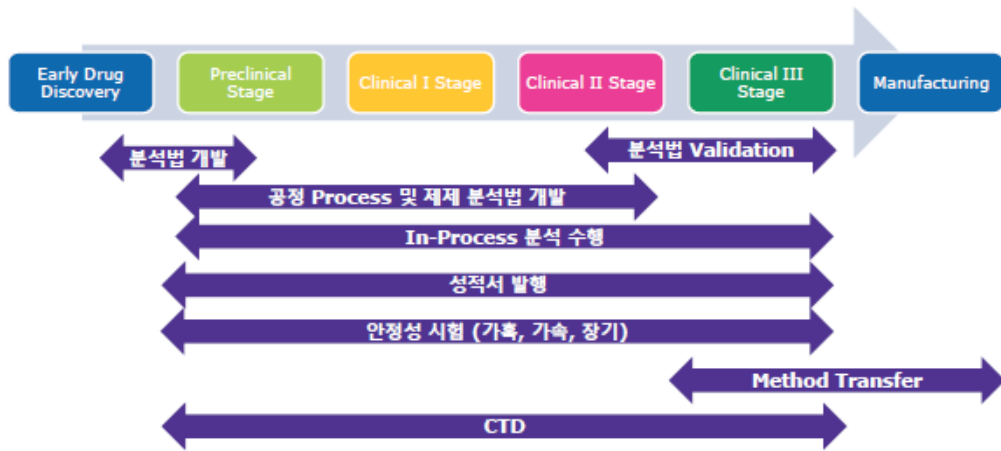
원료시험 (Raw material control)
For API synthesis and drug formulation

불순물 시험 (Impurity Testing)
of drugs arising in production,
packaging, transport and storage

수분함량 (Water Content)
of drug formulations (pills, tablets, gels,
creams, etc.)

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Drug Development Stages – 비임상



37 Title of Presentation | DD.MM.YYYY



의약품 개발 과정



Preformulation

후보물질의 물리화학적 및 생물학적 특성의 확인 필요

Physicochemical property
solubility, chemical stability, solid state property, formulatability

Biological/biopharmaceutical property
permeability/efflux, metabolism, clearance, safety

Preformulation 의약품 개발 과정

Preformulation

후보물질의 물리화학적 특성 (physicochemical properties)의 확인 필요

Physicochemical property
solubility, chemical stability, solid state property, formulatability (제제성)

- Solubility: Kinetic solubility 등
- Chemical stability: salt별, 결정형 별 stability 등 확인
- Solid state property
- Formulatability: 흐름성등을 파악하여 제제성을 확인

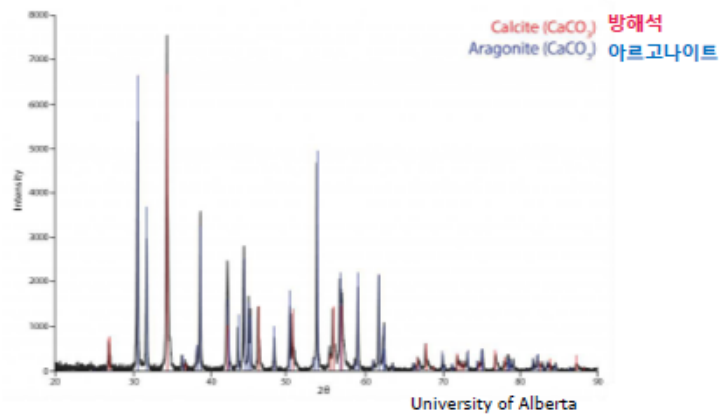
Biological/biopharmaceutical property
permeability/efflux, metabolism, clearance, safety

- Permeability/efflux: 투과율 등 (e.g. BBB 투과율)
- Metabolism: 대사 확인
- Clearance: CL, 몸에서 사라지는 제거율
- Safety: 안전역(Margin of safety) 계산 등, 독성과 약효의 평가

Preformulation

Supelco.

Crystallization



41 SYNTHETIC DRUG DEVELOPMENT PROCESS

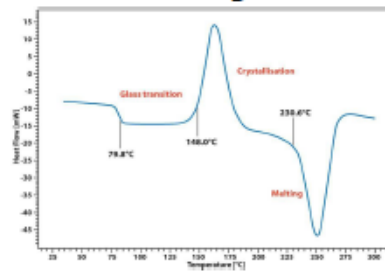
MERCK

Preformulation

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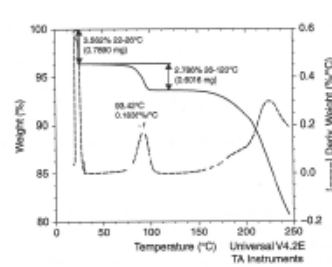
Crystallization

DSC Thermogram



42 SYNTHETIC DRUG DEVELOPMENT PROCESS

TGA Thermogram

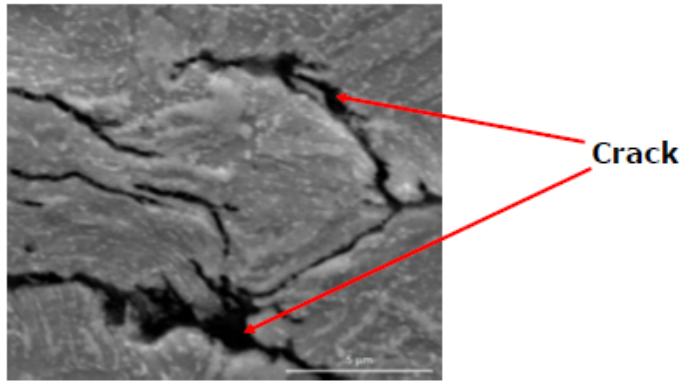


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Preformulation

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SEM - Crack



University of Alberta

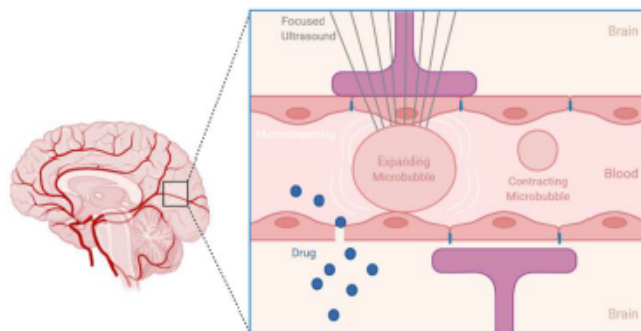
43 SYNTHETIC DRUG DEVELOPMENT PROCESS

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Preformulation

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blood brain barrier permeability



44 SYNTHETIC DRUG DEVELOPMENT PROCESS

MERCK

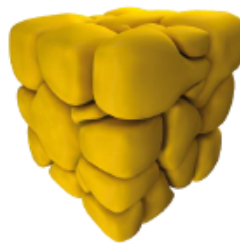
06. Q&A

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머크 생명과학에 대한 최신 정보를 온라인에서 만나보세요!

sigmaaldrich.com

1



2



Kakao Talk Channel



3

YouTube



전상설 (Sang-seol Jeon)
Product Marketing Manager
Science & Lab Solutions Life Science
Sang-seol.jeon@merckgroup.com

MERCK

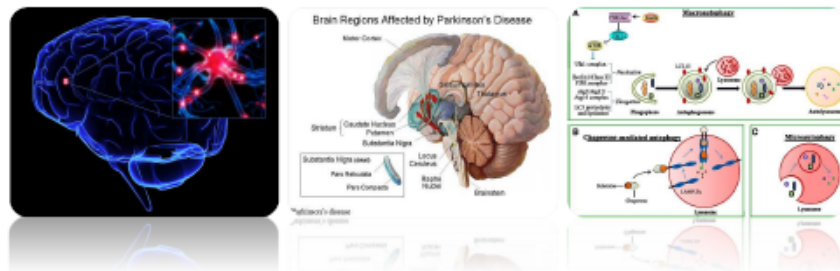
2023.05.17.

우수연구자 초청세미나

차의과대 약학대

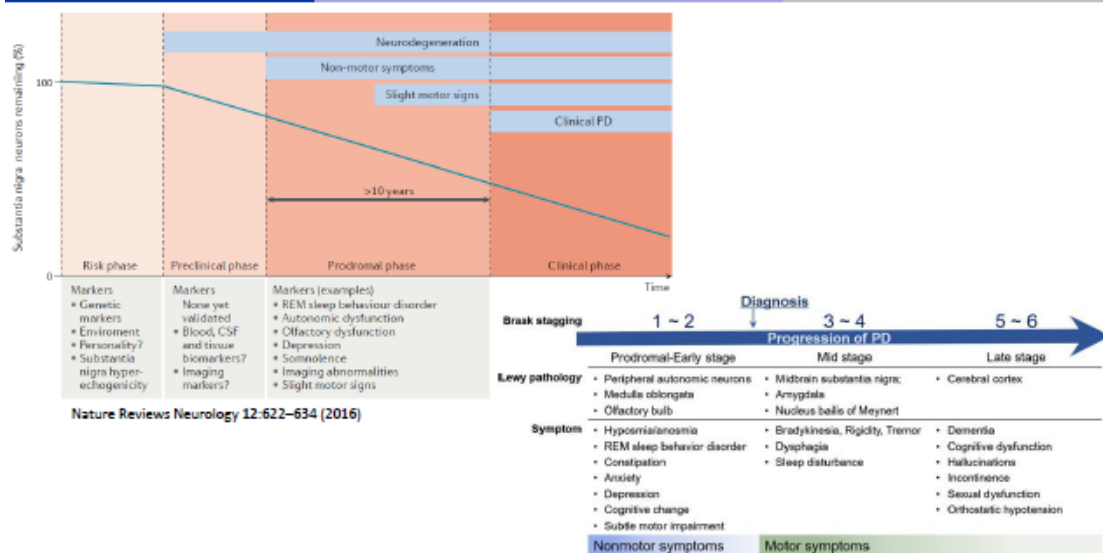
최현진 교수님

핵수용체 매개 neurogenesis 조절: 파킨슨병 수반 우울증 치료 신규 표적



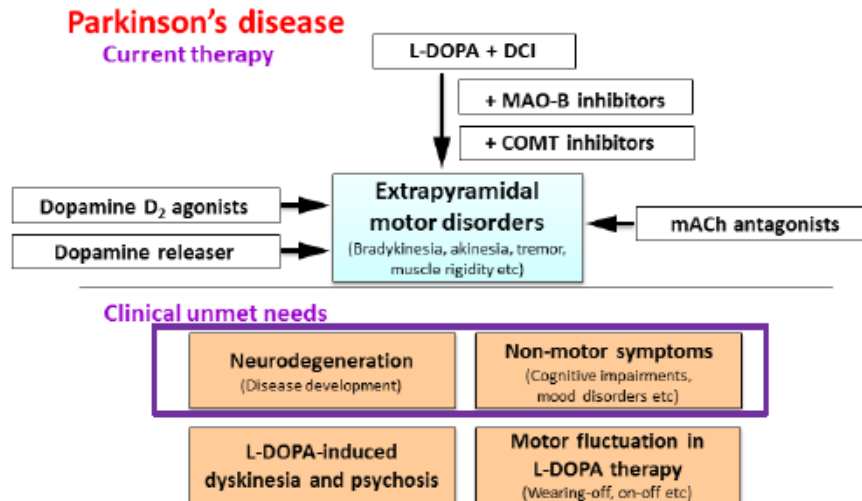
차의과학대학교 약학대학
최 현 진

파킨슨병의 non-motor symptoms



- ✓ When people are diagnosed with Parkinson's disease, they have usually lost approximately 50-60% of the dopamine neurons in an area of the brain called the substantia nigra.

파킨슨병 치료현황 및 한계



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파킨슨병 약물 개발 타겟

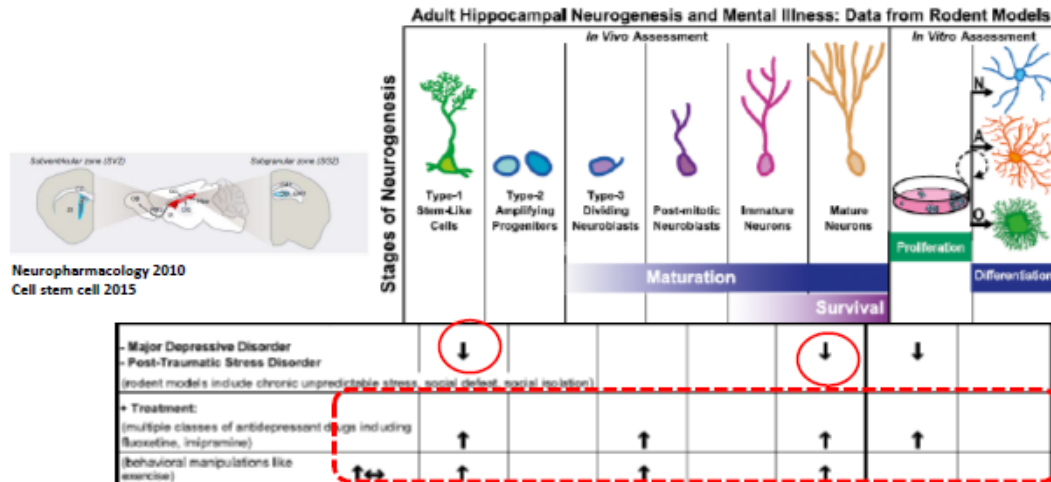
DISEASE-MODIFYING	MOTOR SYMPTOM	NON-MOTOR SYMPTOM
Aim to prevent, slow or halt the progression of PD	The cardinal symptoms of PD affect mobility	For quality of life
• Antioxidant • Anti-inflammatory drugs • Aggregates reducers: autophagy regulators • Neurotrophic factors	• Dopamine availability regulation	• Anxiety/Depression • Constipation • Swallowing problems • Cognitive decline
• Stem Cell Therapy		



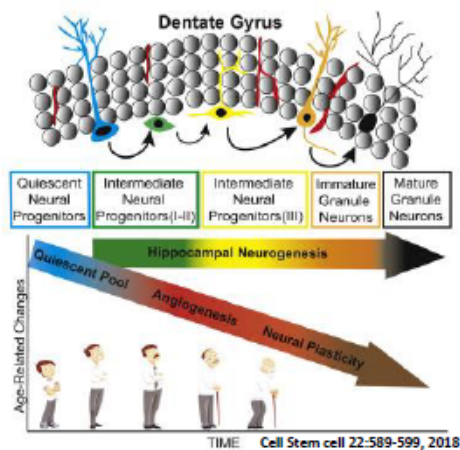
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Adult hippocampal neurogenesis & mental illness

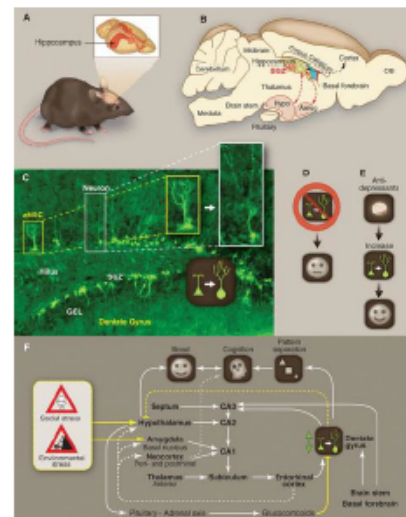
신경생성 및 발달기능 이상 → 신경정신질환 발병 및 진행 핵심 공통 기전



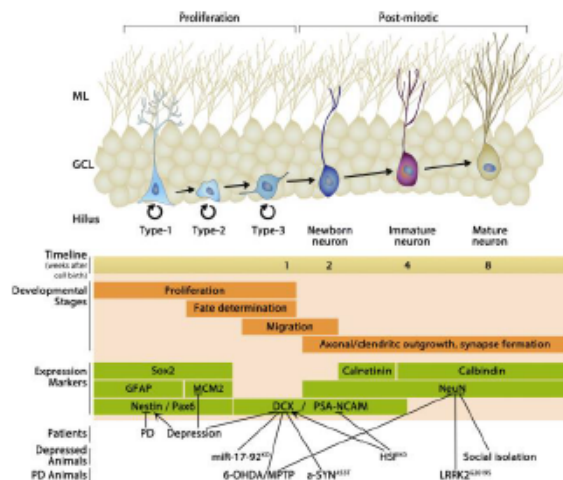
Adult hippocampal neurogenesis declines in aging



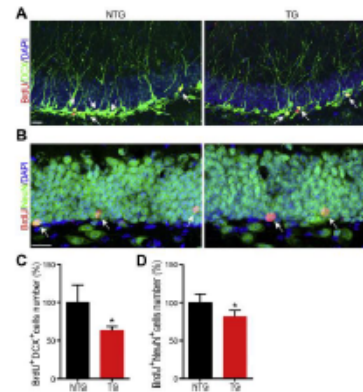
- ✓ Adult hippocampal neurogenesis is correlated with aging, epilepsy, stroke, neurodegenerative disorders, and psychiatric disorders.



Impaired hippocampal neurogenesis in PD



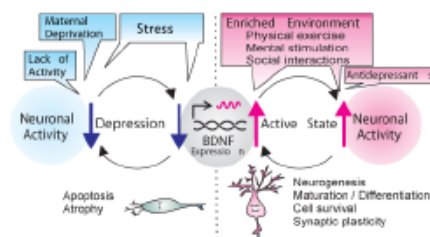
Abnormal hippocampal neurogenesis in Parkinson's disease: relevance to a new therapeutic target for depression with Parkinson's disease
Arch Pharm Res 41, 943 (2018)



Impaired neurogenesis in the hippocampus of an adult VPS35 mutant mouse model of Parkinson's disease
Neurobiol Dis 153, 105313 (2021)

BDNF & neurogenesis

- ✓ BDNF is a member of the neurotrophin family
- ✓ In the CNS, BDNF plays a key role in the neuronal growth and differentiation, neurogenesis, synapse formation, and neuronal survival.



Major Depressive Disorder

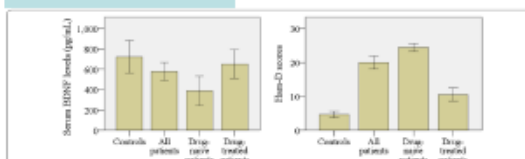
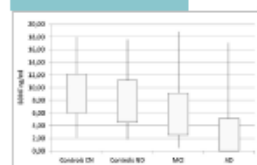


Fig. 1. Comparison of serum brain-derived neurotrophic factor (BDNF) levels and BDNF mRNA expression using quantitative real-time PCR among the different study populations. A significant difference between patient and control groups at a 95% confidence interval.

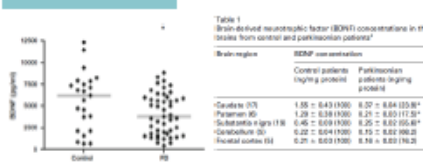
Emon et al., BMC Res Notes, 2020

Alzheimer's Disease



Siude et al., Neurol Neurochir Pol, 2016

Parkinson's Disease



Scalzo et al., J Neurol, 2010

Mogi et al., Neurosci Lett, 1999

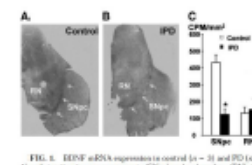
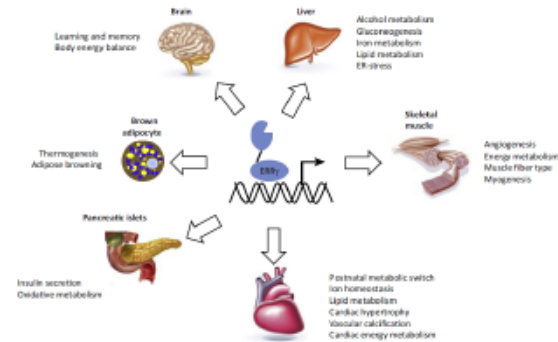


FIG. 1. BDNF mRNA expression in control (n = 10) and PD (n = 10) mice. BDNF mRNA expression was significantly reduced in PD mice compared to controls.

Howells et al., Exp Neurol, 2000

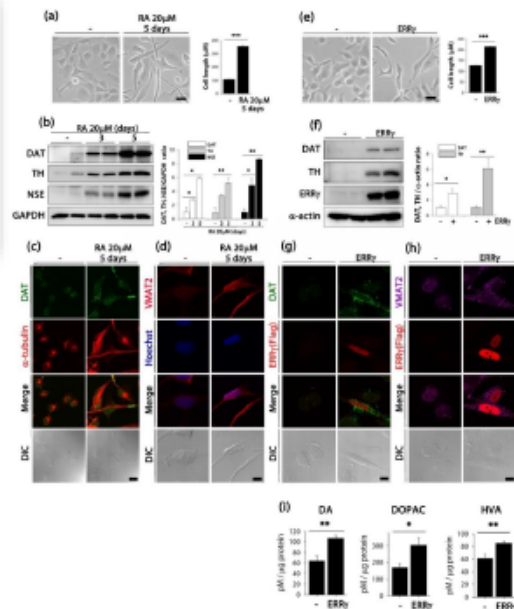
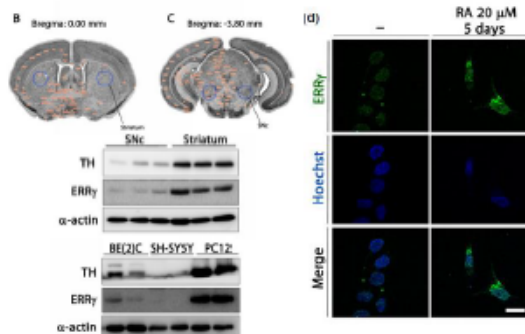
ERR γ (Estrogen-Related Receptor γ)

- ✓ 내인성 리간드가 밝혀지지 않은 orphan nuclear receptor
- ✓ constitutive active transcription factor
- ✓ estrogen receptor와 유사하나, estrogen과 결합하지 않음
- ✓ Tissue-specific expression (high metabolic demand)
- ✓ 현재 발굴된 리간드는 E6(agonist of ERR γ)와 GSK4716 (agonist of ERR β/γ) 등 (주로 암 및 대사성 질환에 연구)

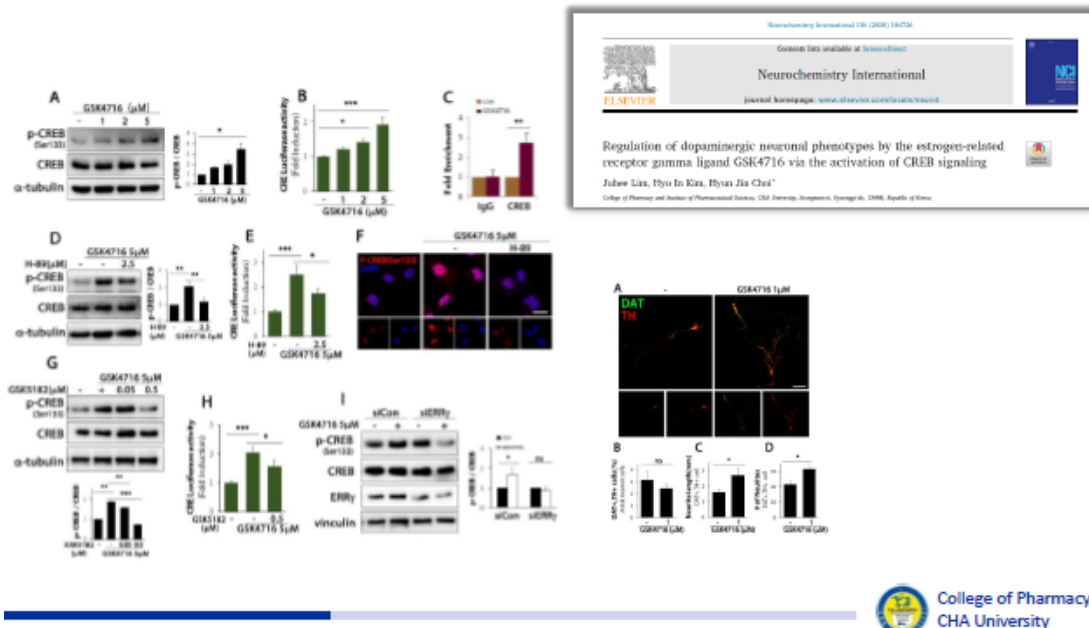


Thanks to Endocrinology & Metabolism

Role of ERR γ in the CNS

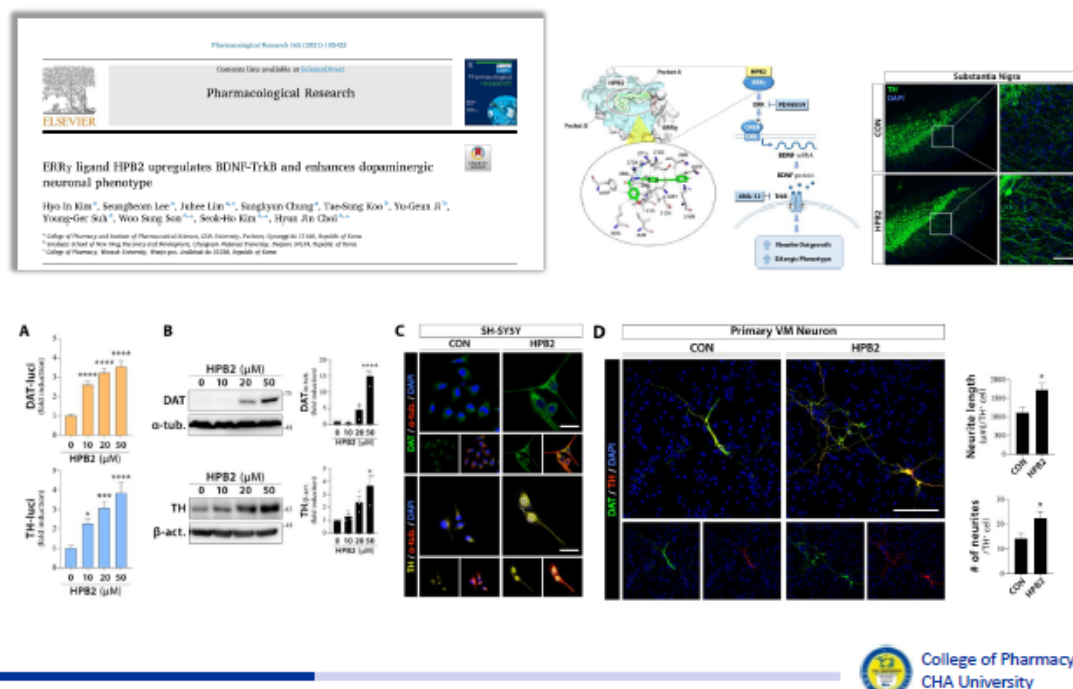


Role of ERR γ ligand in neuronal differentiation



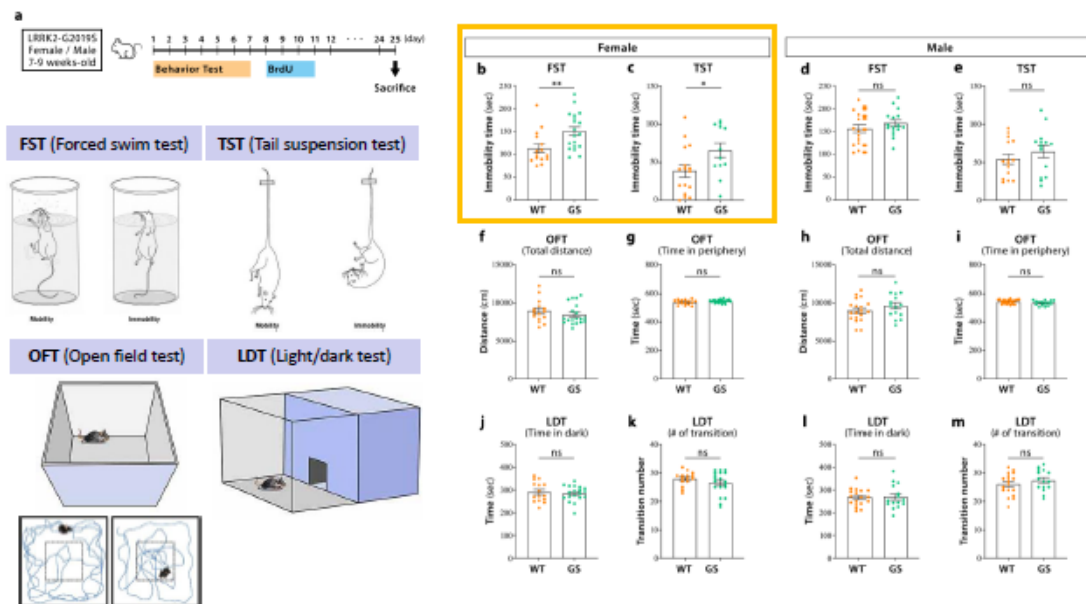
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Role of ERR γ ligand in neuronal differentiation

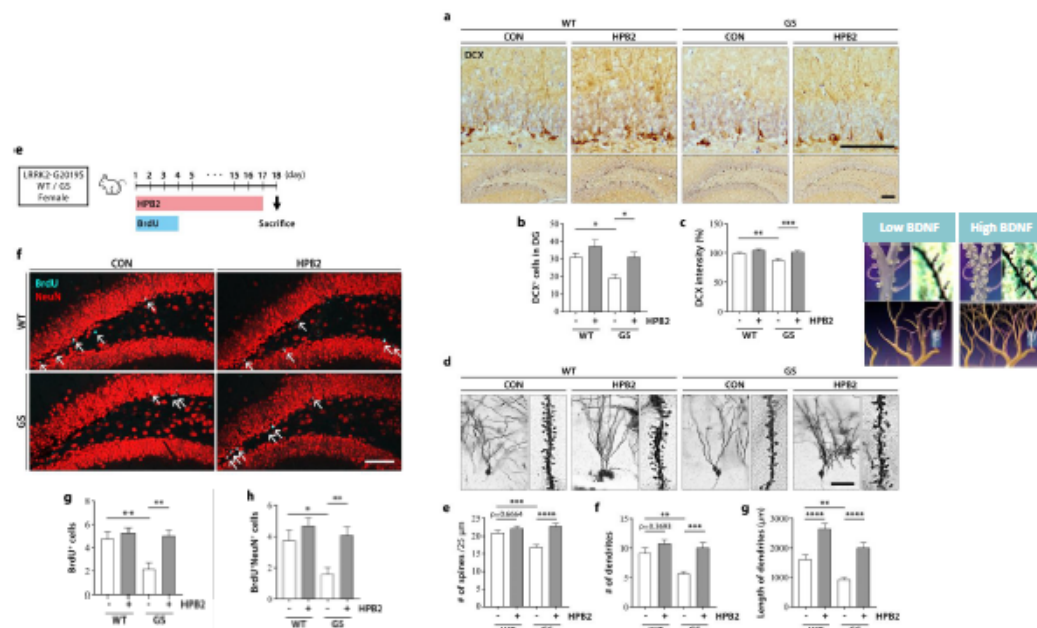


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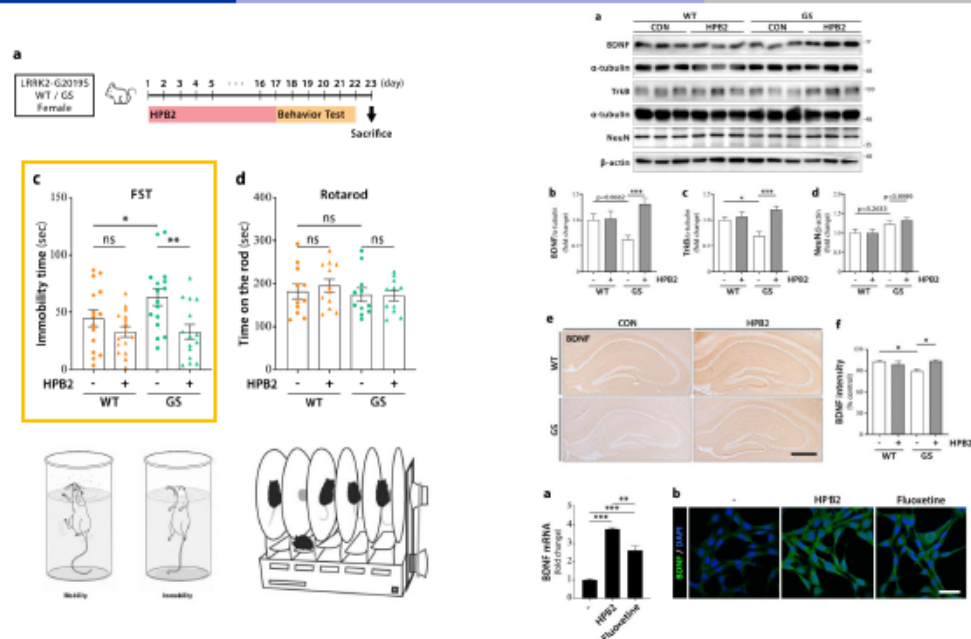
Young female LRRK2-G2019S mice show depressive behavior in FST and TST



ERR γ ligand enhances hippocampal neurogenesis of LRRK2-G2019S female mice

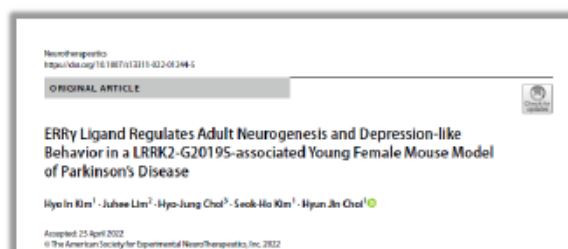


ERR γ ligand HPB2 ameliorates depressive behavior with BDNF upregulation

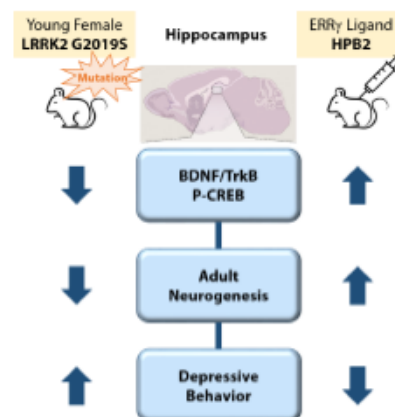


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Role of ERR γ in regulation of adult neurogenesis and PD-related depressive behavior



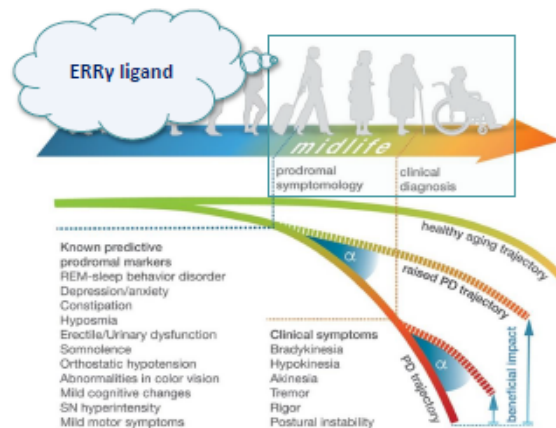
- ✓ ERR γ plays a role in the regulation of BDNF/TrkB pathway signaling and neurogenesis in the hippocampus.
- ✓ ERR γ ligand has potential in ameliorating dysregulated neurogenesis and depressive behavior.



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Neurogenesis 조절 신규 표적으로 핵수용체 ERR γ

- ✓ In the CNS, BDNF plays a key role in growth and differentiation of newly produced neurons, formation of synapses, and neuronal survival.
- ✓ ERR γ plays a role in the regulation of BDNF/TrkB pathway signaling and adult neurogenesis.
- ✓ ERR γ could be a new therapeutic target for regulating depressive symptom in MDD, as well as PD-associated depression prior to the onset of motor dysfunction.



감사합니다



Acknowledgement

Dr. Hyo In Kim
Prof. Juhee Lim

Prof. Seok-Ho Kim
Prof. Young-Ger Suh
Prof. Woo Sung Son
Prof. Min-Soo Kwon
Prof. Young Eun Huh

1/12- 05-24-

생화학 전공 대학원생 발표 세미나

김길환 학생

Role of PAK4 in HSC activation and liver fibrosis

School of Pharmacy

김 길환(석박통합)

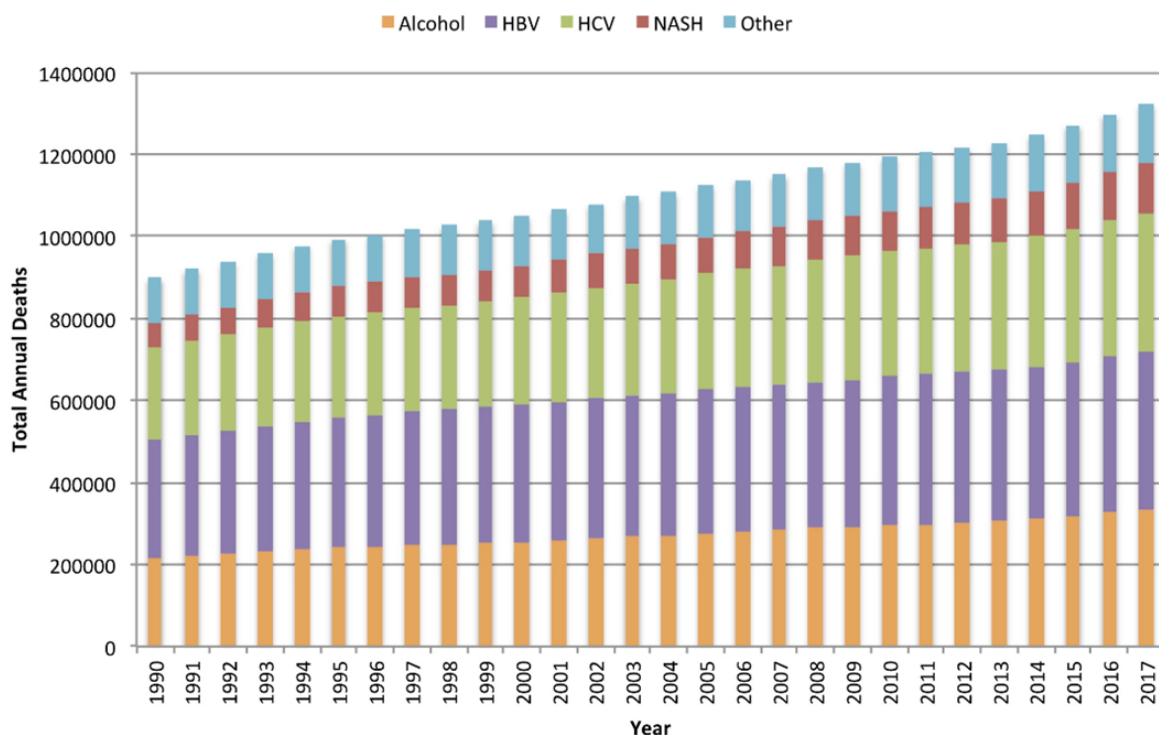


전북대학교
JEONBUK NATIONAL UNIVERSITY



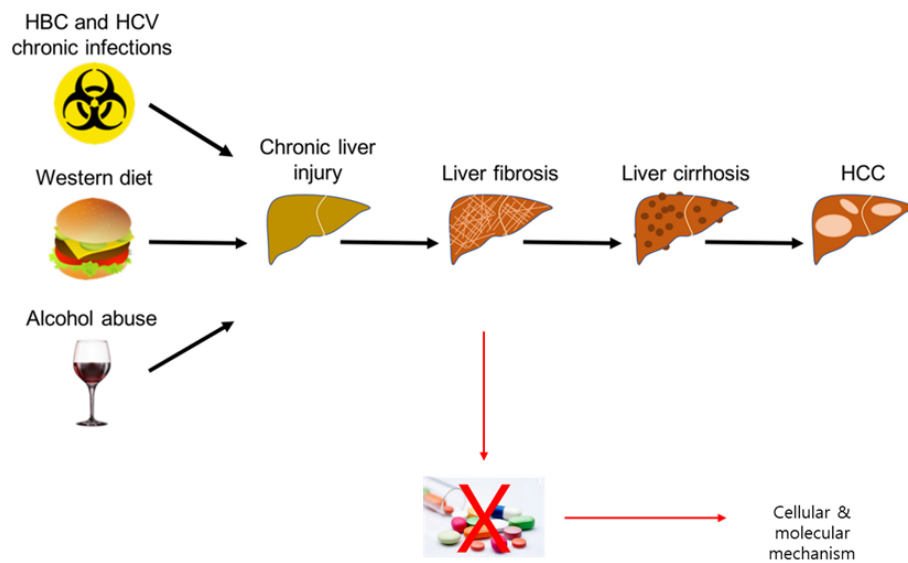
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Liver fibrosis patient in global



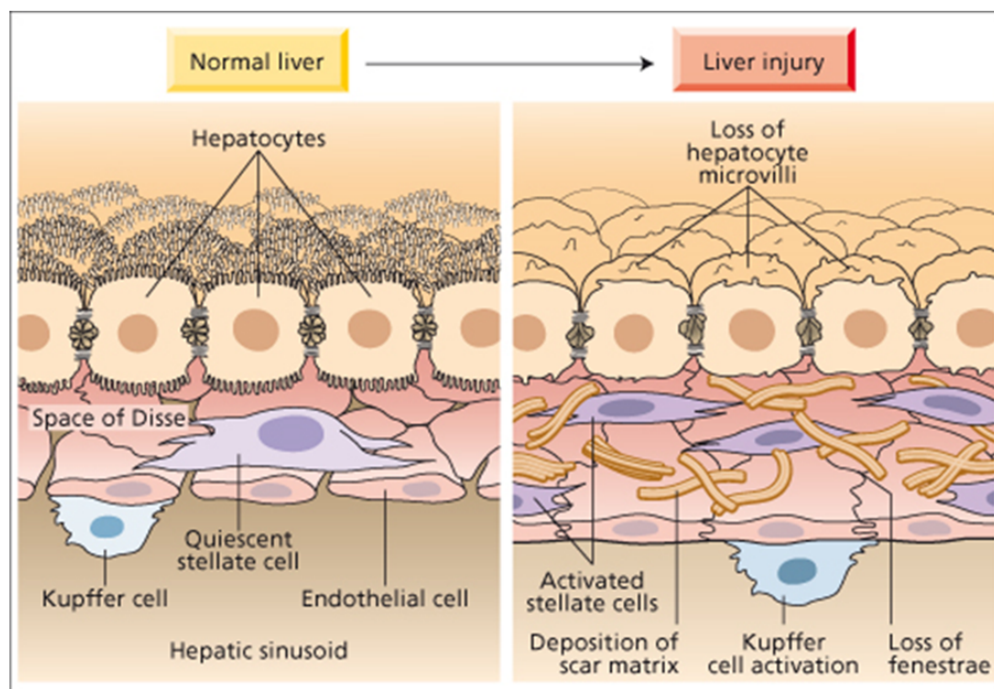
Shantan Cheemerla ClinicaI liver Disease, voll 17, nO 5, MaY 2021

Liver disease



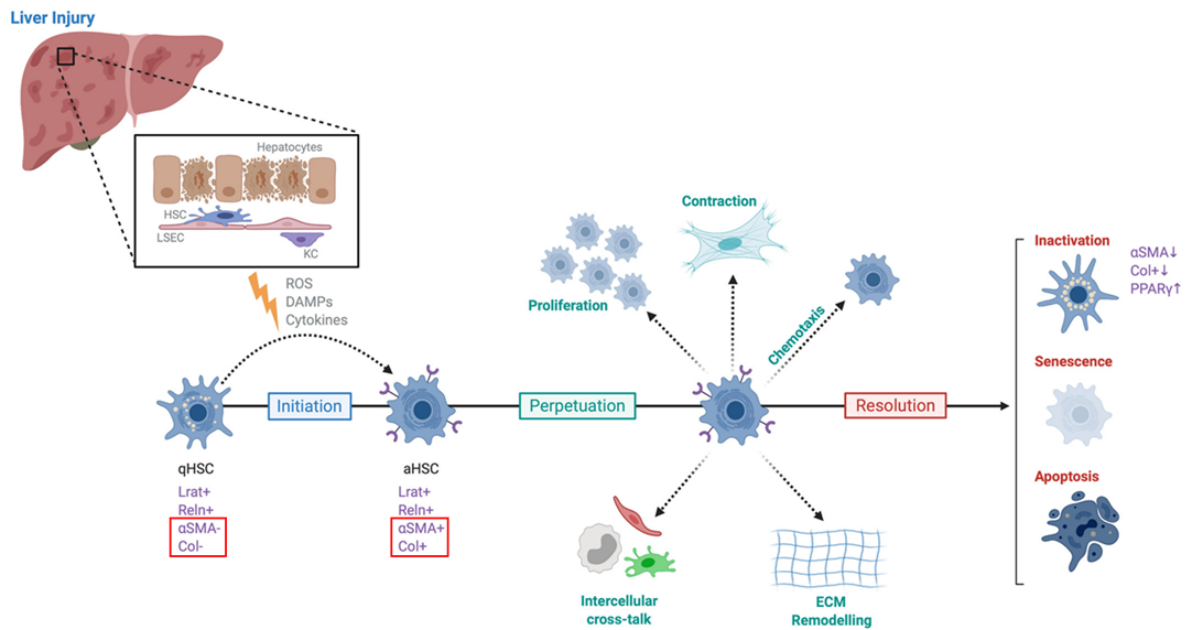
Fernando Bessone et al.
Cellular and Molecular Life Sciences Jan 2019

Role of hepatic stellate cell in liver fibrosis



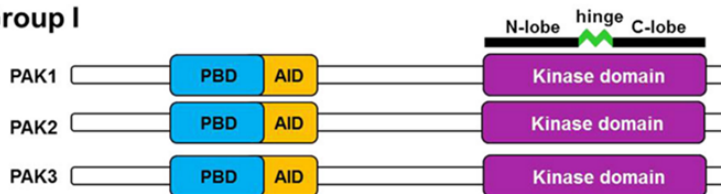
GASTROENTEROLOGY Jun 4, 2016

Activation of hepatic stellate cell



P21-Activate kinase family

Group I



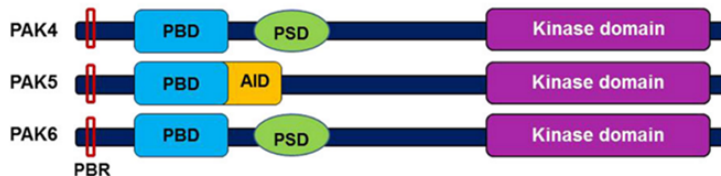
PAK (p21 activated kinase)

Group I (1,2,3), Group II (4,5,6)

Initially studies on PAK, role in reorganization of the cytoskeleton. Control cellular morphology, adhesion, and migration.

Later, expanded to cellular functions. Cell proliferation and survival.

Group II



PBD p21-binding domain AID Autoinhibitory domain PSD Pseudosubstrate domain

Won et al. *Experimental & Molecular Medicine* (2019)

Research for PAK4 in liver

Cancer Letters 402 (2017) 71–80

Contents lists available at ScienceDirect
Cancer Letters
journal homepage: www.elsevier.com/locate/canlet

Original Article

Zic2 promotes tumor growth and metastasis via PAK4 in hepatocellular carcinoma

Shi-Xun Lu^{a, b, 1}, Chris Zhiyi Zhang^{a, b, 1}, Rong-Zhen Luo^{a, b, 1}, Chun-Hua Wang^{a, b}, Li-Li Liu^{a, b}, Jia Fu^{a, b}, Lanjing Zhang^{c, d}, Huamin Wang^e, Dan Xie^{a, b}, Jing-Ping Yun^{a, b, *}

^a Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Guangzhou 510060, China
^b Department of Pathology, Sun Yat-sen University Cancer Center, Guangzhou 510060, China
^c Department of Pathology, University Medical Center of Princeton, Princeton, NJ, USA
^d Rutgers University, Newark, NJ, USA
^e Department of Pathology, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, USA

Biochemical and Biophysical Research Communications 503 (2018) 3003–3010

Contents lists available at ScienceDirect
Biochemical and Biophysical Research Communications
journal homepage: www.elsevier.com/locate/ybbr

Original Article

p21-activated kinase 4 as a switch between caspase-8 apoptosis and NF-κB survival signals in response to TNF-α in hepatocarcinoma cells

Qing Li^a, Xiaoyun Zhang^b, Na Wei^a, Shuwen Liu^c, Yaqin Ling^{d, *, 1}, Hao-Wang^{e, **, 1}

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^c Gastrointestinal Surgery Department, Gansu Provincial Cancer Hospital, Lanzhou, 730050, China
^d Key Laboratory of Ethnomedicine (Minzu University of China), Ministry of Education, Beijing, 100081, China
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Molecular and Cellular Pathobiology

Cancer Research

PAK4 Phosphorylates p53 at Serine 215 to Promote Liver Cancer Metastasis

Hai-Tao Xu¹, Wai-Lung Lai¹, Heong-Fai Liu¹, Leo Lap-Yan Wong¹, Irene Oi-Lin Ng^{2,3}, and Yick Pang Ching^{1,3,4}

HEPATOLOGY



ORIGINAL ARTICLE | [Full Access](#)

p21-activated kinase 4 inhibition protects against liver ischemia/reperfusion injury: Role of nuclear factor erythroid 2-related factor 2 phosphorylation

Yuancheng Mao, Chang Yeob Han, Lihua Hao, Younho Lee, Jung Beom Son, Hwangeun Choi, Mi Rin Lee, Jae Do Yang, Suk Kyun Hong, Kyung-Suk Suh, Hee Chul Yu, Nam Doo Kim ... See all authors

First published: 02 February 2022 | <https://doi.org/10.1002/hep.32384>

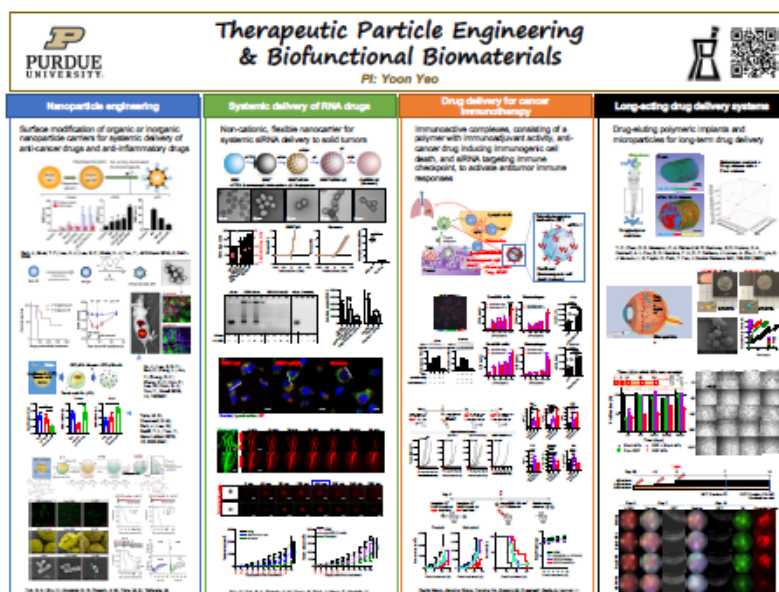
PAK4 research for liver I/R injury

Thank you

2023.05.31.

우수연구자 초청세미나

PURDUE UNIVERSITY
여윤 교수님



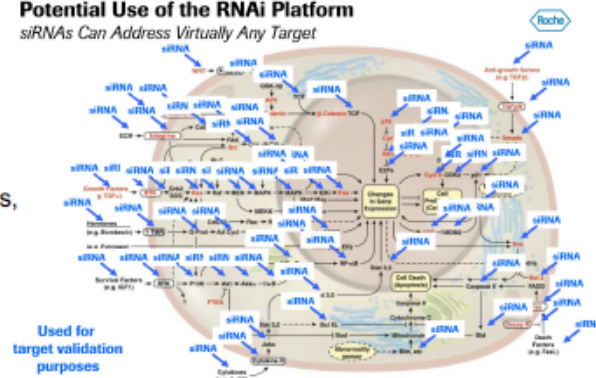
siRNA as a therapeutic

- Silence disease-causing genes, particularly those that encode so-called 'non-druggable' targets.
 - Viral infections
 - Neurodegenerative diseases
 - Autoimmune diseases
 - Cancer (RNAi of carcinogenesis, tumor-host interactions, tumor resistance to chemotherapy)

+ Efficiency, potency, target specificity, availability of synthetic siRNA

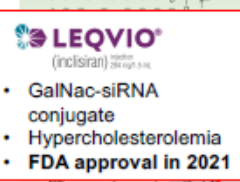
- Instability, off-target silencing, TLR3 activation

Potential Use of the RNAi Platform siRNAs Can Address Virtually Any Target

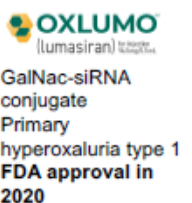




- siRNA covalently linked to a ligand (GalNac) targeting hepatocytes
- Acute hepatic porphyria
- **FDA approval in 2019**



- GalNac-siRNA conjugate
- Hypercholesterolemia
- **FDA approval in 2021**



- GalNac-siRNA conjugate
- Primary hyperoxaluria type 1
- **FDA approval in 2020**



- GalNac-siRNA conjugate
- Hereditary transthyretin-mediated amyloidosis
- **FDA approval in 2022**

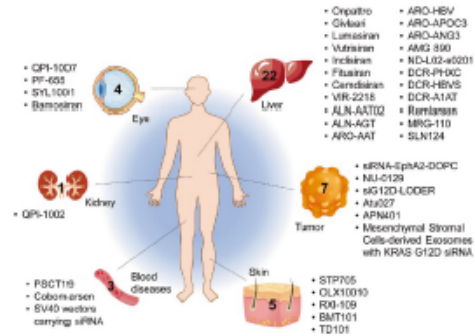


- siRNA encapsulated in a lipid nanoparticle
- Hereditary transthyretin-mediated amyloidosis
- **FDA approval in 2018**

Hu, B.; Zhong, L.; Wang, Y.; Peng, L.; Huang, Y.; Zhao, Y.; Liang, X. J., Therapeutic siRNA: state of the art. *Signal Transduct Target Ther* 2020, 5 (1), 101.

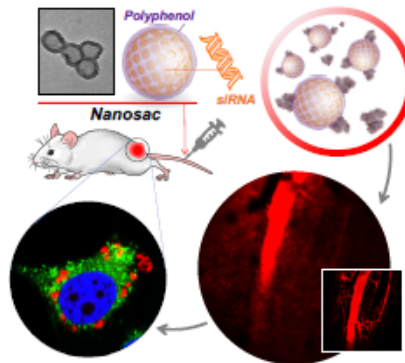
Need for systemic carriers of siRNA

- **Limited success in systemic delivery** to the organs other than the liver (or spleen & lungs)
 - Binds to ApoE and accumulates in the liver via low-density lipoprotein receptor (LNP)
- **Potential issues related to the cationic carriers**
 - Non-specific interaction with serum proteins
 - Proinflammatory properties



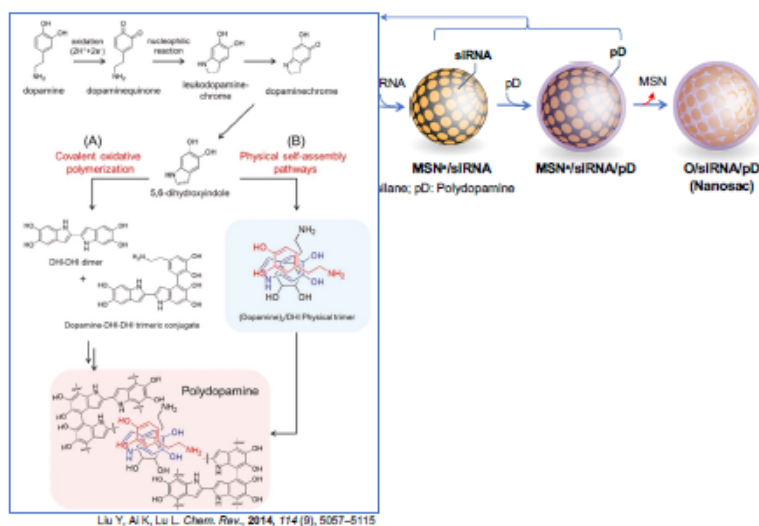
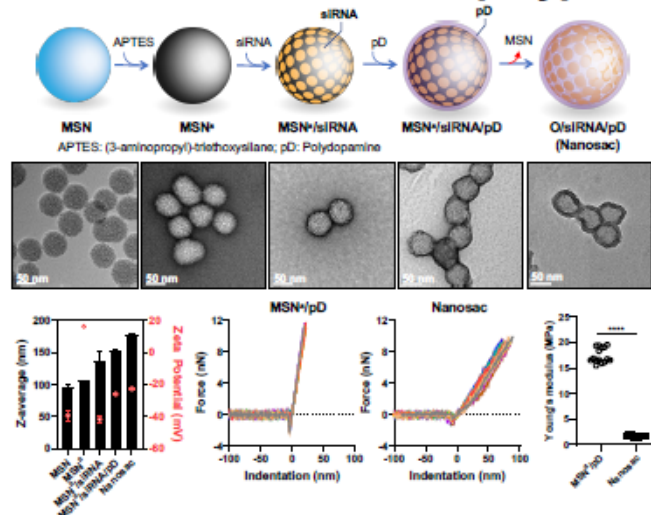
Hu, B.; Zhong, L.; Wang, Y.; Peng, L.; Huang, Y.; Zhao, Y.; Liang, X. J., Therapeutic siRNA: state of the art. *Signal Transduct Target Ther* 2020, 5 (1), 101.

NANOSAC for systemic siRNA delivery to solid tumors

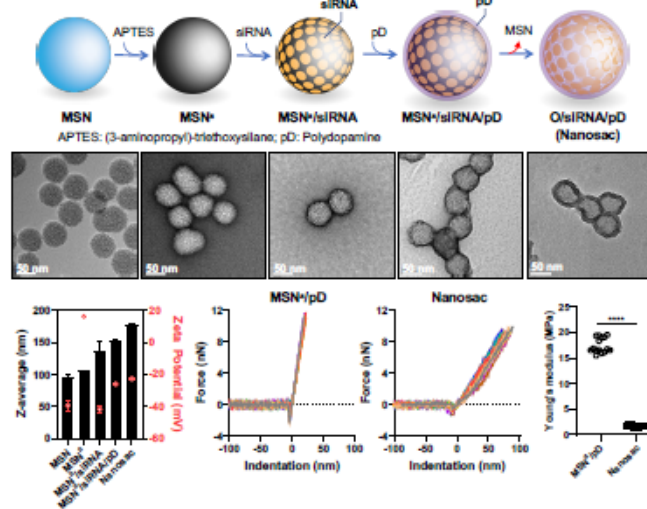


Kim, H.; Yuk, S. A.; Dieterly, A. M.; Kwon, S.; Park, J.; Meng, F.; Gadalla, H. H.; Cadena, M. J.; Lyle, L. T.; Yeo, Y., *ACS Nano* 2021, 15, 3, 4576–4593

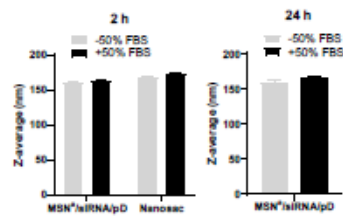
Nanosac, a non-cationic and soft polyphenol capsule



Nanosac, a non-cationic and soft polyphenol capsule

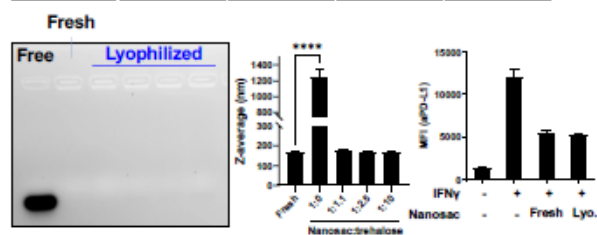
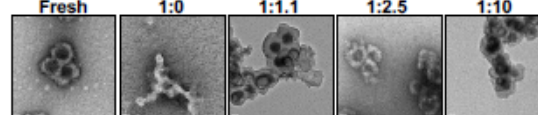


Etching
 - +
 MSN etching process did not affect the stability of siRNA.



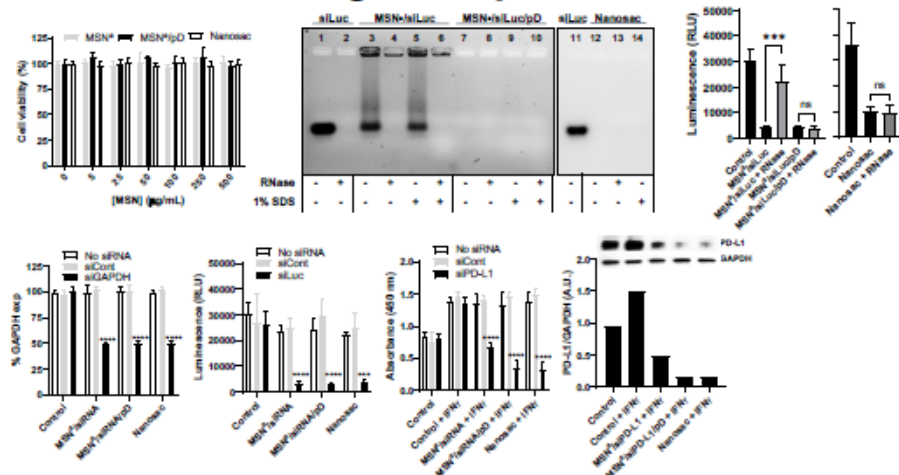
NPs with pD layer remained stable in 50% serum.

Lyophilized at Nanosac:trehalose (w:w):

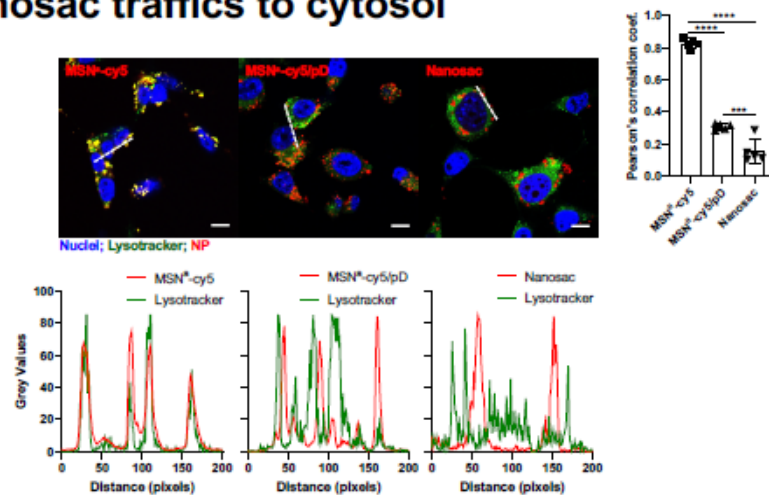


Nanosac can be lyophilized and stored in dry state.

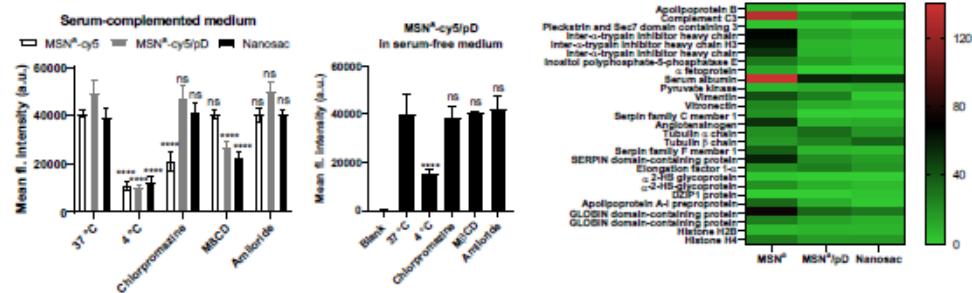
Nanosac is non-toxic, encapsulates and protects siRNA, and silences gene expression in vitro



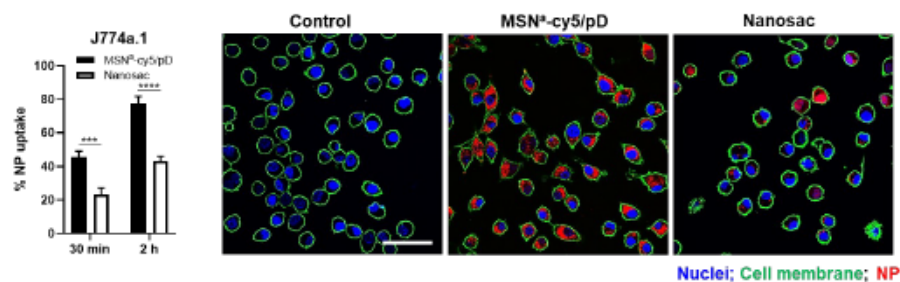
Nanosac traffics to cytosol



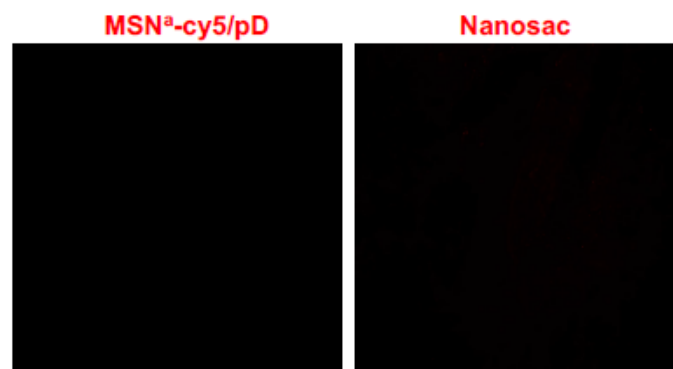
Nanosac, with pD layer, co-opts *serum albumin* and enters CT26 cells via caveolae-mediated endocytosis.



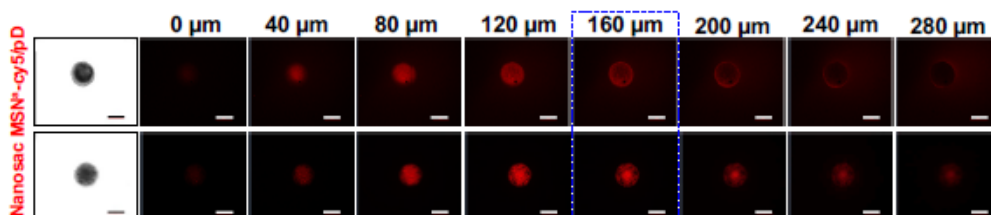
Softness of Nanosac helps reduce macrophage uptake



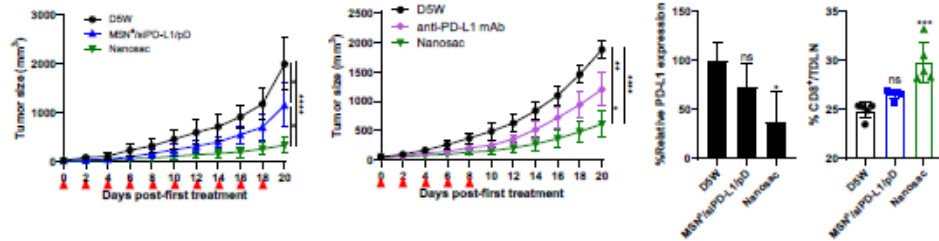
Nanosac extravasates into tumors better than hard counterpart.



Nanosac penetrates into tumor spheroids better than hard counterpart.



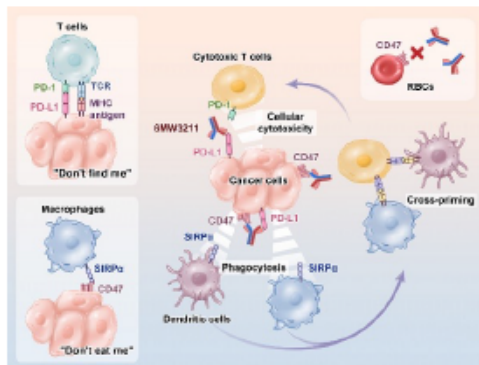
siPD-L1-loaded Nanosac attenuates CT26 tumor growth via immune checkpoint blockade.



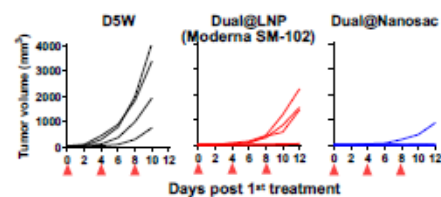
Nanosac outperforms Lipid nanoparticles in dual delivery of siPD-L1 & siCD47



Hytham Gadalla



Wang et al. *Theranostics* 2023; 13(1):148-160



CT26@BALB/c 0.75 mg siRNA/kg q4d x 3

siPD-L1@LNP & siCD47@LNP

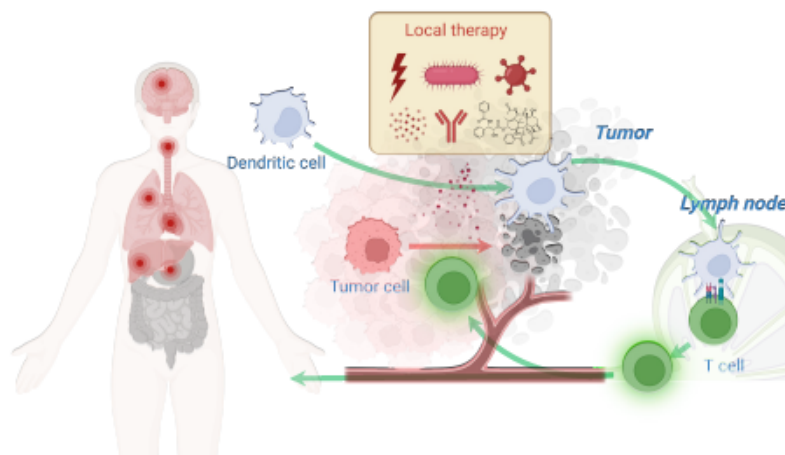
siPD-L1@Nanosac & siCD47@Nanosac

Nanosac for systemic gene delivery

- *Nanosac*, a **non-cationic** and **soft polyphenol capsule**
 - Avoids toxicity, non-specific protein adsorption
 - Improves transvascular and interstitial delivery of siRNA to tumors.
 - Reduces macrophage uptake
- Polydopamine-mediated **albuminylation**
 - Allows for direct delivery of siRNA to cytosol via caveolae-mediated endocytosis.
 - May mediate distinct interactions with cancer cells and endothelial cells.
 - May improve extravasation, leveraging the effect of softness.

Intratumoral Delivery of Immunotherapy

Act locally, Function systemically



Intratumoral Delivery of Immunotherapy

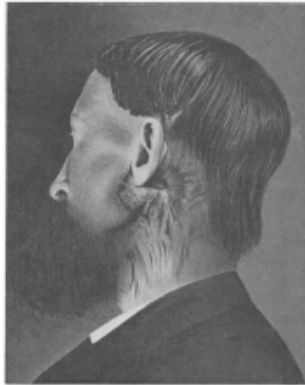


FIG. 1.

Recurrent round-celled sarcoma. Spontaneous recovery following accidental erysipelas. Photograph taken seven years after the cure.

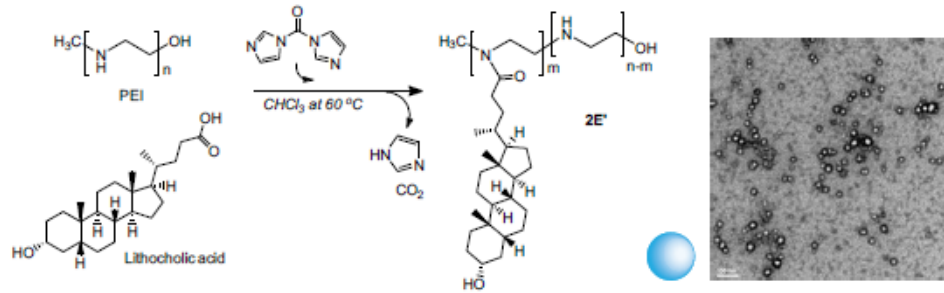
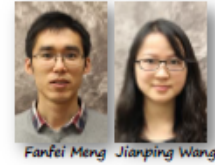
Coley, W. B., The Treatment of Inoperable Sarcoma by Bacterial Toxins (the Mixed Toxins of the Streptococcus Erysipelas and the Bacillus Prodigiosus). *Proceedings of the Royal Society of Medicine* 1910, 3 (Surg Sect), 1-48.

At the fifth operation, in 1884, Dr. Bull found the tumour to involve the deep structures so extensively that it was impossible to remove it, and he gave up the attempt. The case was regarded as absolutely hopeless, when, shortly after the operation, the man developed a very severe attack of accidental erysipelas (infection) in the face and neck, followed two weeks later by a second attack. Within a few days after the beginning of the first attack, the tumour began to soften and decrease rapidly in size.

Carrier consideration

- Retain immunotherapeutic agents locally to maximize their pharmacological effects in tumors and prevent systemic side effects
- Co-deliver multiple drugs, which share little physicochemical features and would otherwise not colocalize
- Stimulate antitumor immunity to leverage immunostimulatory effects of therapeutic agents

Immunoactive complexes (IMAX)

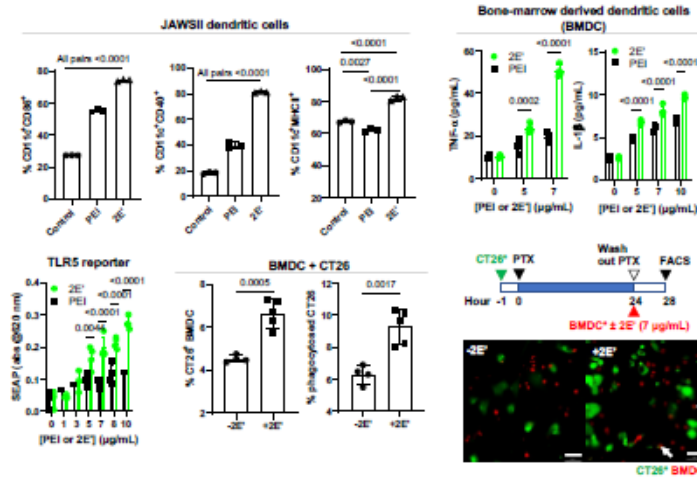


Fanfei Meng, Jianping Wang, Yanying He, Gregory M. Cresswell, Nadia A. Larman, L. Tiffany Lyle, Timothy L. Ratliff, Yoon Yeo. *PNAS*. 2022 (119) 22: e2122595119

Polyethylenimine derivative (2E')



2E' serves as an immunoadjuvant and enhances cancer cell uptake by antigen presenting cells



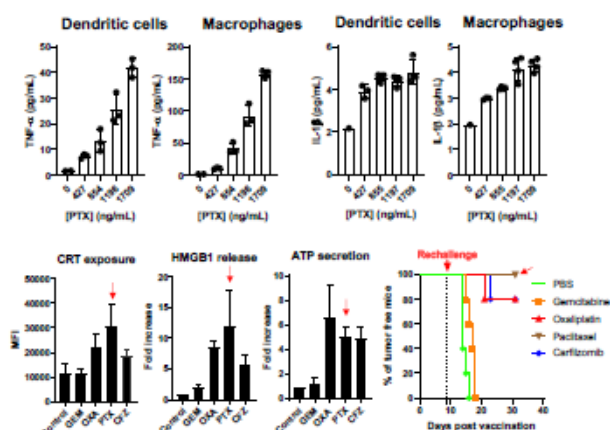
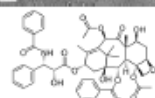
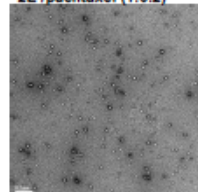
Fanfei Meng, Jianping Wang, Yanying He, Gregory M. Cresswell, Nadia A. Larman, L. Tiffany Lyle, Timothy L. Ratliff, Yoon Yeo. *PNAS*. 2022 (119) 22: e2122595119

Polyethyleneimine derivative (2E')



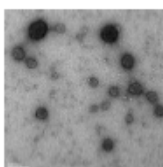
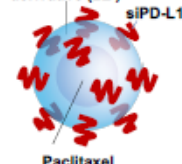
Paclitaxel (PTX) stimulates antigen-presenting cells and induces immunogenic death of tumor cells

2E'/paclitaxel (1:0.2)



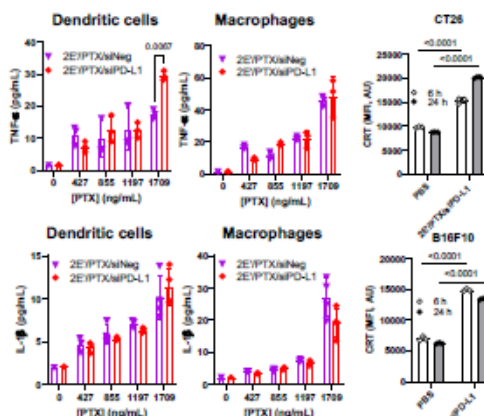
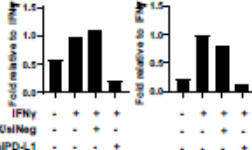
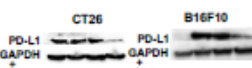
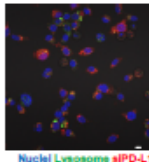
Soonbum Kwon

Polyethyleneimine derivative (2E')

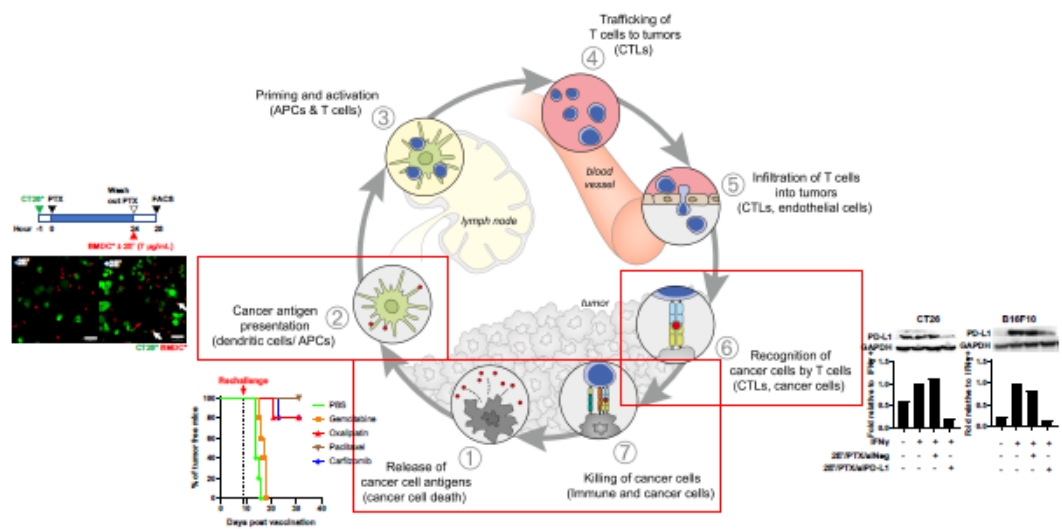


2E' carries paclitaxel and siPD-L1, retaining its immunostimulatory effects.

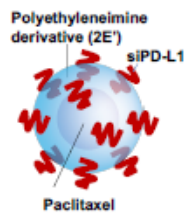
2E'/PTX/siPD-L1



Fanfei Meng, Jianping Wang, Yanying He, Gregory M. Cresswell, Nadia A. Lanman, L. Tiffany Lyle, Timothy L. Ratliff, Yoon Yoo. *PNAS*. 2022 (119) 22: e2122595119



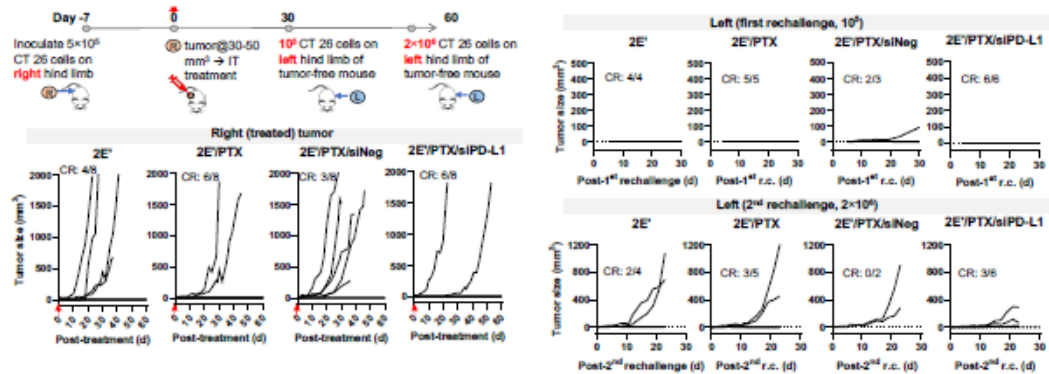
Chen, D. S. & Mellman, I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* **39**, 1-10 (2013).



Single local administration of 2E'/PTX/siPD-L1 induces immediate regression of large established tumors

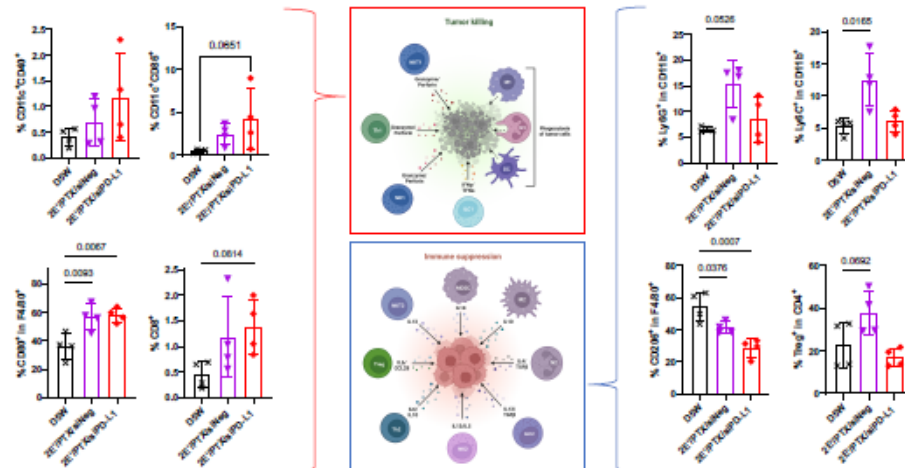


Single intratumoral injection of 2E'/PTX/siPD-L1 induces tumor regression and protects animals from repeated tumor challenge in CT26@Balb/c model.

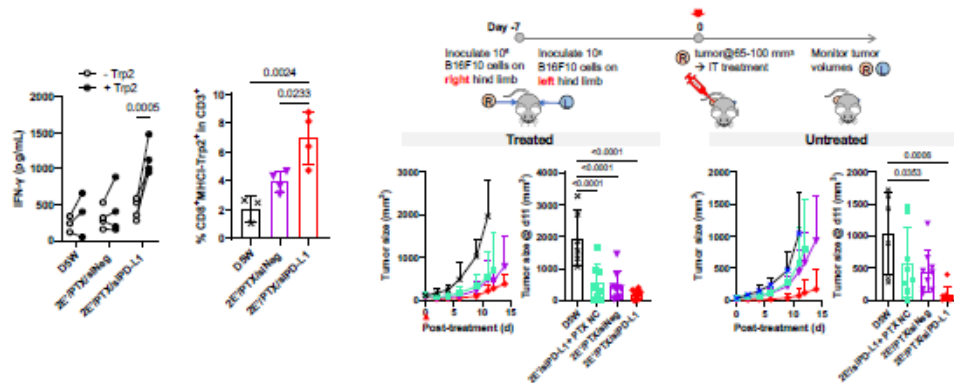


Fanfei Meng, Jianping Wang, Yanying He, Gregory M. Cresswell, Nadia A. Lanman, L. Tiffany Lyle, Timothy L. Ratliff, Yoon Yoo. *PNAS*. 2022 (119) 22: e2122595119

Single intratumoral injection of 2E'/PTX/siPD-L1 induces and maintains immunoactive phenotype in poorly immunogenic B16F10 tumors.

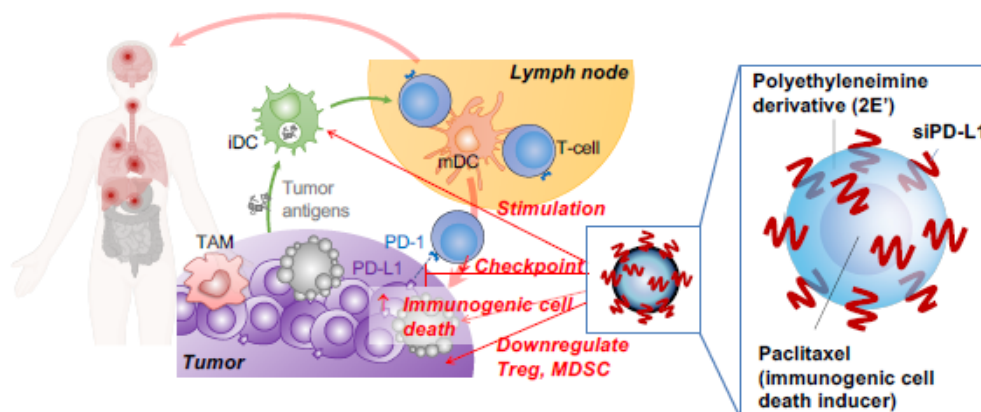


Single intratumoral injection of 2E'/PTX/siPD-L1 primes systemic antitumor immunity in B16F10 tumors.



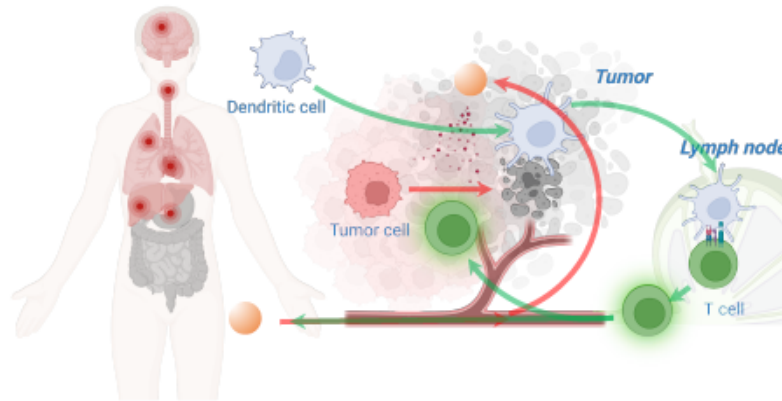
Fanfei Meng, Jianping Wang, Yanying He, Gregory M. Cresswell, Nadia A. Lanman, L. Tiffany Lyle, Timothy L. Ratliff, Yoon Yoo. *PNAS*. 2022 (119) 22: e2122595119

Immunoactive complexes (IMAX)



Systemic Delivery of Immunotherapy

Enter systemically, Act locally, Function systemically



Summary

- An **immunoactive nanoparticle (IMAX)** induced immediate regression of tumors upon a **single local administration** and enhanced the activation of antitumor immunity.
- In IMAX, **2E'** activated innate immunity against tumor due to the immunoadjuvant effect, and **PTX** induced immunogenic cell death, which then activates T-cell immunity against tumors. **siPD-L1** prevented tumor expression of PD-L1 that would otherwise engage in immune checkpoint interaction and MDSC and Treg recruitment.
- **Systemic delivery of PTX by ATP-conjugated ("find-me") nanoparticles** induced regression of CT26 tumors and generated systemic antitumor immunity.
- Addition of **anti-PD-1 antibody** counteracts the resistance to PTX-loaded Find-me nanoparticles, leading to complete remission of CT26 tumors.

Current; Past

Grad students	Post-docs & Visiting scholars	Collaborators	Funding
- Dhawal Chobisa - Jianping Wang - Hytham Gadalla - Yanying He - Yongzhe Li - Zhongyue Yuan - Soonbum Kwon - Simseok Yuk - Maie Taha - Joonyoung Park - Jun Xu - Yihua Pei - Hyesun Hyun - Bo Sun - Sara Abouelmagd - Karen Liu - Hillary Holback - Basma Ibrahim - Emily Gullotti - Zohreh Amoozgar	- Fanfei Meng - Woojun Kim - Marwa Elmaghrabi - Hassan Tamam - Karen Gutierrez - Ly Ky - Sooyoung Kim, Pharm.D., Ph.D. - Sherryhan Ahmed - Hyunjun Kim - Nisar Khalil - Simseok Yuk - Kunyu Jiang - Yihua Pei - Jinho Park - Kyung-Oh Doh - Eun Jung Cho - Gaurav Bajaj - Peisheng Xu - Yuanfen Liu - Ning Han - Bleong-ki Kim* - Ruoci Hu - Hyesun Hyun - Jianping Wang - Liang Ping - Yun-Chu Chen	- Tim Ratliff (Purdue) - Debbie Knapp (Purdue) - Melissa Fishel (Indiana Univ) - Tim Corson (Indiana Univ) - Woolin Lee (SNU) - Steve Kron (U Chicago) - Chang H. Kim (Michigan) - Mohamed Seleem (Virginia Tech) - Tiffany Lyle (Purdue) - Ick Chan Kwon (KIST) - Pilhan Kim (KAIST)	- NIH/NCI (R01 CA232419) - NIH/NCI (R01CA258737) - NIH/NCI (R01CA199663) - Purdue Institute for Drug Discovery - Purdue Research Foundation - Eli Lilly - NIH/NIBIB (R01EB017791) - NIH/NIAID (R21AI119479) - NSF-DMR - NSF/CAREER - LIFA fellowship - Showalter Trust - Purdue Center for Cancer Research



2023.06.07

분자면역학 전공대학원생 발표 세미나

한지희 학생

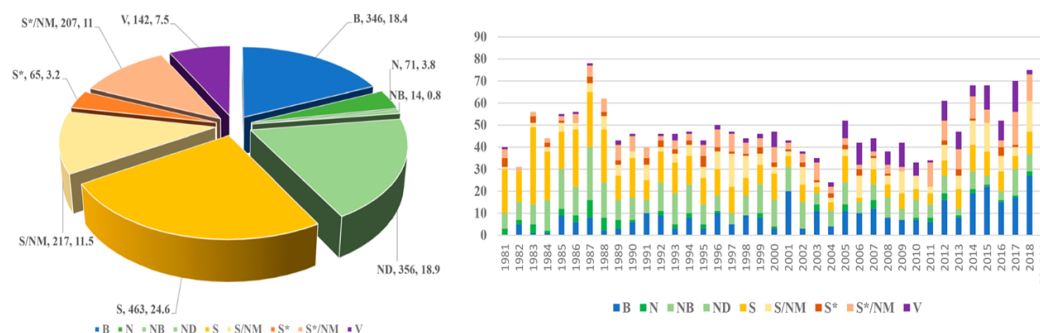
Total synthesis and structural confirmation of gosodesmine

JEONBUK NATIONAL UNIVERSITY

Jeonbuk National University School of Pharmacy
Medicinal Chemistry and Organic Synthesis Lab
Jihee Han

Contents

- Introduction
- Retrosynthesis
- Synthesis
- Conclusion
- Plan



B : Biological macromolecule **N** : Unaltered natural product **NB** : Botanical drug (defined mixture) **ND** : Natural product derivative
S : Synthetic drug **S*** : Synthetic drug (NP pharmacophore) **V** : Vaccine **/NM** : Mimic of natural product

J. Nat. Prod. 2020, 83, 770.

Natural product-based drugs



Penicillin

- the most representative antibiotic, was first isolated from the *Penicillium* mold
- inhibits the synthesis of bacterial cell walls, thereby suppressing the growth and reproduction of bacteria.



Aspirin

- initially developed based on salicylic acid, an extract from the bark of the willow tree
- As nonsteroidal anti-inflammatory drug, used to reduce pain, fever, and inflammation



Stillen

- The flavonoid eupatilin, which is found in *Artemisia asiatica* Nak. (Ae-yeop or Korean mugwort), has been reported to possess anti-ulcer effects
- Effect of improving mucosal lesions

Chemical defense secretion of Millipedes



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Defensive Secretion of the Millipede *Floridobolus peneri*

Athula B. Attygalle, Shang-Cheng Xu, Jerrold Meinwald, and Thomas Eisner

Cite this: *J. Nat. Prod.* 1993, 56, 10, 1700–1706

Publication Date: October 1, 1993

<https://doi.org/10.1021/np50100a007>

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Journal of Natural Products



RESEARCH ARTICLE

Chemical Defence in a Millipede: Evaluation and Characterization of Antimicrobial Activity of the Defensive Secretion from *Pachyiulus hungaricus* (Karsch, 1881) (Diplopoda, Julida, Julidae)

Staviša Stanković^{1*}, Ivica Dimkić¹, Ljubodrag Vujić², Sofija Pavković-Lučić³, Zvezdana Jovanović⁴, Tatjana Stević⁵, Ivana Sofrenić², Bojan Mitlić⁴, Vladimir Tomić⁴

¹ Department of Microbiology, University of Belgrade – Faculty of Biology, Belgrade, Serbia, ² Department of Organic Chemistry, University of Belgrade – Faculty of Chemistry, Belgrade, Serbia, ³ Department of Genetics and Evolution, University of Belgrade – Faculty of Biology, Belgrade, Serbia, ⁴ Department of Animal Development, University of Belgrade – Faculty of Biology, Belgrade, Serbia, ⁵ Institute for Medicinal Plants Research “Dr. Josif Pančić”, Belgrade, Serbia



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HOME > SCIENCE > VOL. 135, NO. 3539 > SECRETION OF BENZALDEHYDE AND HYDROGEN CYANIDE BY THE MILLIPEDE *PACHYDESMUS CRASSICUTIS* (WOOD)

REPORT

Secretion of Benzaldehyde and Hydrogen Cyanide by the Millipede *Pachydesmus crassicutis* (Wood)

MURRAY S. BLUM AND J. PORTER WOODBINE [Authors Info & Affiliations](#)

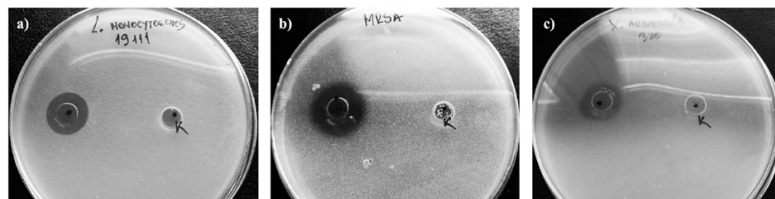
SCIENCE • 26 Oct 1962 • Vol 135, Issue 3539 • pp. 512-513 • DOI:10.1126/science.135.3539.512

J. Nat. Prod. 1993, 56, 1700–1706.

SCIENCE. 1962, 138, 512–513.

PLoS One. 2016, 11, e0167249.

Chemical defense secretion of Millipedes



In vitro effects of the defensive secretion against *L. monocytogenes* (a), methicillin-resistant *S. aureus* (b) and *X. arboricola* (c). K) Negative control of solvent.

Name	ATCC No.	Zone of inhibition (mm) ^b	MIC (mg/ml)	MBC (mg/ml)	MIC of rifampicin (mg/ml)	MBC of rifampicin (mg/ml)
<i>Aeromonas hydrophila</i>	49140	16	0.20	0.40	0.10	0.20
<i>Pseudomonas aeruginosa</i>	15442	10	0.60	0.80	0.20	–
<i>Escherichia coli</i>	25922	19	0.60	1.00	0.20	0.40
<i>Xanthomonas arboricola</i>	IZB 301 ^a	14	0.40	0.60	0.20	0.40
<i>Listeria monocytogenes</i> 1/2a	19111	16	0.20	0.40	0.40	–
<i>Staphylococcus aureus</i>	25923	25	0.40	0.60	0.20	0.40
Methicillin resistant <i>Staphylococcus aureus</i> (MRSA)	33591	19	0.25	0.50	0.12	0.18
<i>Bacillus subtilis</i>	6633	25	0.60	1.00	0.080	0.20

^a The strain *X. arboricola* was isolated from walnut fruit.

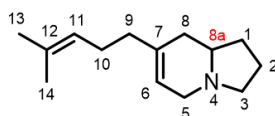
^b Mean values of three experiments, expressed as the diameter of the inhibition zone in mm includes the diameter of well (5 mm).

Antibacterial activity of defensive secret extract from *Pachyiulus hungaricus*

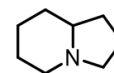
PLoS One, 2016, 11, e0167249.



Gosodesmus claremontus



Gosodesmine



Octahydroindolizine

- ◆ Isolated from the chemical defense secretions of the millipede *Gosodesmus claremontus* Chamberlin
- ◆ Unique 7-substituted hexahydroindolizine alkaloid
- ◆ **Absolute configuration at C_{8a} was not elucidated**
- ◆ Any biological activities were not reported

J. Nat. Prod. **2020**, 83, 2764.

경청해 주셔서 감사합니다

JEONBUK NATIONAL UNIVERSITY

2023.06.07

미생물 및 면역학 전공대학원생 발표 세미나

이도현 학생

Studies Toward the Total Synthesis of Bananamides D-F

2023.06.07.

이 도 현

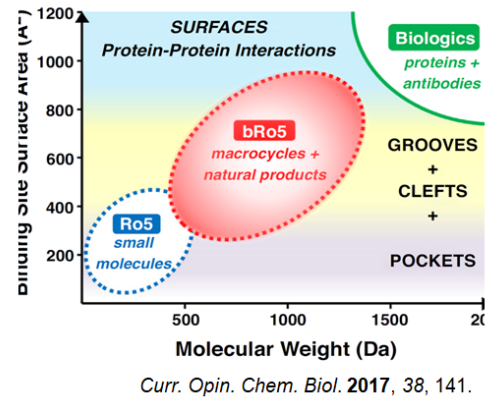
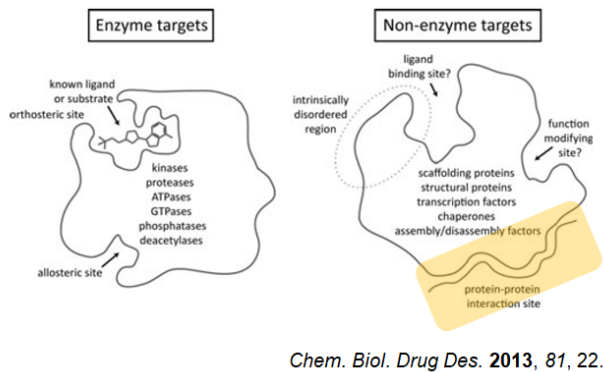
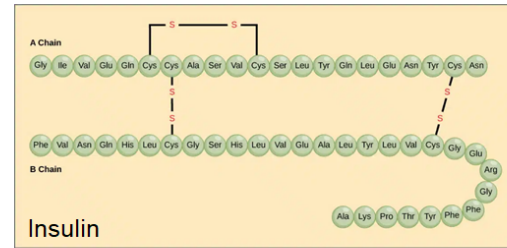
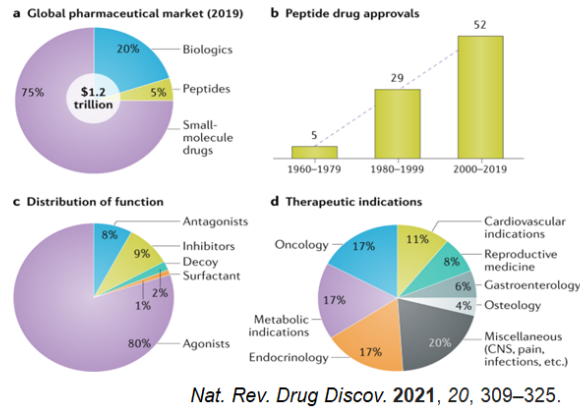
전북대학교 약학대학



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- Introduction
- Retrosynthesis
- Synthesis
- Summary and Future Plan

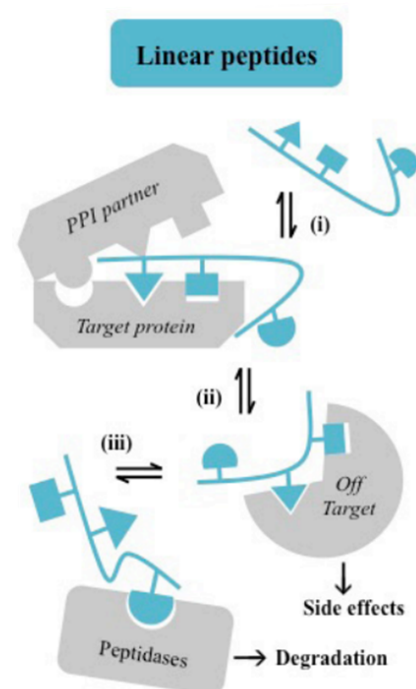
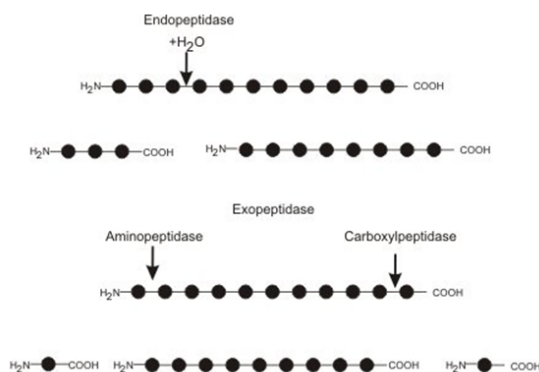


Disadvantages of Linear Peptide Drugs

Table 1 Advantages and disadvantages of therapeutic peptides (Adopted from [25])

Advantages	Disadvantages
High potency of action	Metabolic instability
High target specificity and selectivity	Poor membrane permeability
Wide range of targets	Poor oral bioavailability
Low toxicity	Poor solubility
Fewer side effects	Rapid clearance
Low accumulation in tissues	High manufacturing cost
High biological and chemical diversity	Poor activity

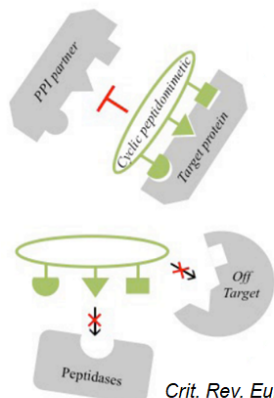
Journal of Biomedical Science **2017**, *24*, 21



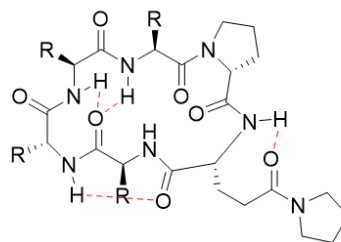
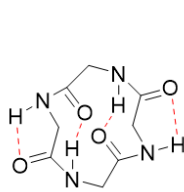
Crit. Rev. Eukaryot. Gene Expr. **2016**, *26*, 199–221

Advantages of Cyclic Peptide Drugs

Cyclic peptidomimetics

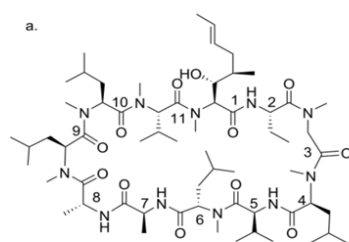


Crit. Rev. Eukaryot. Gene Expr. **2016**, *26*, 199-221

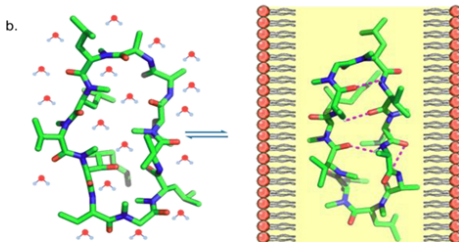


J. Am. Chem. Soc. **2006**, *128*, 14073–14080

J. Med. Chem. **2022**, *65*, 5072–5084



cyclosporin A (CsA)

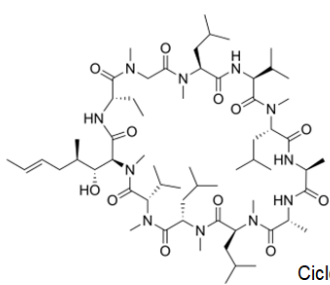


"open"-conformation

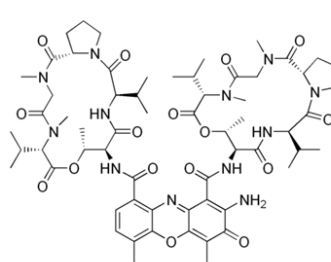
"closed"-conformation

"open"-conformation
Mar. Drugs **2021**, *19*, 311

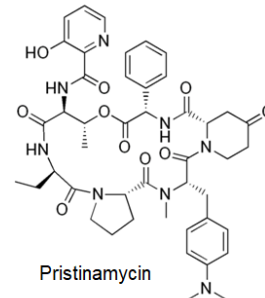
Cyclic Peptide Drugs



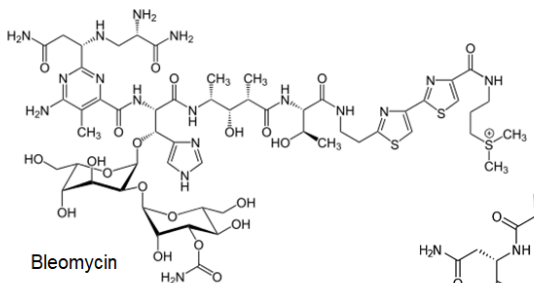
Ciclosporin



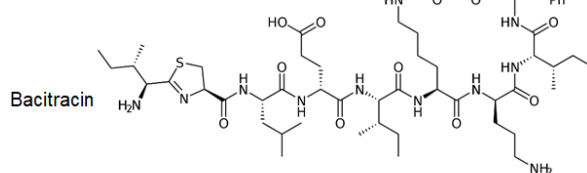
Actinomycin D



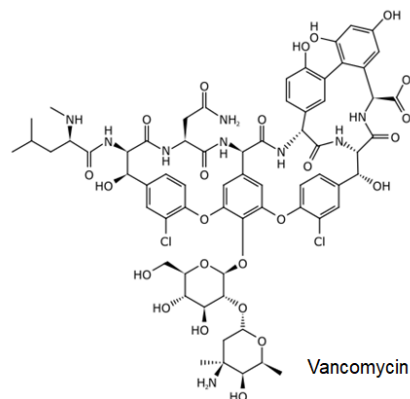
Pristinamycin



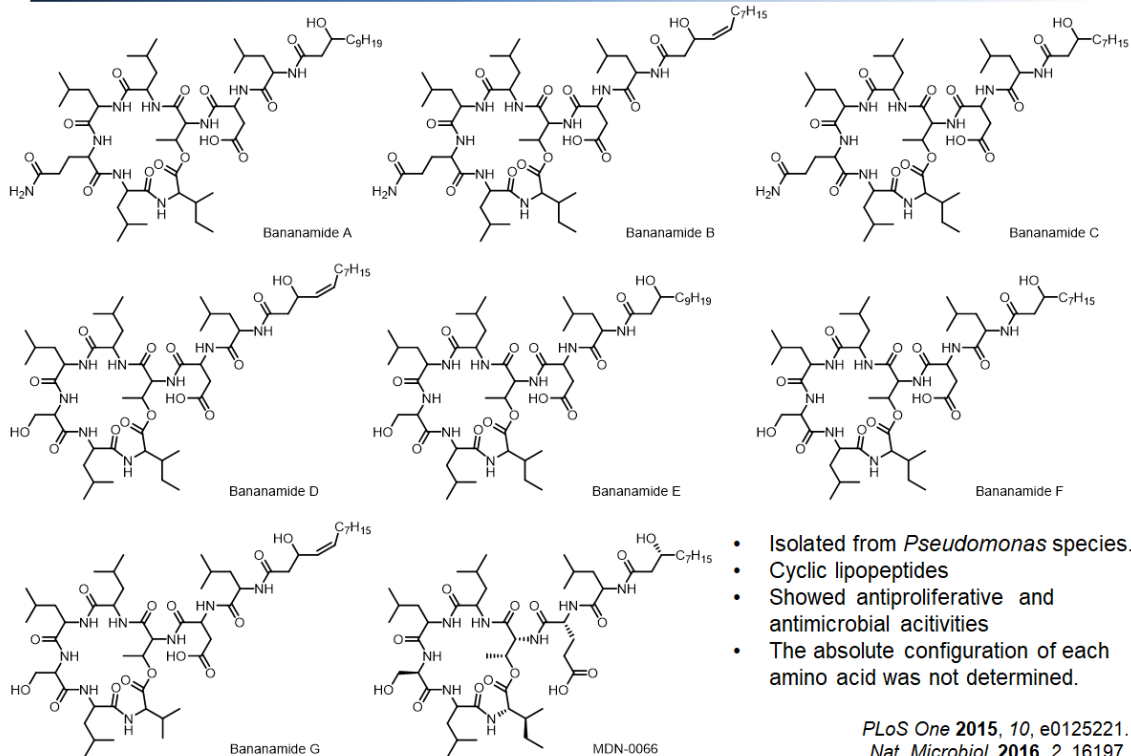
Bleomycin



Bacitracin



Vancomycin



감사합니다

2023.06.14.

우수연구자 초청 세미나

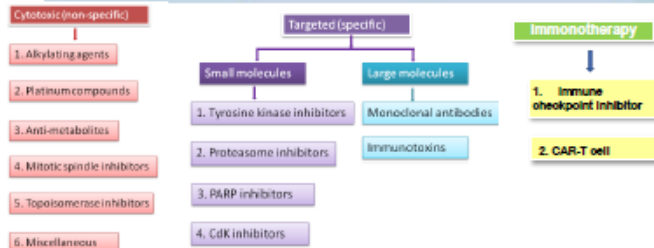
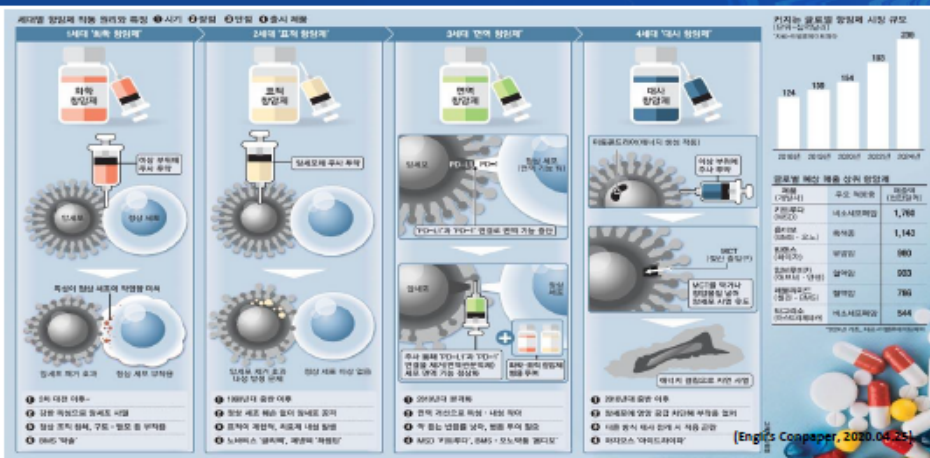
목포대 약대

박은영 교수님

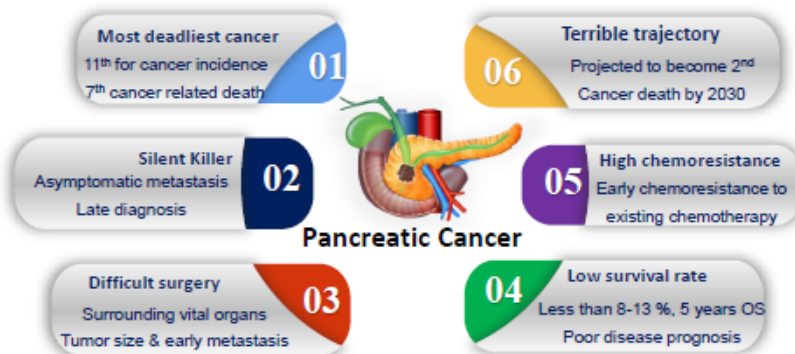
Development of new anti-cancer drugs through sphingolipid signaling regulation

Mokpo National University
Eun-Young Park

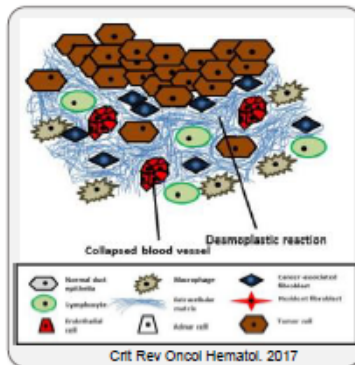
Anticancer drugs by generation



Why pancreatic cancer ?



Difficulties in improving treatment efficiency of pancreatic cancer



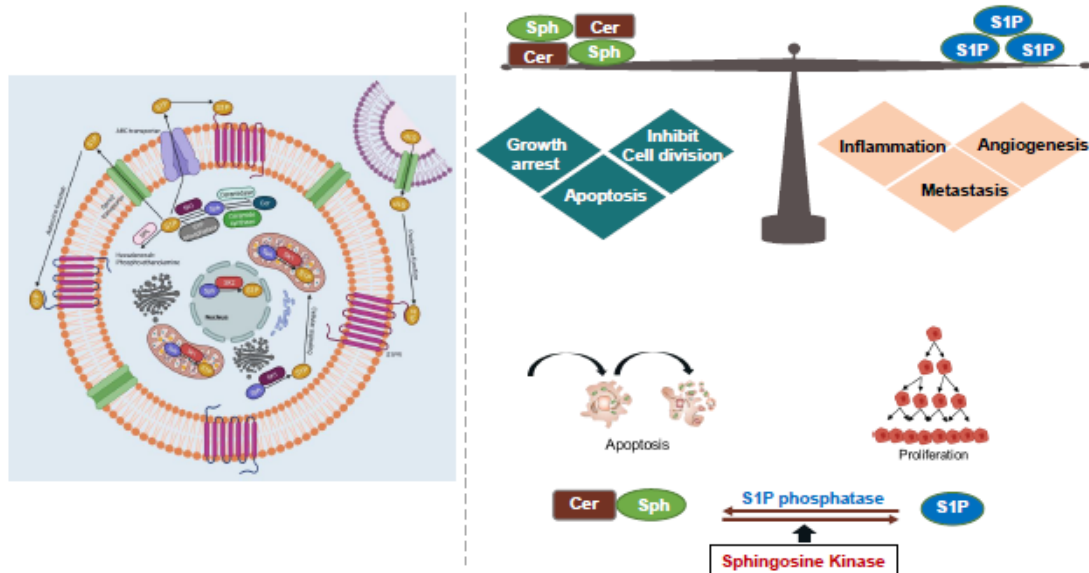
First-line treatment option

- 1) FOLFIRINOX
(fluorouracil, folinic acid, irinotecan, and oxaliplatin)
 - 2) Gemcitabine + nab-paclitaxel
- Olaparib :PARP (Poly ADP-ribose polymerase) inhibitor
 - Pembrolizumab

Urgent need of developing novel targeted therapy for the treatment of pancreatic cancer.

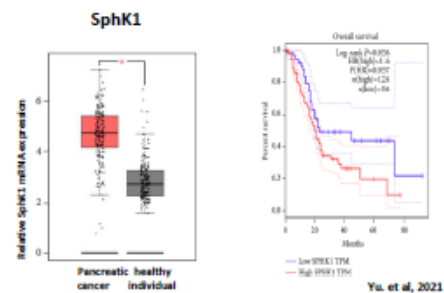
Sphingolipid rheostat and cancer

A balance to decide cellular fate

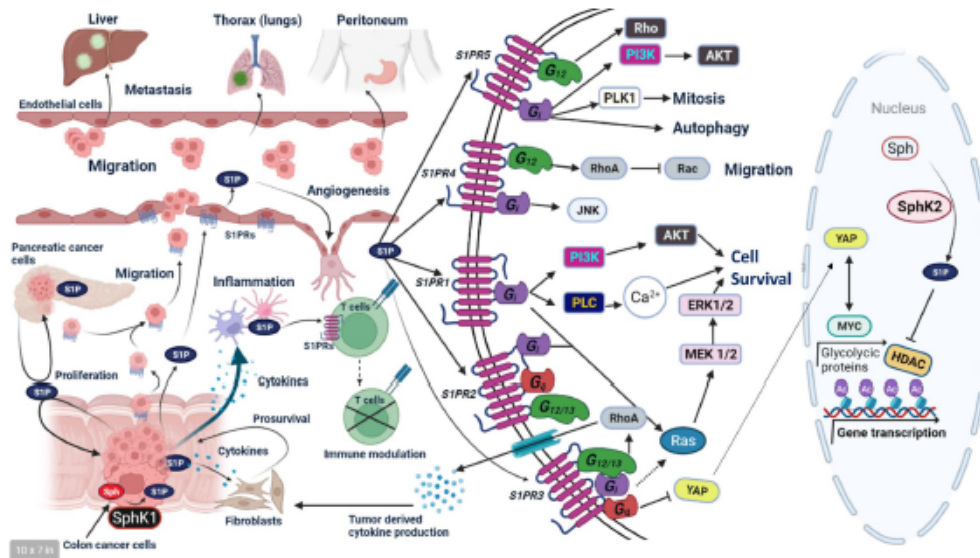


SphK1/2 upregulated in Pancreatic cancer

- SphK1 and SphK2 is **overexpressed** in PDAC compared to normal tissue
- Tumor and serum S1P level elevated in pancreatic cancer patients
- Increased **S1P level** in **PDAC** correlated to cancer progression, metastasis, poor prognosis
- Increased **S1P/ceramide** ratio induce chemoresistance in PDAC
- SphK2 inhibitor have anticancer effect to PDAC
- SphK2 inhibition increased Gemcitabine sensitivity



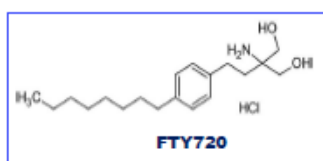
Role of S1P in cancer progression



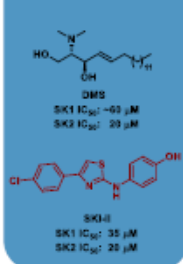
Are inhibition of SphK activity and lowering S1P levels promising approaches in pancreatic cancer therapy?



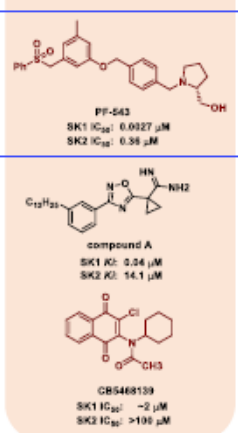
Reported sphingosine kinase inhibitors



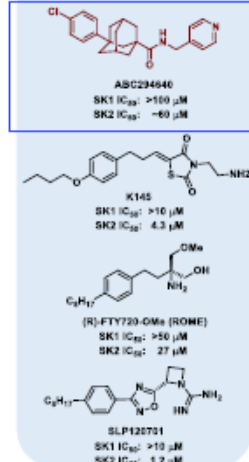
Non selective



SK1 selective

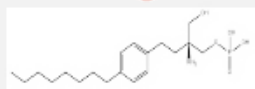


SK2 selective



Problems of existing SphK inhibitors to use as anticancer therapy

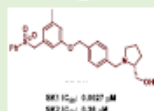
01



FTY720

- Strong anticancer efficacy
in-vitro and *in-vivo*
- Converted to FTY720-p by SphK2 and binds to S1PR1
- Immune suppressive effect through modulation of T-cell function

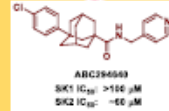
02



PF543

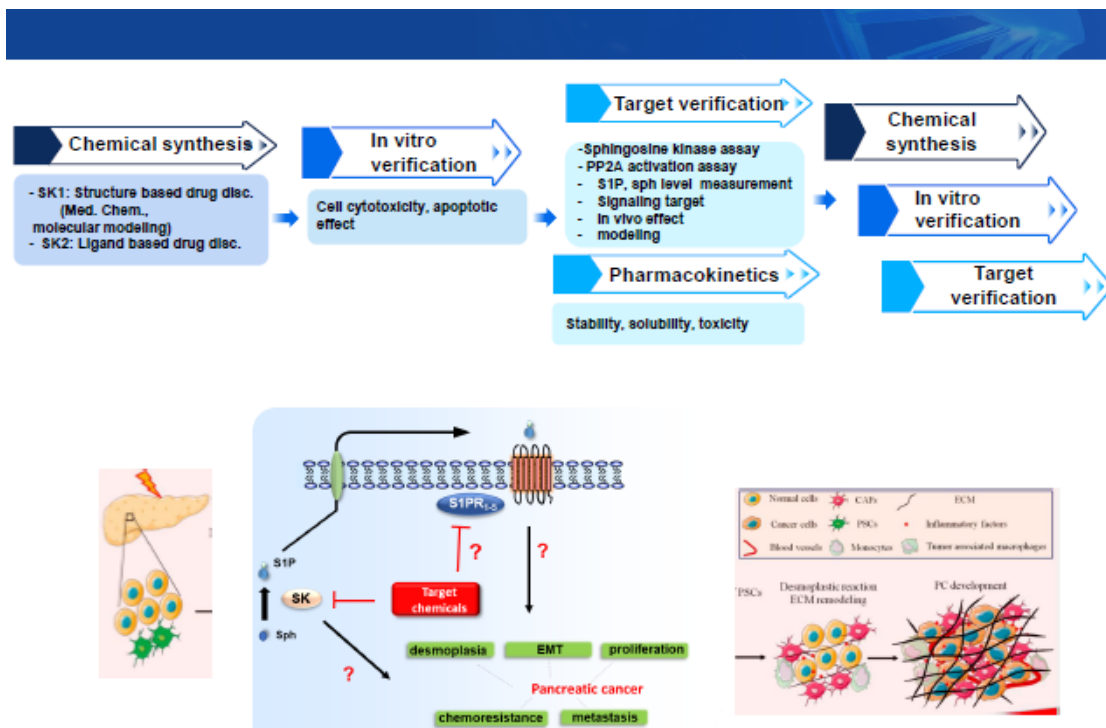
- Strong SphK1 inhibitor
- Highest selectivity (300 fold) to SphK1 over SphK2
- Poor *in-vitro* and *in-vivo* anticancer activity

03

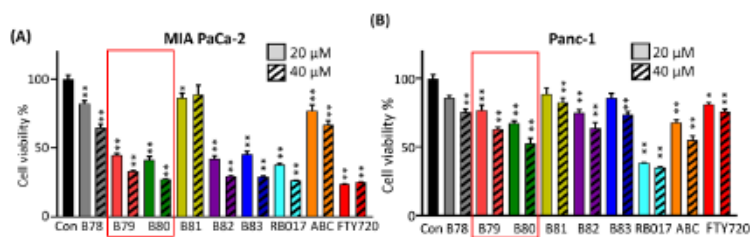
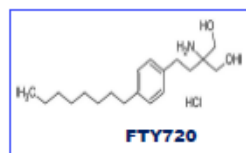


ABC294640

- Best known SphK2 inhibitor
- Weak *in-vitro* anticancer effect good *in-vivo* anticancer effect
- The potency of SphK2 Inhibition is poor (10 μM)

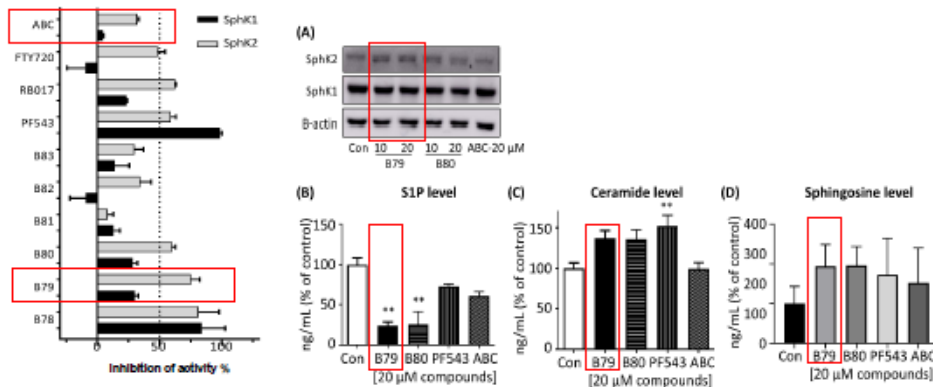


Cytotoxic effects of new synthetic compounds in pancreatic cancer cells



Compound **B79** and **B80** showed significant cytotoxic effect in pancreatic cancer

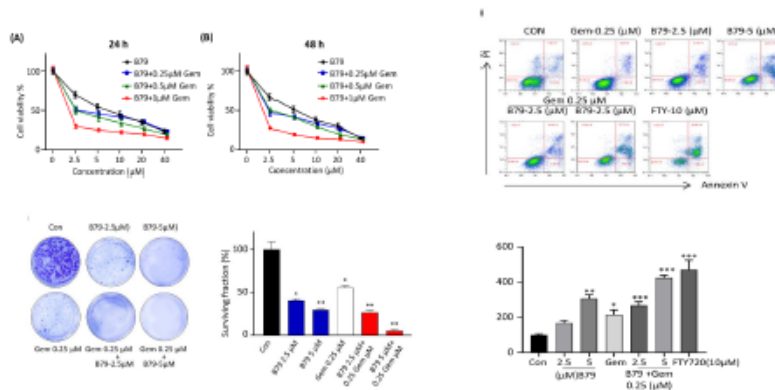
Target verification of B79 and B80



- Compound B78 have high SphK inhibitory effect with no selectivity, B79 showed selective SphK2 inhibitory effect.
- Compound B79 have no effect in SphK1/2 protein expression, decreased S1P level, increased ceramide and Sph level.

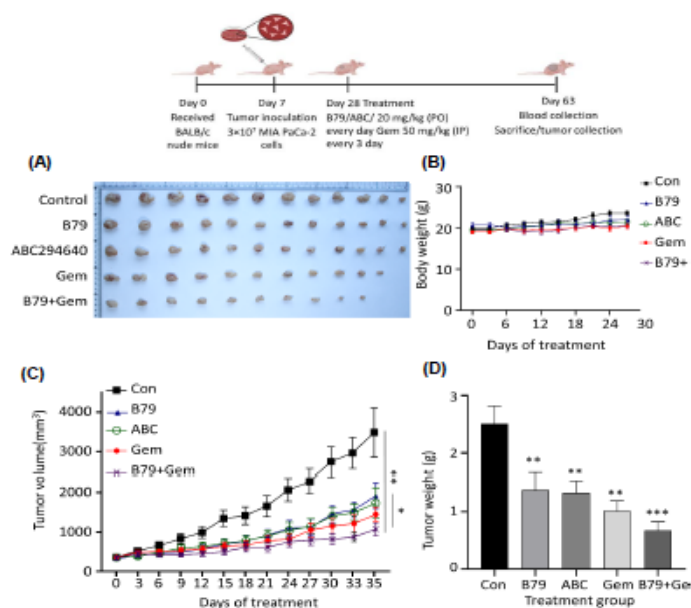
B79 improves gemcitabine sensitivity for anticancer effect in pancreatic cancer *in-vitro*

Suppression of c-Myc and RRM2 expression in pancreatic cancer cells by the sphingosine kinase-2 inhibitor ABC294640.
Lewis et al. 2016 oncotarget

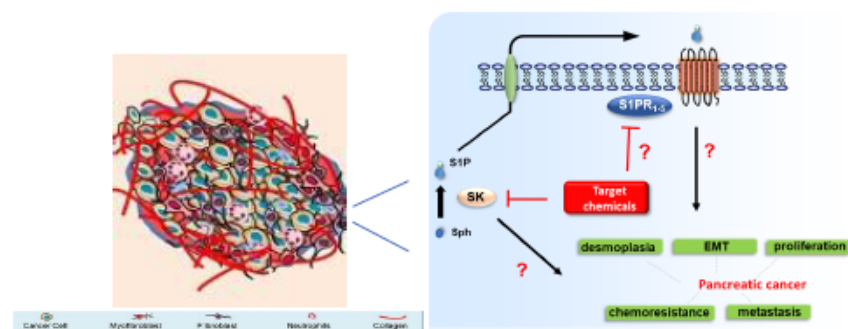


The combination of B79 and gemcitabine increased apoptosis in MIA PaCa-2 cells more than single treatment

MIA PaCa-2 tumor xenograft study in BALB/c mice



B79 treatment reduced xenograft tumor volume and weight in a similar way as ABC294640 treatment.



Inhibition of SphK activity is a promising approach in colorectal cancer therapy



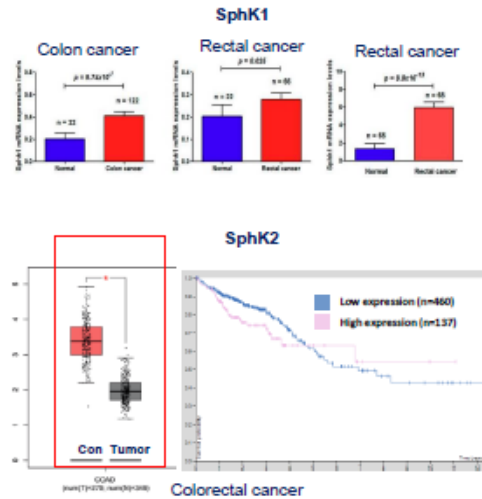
Colorectal cancer

2022 NCCN

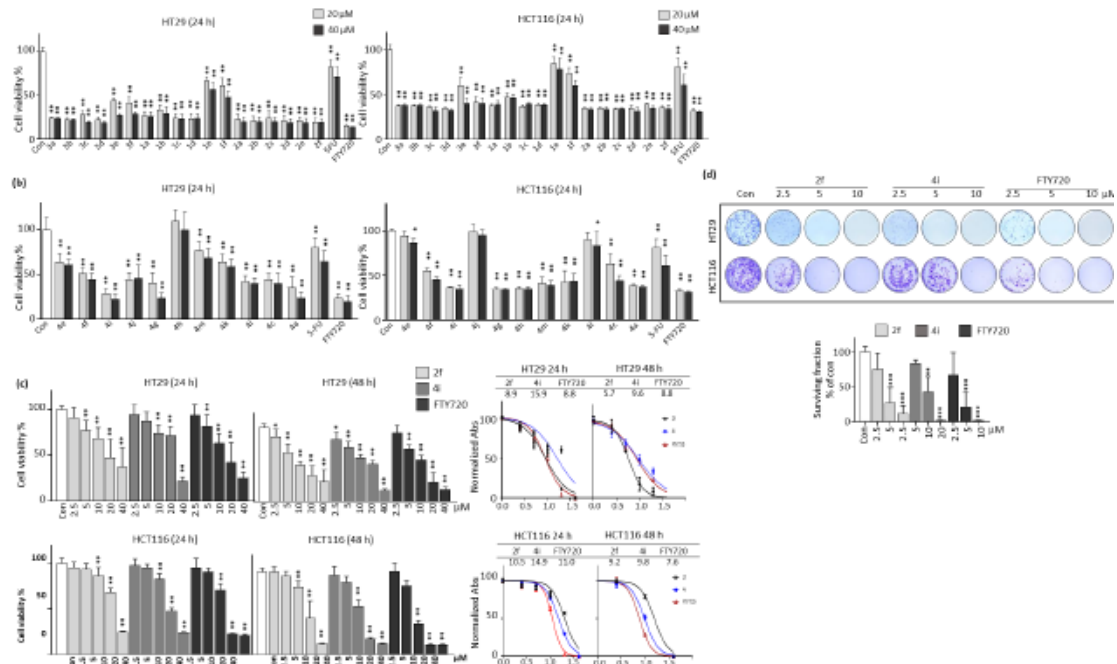
1 st line	FOLFOX or CapeOX ± bevacizumab FOLFOX ± cetuximab or panitumumab (KRAS, NRAS wild only) FOLFIRI ± bevacizumab FOLFIRI ± cetuximab or panitumumab (KRAS, NRAS wild only) 5-FU/LV or capecitabine ± bevacizumab
2 nd line	FOLFIRI ± bevacizumab FOLFIRI ± cetuximab or panitumumab (KRAS, NRAS wild only) FOLFOX or CapeOX ± bevacizumab FOLFOX ± cetuximab or panitumumab (KRAS, NRAS wild only) Irinotecan ± bevacizumab, Irinotecan+oxaliplatin ± bevacizumab
3 rd line	Irinotecan ± cetuximab or panitumumab (KRAS, NRAS wild only) cetuximab or panitumumab (KRAS, NRAS wild only) Regorafenib or trifluridine, dMMR/MSI-H : Nivolumab, Pembrolizumab

SphK1/2 upregulated in Colorectal cancer

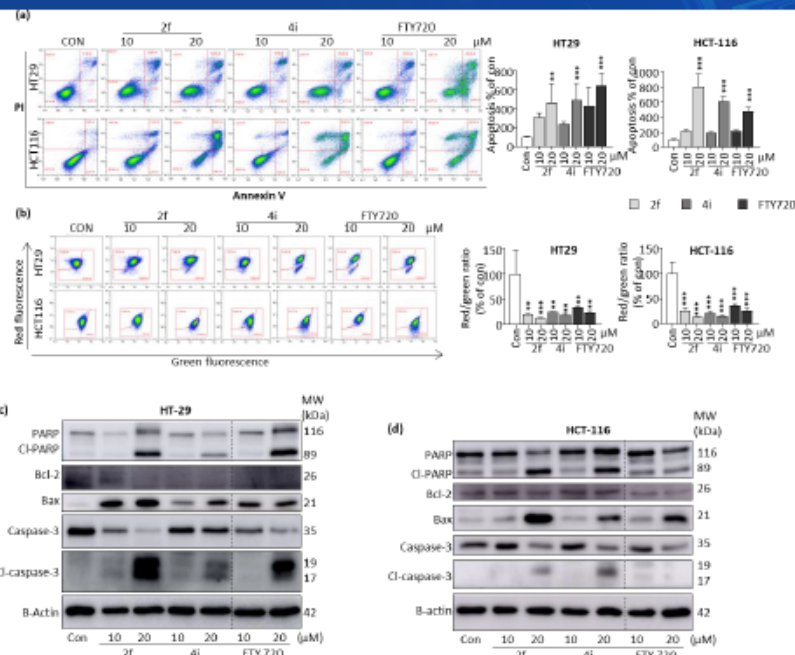
- SphK1 and SphK2 is overexpressed in colorectal cancer compared to normal tissues
- Tumor and serum S1P level elevated in colorectal cancer
- Increased SphK expression correlated to cancer progression, metastasis, low OS
- Increased SphK2 in CRC enhance chemo-resistance to 5FU
- SphK1 inhibition have significant anticancer efficacy and increased sensitivity toward 5FU



Cytotoxicity and anti-proliferative effect of selective compounds in

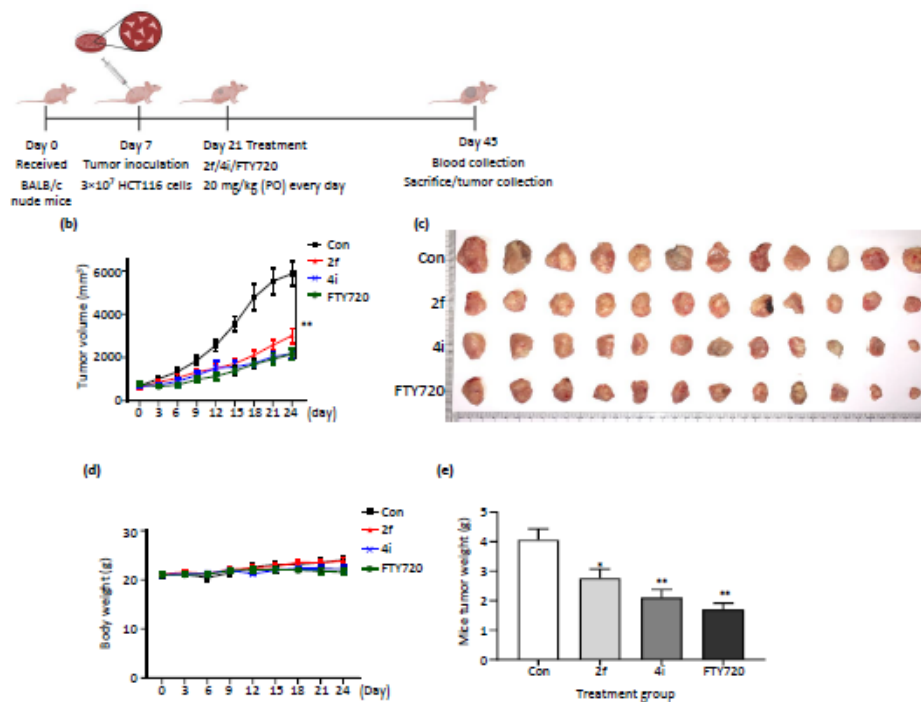


(a)



Compounds 2f and 4i induce apoptosis via mitochondrial intrinsic pathway in HT29 and HCT116 cells

(a)





감사합니다.