한국발생생물학회
Korean Society of Developmental Biology

KNU G-LAMP

제43회
한국발생생물학회
정기학술대회

Cellular Dynamics and Evolutionary Aspects in Developmental Biology

기조 강연

발생생물학과 양식의 하모니 (이영돈, 제주대학교)

시가 사람을 살립니다 (나태주, 시인)

2024.08.22.(목)~08.24.(토)

경북대학교 글로벌플라자 1층 경하홀

주 최: (사)한국발생생물학회
경북대학교 G-램프(LAMP) 사업단

후 원: 자이스코리아, 에비던트코리아, 남북메디칼, 라이노바이오, 지오리서치, 가영메디칼, 브니엘바이오, 파스칼바이오사이언스, 바이오드림, LG 화학, 특허법인 SRB, 바이오솔빅스, 한미사이언스, 스페바이오, 신풍제약, 아름사이언스, 한국오가논, 옵티젠, 성우라이프사이언스

INSTRUMENTS

생명과학 연구용제품 전문공급처
브니엘바이오

Attune™ NxT
Acoustic Focusing Cytometer

Acoustic Focus, Cell 이 지나갈 때 강한 진동으로 한줄로 정렬해서 Damage 없이 정확하고 빠르게 측정 가능.

EVOS™ M5000
Imaging System

머신러닝 기반의 세포정량 및 confluency분석
자동초점조절기능, Z-stack, 멀티채널 형광
자동화, 고해상도의 흑백 카메라와 LED컬러
조명모드

EVOS™ M7000
Imaging System

빠른 속도, 흑백 & 컬러 카메라, stitching
Time-lapse live-cell Imaging(incubator 장착)
자동초점 조절, 빠른 스테이지 움직임
Celleste 이미지 분석 소프트웨어

CellInsight CX5/CX7
High Content Screening Platform

형광 현미경, 영상 처리, 자동화된 세포 측정, 정보과학 분석 툴의 결합으로 세포 이미징과 동시에 여러 가지 조건의 측정 및 정량화 가능

Neon ™
Transfection System

Primary cell, Stem cell 및 Transfection이 어려운 세포 등 모든 포유류 세포 유형에 핵산을 빠르고 효율적으로 전달 가능

Countess™ 3 &
FL Automated Cell Counter

Automated Cell Counter, 일회용 슬라이드
Cell size와 Viability 동시 측정 가능
FL은 형광큐브장착, 재사용슬라이드 가능

CTS Rotea Counterflow
Centrifugation

완벽한 폐쇄형 세포 공정 시스템
CTS Rotea 소프트웨어
멸균 일회용 키트 사용

KingFisher™
Purification system

DNA/RNA/Protein 추출정제 및 IP
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High-speed, High-throughput
(Duo Prime, Flex, Apex, Presto)

Bandmate™ Automated
Western Blot Processor

Blot rocking system(Blocking, washing, antibody incubation 자동 진행)
2개의 Midi blot 또는 4개의 Mini blot 가능

iBright™ Imaging Systems
CL750/CL1500/FL1500

Chemiluminescence blot, Protein gel, Nucleic acid gel,
Fluorescence blot(FL1500만 가능)
분석 tool제공

Multiskan™ FC
Microplate Photometer

Filter type(basic : 405,450,620nm)
Assay range : 340-850nm
Quarts-Halogen Lamp

Multiskan SkyHigh Microplate
Spectrophotometer

MonoChromator
Assay range : 200-1000nm
µDrop Plate 또는 Cuvette를 이용 핵산 정량

Varioskan™ LUX multimode
microplate reader

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Filter 2개 장착, 2개 옵션

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Real-Time PCR System

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*브니엘바이오는 Thermo scientific, Invitrogen TaKaRa의 대구, 경북 공식 대리점입니다.

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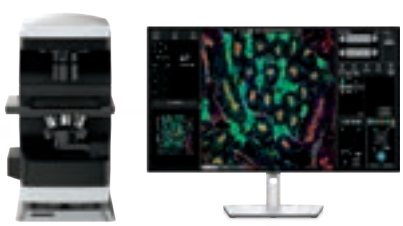

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IPTG	I373	5g	70,000
Cefotaxime	C380	25g	500,000
Cefotaxime	C380	5g	120,000
Cabenicillin	C346	25g	350,000
Cabenicillin	C346	5g	95,000
DTT	D259	25g	270,000
DTT	D259	5g	70,000
MS medium(vitamin)	M519	50L	50,000
Chu N6 medium(vitamin)	C167	50L	50,000
Hygromycin B	H397	1g	160,000
Hygromycin B	H385	10ml	160,000
X-gal	X874	1g	87,000
X-Gluc	X877	100mg	66,000
Gellan gum powder(Gellite)	G434	1kg	200,000
CHAPS	C526	10g	160,000
CHAPS	C526	5g	100,000

생명공학관련 실험용 기자재 및 시약, 소모품
바이오드림㈜
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762-8459
Fax) 055-755-0336
E-mail : bio-dream@hotmail.com

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- Moisture : ≤ 10%
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주문정보

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E-mail: bio-dream@hotmail.com

이화학 장비, 실험실 관련 소모품, 시약 모두 취급 합니다. 견적을 원하시면 언제든 연락주세요

제43회 한국발생생물학회
정기학술대회

Cellular Dynamics and Evolutionary Aspects in Developmental Biology

2024년 8월 22일(목) ~ 8월 24일(토)
경북대학교
글로벌플라자 경하홀

주　　최: (사)한국발생생물학회
경북대학교 G-LAMP 사업단

후　　원: 자이스코리아, 에비던트코리아, 남북메디컬, 라이노바이오, 지오리서치, 가영메디칼, 브니엘바이오, 파스칼바이오사이언스, 바이오드림, LG 화학, 특허법인 SRB, 바이오솔빅스, 한미사이언스, 스페바이오, 신풍제약, 아름사이언스, 한국오가논, 옵티젠, 성우라이프사이언스

제43회 한국발생생물학회 정기학술대회 프로그램

Cellular Dynamics and Evolutionary Aspects in Developmental Biology

주최: (사)한국발생생물학회, 경북대학교 G-LAMP 사업단
장소: 경북대학교 글로벌플라자 경하홀
일자: 2024년 8월 22일(목)~8월 24일(토)

2024년 8월 22일 (목) 12:00-18:00			좌장
12:00-13:00	등록		
13:00-13:30	**개회식** **(한국발생생물학회 회장 개회사, 경북대학교 총장 축사)**		(사회) 학술위원장 **허성표**
13:30-14:10	기조강연 I	발생생물학과 양식의 하모니 **이영돈 교수** (제주대학교)	회장 **이현식**
14:10-14:20		공로패 증정	
14:20-14:50	정회원 강연	미세플라스틱 노출에 따른 어류 독성생리 연구 **김준환 교수** (제주대학교)	**권태준** **허성표**
14:50-15:20		Zebrafish genetic tauopathy models to unveil the underlying mechanisms of Tau clearance and to identify modifiers *in vivo* **이정수 박사** (한국생명공학연구원)	
15:20-15:30		**Coffee Break & Booth Tour**	
15:30-16:00		Liver regeneration in the zebrafish, which may contribute to the modeling of human diseases **최태영 교수** (원광대학교)	
16:00-16:30		우리나라 양식 참다랑어의 성성숙과 산란가능성 **조정현 박사** (국립수산과학원)	
16:30-17:00		Coordination of cell cycle and morphogenesis during organ formation **정세연 교수** (Louisiana State University)	
17:00-17:10	**Coffee Break & Booth Tour**		
17:10-17:50	기조강연 II	시가 사람을 살립니다 **나태주 시인** (나태주풀꽃문학관)	회장 **이현식**
18:00	**환영 리셉션**		

2024년 8월 23일 (금) 14:00-20:00			좌장
14:00-14:40	기조강연 III	Stem cells and biomaterial tech for endometrial regeneration **이경욱 교수** (고려대학교 의과대학)	**김성훈**
14:40-15:00	심송 신진과학자 강연	Nuclear structure and chromatin involvement in hematopoietic cell development **차혜지 교수** (단국대학교)	**권우성** **최태영**
15:00-15:20		Organoid modelling of human fetal lung development **임경태 교수** (고려대학교)	
15:20-15:40		Microalgae biomass cultivation strategies overcoming the climatic disadvantages of Korea **이원규 박사** (한국해양과학기술원)	
15:40-16:00		Positional information during primitive streak formation in the chick embryo **이형철 교수** (전남대학교)	
16:00-16:10	**Coffee Break & Booth Tour**		
16:10-16:30	신진과학자 강연	Extending the application of tissue clearing techniques in developmental biology **우지원 교수** (연세대학교)	**류홍열** **문성환**
16:30-16:50		Dynamic sperm translation promotes the functional capacitation of spermatozoa to acquire fertility **박유진 박사** (중앙대학교)	
16:50-17:10		Non-clinical developmental toxicity studies of hazardous materials and new drug candidates with laboratory animals **이진수 교수** (충남대학교)	
17:10-17:30		Implication of CpG-mediated dual-mode gene regulation **이준영 교수** (경북대학교)	
17:30-17:50		Expanding the knowledge about teleost intestinal immunity by exploiting intestinal organoids **박영진 교수** (선문대학교)	
17:50-18:50	**포스터 세션 / 이사회**		사무총장 **박태주**
18:00	**저녁 식사**		

2024년 8월 24일 (토) 09:00-12:30			좌장
09:00-09:15	젊은과학자 강연	Genomic prediction for genetic improvement of low-fishmeal diet adaptability in Olive flounder (*Paralichthys olivaceus*) **Hanchapola Appuhamillage Chanuka Ravindu Hanchapola** (제주대학교)	김기범
09:15-09:30		Elucidating Dopaminergic Neuron Development Using Pig Embryonic Stem Cells In Vitro **최혜린** (충북대학교 수의과대학)	
09:30-09:45		Drosulfakinin signaling encodes early-life memory for adaptive social plasticity **정지원** (UNIST/KAIST)	
09:45-10:00		Myo-inositol improves the oxidative stress and mitochondrial dysfunction in porcine embryos after parthenogenetic activation **Ali Jawad** (충북대학교 수의과대학)	
10:00-10:15		Effect of regional characteristics of spawning and growing sites on growth of Pacific oyster, Crassostrea gigas **문지성** (부경대학교)	
10:15-10:30		**Coffee Break & Booth Tour**	
10:30-10:45		Identification of the functional role of Dorsal switch protein (DSP1) in Drosophila **백시은** (경북대학교)	염은별
10:45-11:00		The PI3K/PDK1/AKT Signaling Pathway During Capacitation: Application as a Biomarker for Reproductive Toxicity **이우진** (경북대학교)	
11:00-11:15		Clock gene expression in response to photo-periodic changes and absence of moonlight in eel retina and hypothalamus **안태영** (제주대학교)	
11:15-11:30		Evaluation of cardiotoxicity using human cardio-myocytes **박윤귀** (중앙대학교)	
11:30-12:00	**시상 및 경품 추첨**		학술위원장 **허성표**
12:00-12:30	**총회 및 폐회**		사무총장 **박태주**

제43회 한국발생생물학회 정기학술대회 초록

Cellular Dynamics and Evolutionary Aspects in Developmental Biology

목 차

기조강연

정회원 강연

심송 신진과학자 강연

신진과학자 강연

젊은과학자 강연

포스터 초록 A (자연분과)

포스터 초록 B (수산분과)

포스터 초록 C (수의/축산분과)

포스터 초록 D (의학분과)

기조강연

발생생물학과 수산양식의 하모니
(Harmonizing of Developmental Biology and Aquaculture)
- 되겠습니까? 어렵지 않습니까? 그래, 한번 해보자 -

이영돈
제주대학교 기당해양과학원
leesisu@hanmail.net

- 지식과 정보를 탐구하고, 아이디어를 모아서 프로젝트를 기획하며 수행
- 수행한 결과와 경험치를 토대로 사업현장에 적용하고 상용화에 주력

되겠습니까?

- 넙치 수정란 연중생산시스템 구축: 제주의 염지하수(용암해수)와 광주기 조절을 넙치 어미사육에 적용하여 가을에 수정란 생산(1989)
- 제주용암해수사업(2005~2008)
- 오분자기의 생식주기 특성과 종패 방류사업(2017~2021)

어렵지 않습니까?

- 케뉼레이션으로 생식소 발달을 조사하는 방법 그리고 바리과 어류의 성 성숙제어와 수정란 생산 그리고 종자생산(2000~현재)
- 붉바리 GSP사업(2013~2021)

한번 해보자

- 제주연안 서식하는 어패류의 성과 생식주기 그리고 산란생태특성(1991~2019)
- 군소의 성 성숙제어와 수정란 생산 그리고 종자생산과 변태유도(2002~2008)
- 돌멍게(리테르게멍게)의 발생과정(2002~2003)
- 자주복의 성 성숙제어와 수정란과 종자생산(2003~2006)
- 광어RIS사업(2009~2015)

사단법인 한국발생생물학회는 저에게 학문과 연구자의 발전적 기회를 제공해주셨습니다. 이제 교수로 정년을 맞아 퇴직을 준비합니다. 퇴직은 사회생태의 순환과정이고, 후학과 제자들에게 새로운 공간을 제공하며, 새로운 시작이라고 봅니다.

사단법인 한국발생생물학회의 무궁한 발전을 기원합니다.

감사합니다.

시를 통해 헤아리는 삶의 지혜

나태주

나태주풀꽃문학관

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- 우리는,
- 시비와 호오
- 행복감

● 사람을 응원하는 詩

- 왜?
- 시의 이로움
- 혼자서

● 사람을 살리는 詩

- 나태주풀꽃문학관
- 좋은 약

● 시로 배우는 예쁜 말

- 윤동주 : 서시
- 김소월 : 진달래꽃
- 나태주 : 시

● 하고 싶은 말

- 꿈과 詩 그리고 그리움
- 성공 = 열정 (그릿 GRIT) + 행복
- 자신을 소중하게 ('풀꽃 詩'처럼)

Stem cells and biomaterial tech for endometrial regeneration

이경욱

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여성의 가임기에서 자궁내막은 내인성 성호르몬 분비와 변화에 의해 주기적인 증식, 탈락, 재생을 반복하는 역동적인 장기이다. 임신 과정에 있어 자궁내막은 황체기에 다양한 생체물질을 분비함으로써 수정된 배아의 착상을 유도하는 역할을 하며, 이는 성공적인 임신을 위한 중요한 단계이다. 따라서, 자궁내막의 구조적 또는 기능적 이상이 있는 경우에는 반복적인 착상 실패로 인한 난임의 원인이 되며 아직 치료가 어려운 난치성 질환이다. 임상적으로 자궁내막 손상은 자궁내막유착(또는, 아셔만 증후군)이나 얇은 자궁내막 등이 있다. 자궁내막 손상의 병태기전으로는 자궁에 시술이나 수술 등으로 인한 의한 물리적 손상, 또는 자궁내 감염 등에 의한 자궁내막에 국소적 염증이 생기고, 이로 인해 내막조직의 유착이나 섬유화가 일어나게 되어 호르몬 영향에도 자궁내막의 증식과 성장이 잘 이루어지지 않게 된다.

줄기세포의 특성인 자가복원능 및 분화능을 이용하여 손상된 자궁내막을 재생하고자 하는 연구들이 시도되고 있다. 전임상 동물실험에서는 다양한 기원 (지방, 골수 등)의 성체줄기세포을 분리 및 배양하여 이를 자궁내막 손상 모델에서 처치 시 자궁내막 재생에 효과가 있다고 보고된다. 골수줄기세포의 경우, 자궁내막 손상이 있는 환자를 대상으로 한 몇몇 clinical trials에서 자궁내막 성장의 회복과 조직 재생에 유의미한 효과가 관찰되었다.

최근에는 조직공학기술을 이용하여 자궁내막 재생의 치료 효과를 증대하고자 하는 시도들도 있다. 콜라젠, 젤라틴, 하이알유론산 등과 같은 생체스캐폴드는 손상된 장기에 적용 시 세포외기질의 지지를 통해 혈관 생성, 세포 성장 등 세포기능을 향상시켜 조직 재생에 효과가 있으며, 다중층, 다공성 구조로 되어 있어 줄기세포 또는 다양한 생체인자를 탑재하여 조직재생효과를 보다 증대시킬 수 있을 것으로 생각된다. 따라서, 자궁내막 손상에서 조직학적, 기능적 재생 효과와 함께, 궁극적으로 착상률의 개선에 기여할 수 있을지에 대해 지속적인 연구가 필요하다.

Key words: 난임, 자궁내막 손상, 조직공학, 줄기세포, 착상

정회원 강연

Toxic physiology and toxicity standard in fish by microplastic exposure

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Plastic production is steadily increasing in various fields, including agriculture, industry, and medical treatment, owing to the excellent properties of plastics (e.g., low cost, lightweight, durability, and waterproofness). Global plastic production was approximately 360 million tons in 2018 and is expected to increase to 1.1 billion tons by 2050. With increasing plastic production, a large amount of plastic waste is being discharged into aquatic ecosystems. Plastic waste in aquatic environments is broken down into small particles by various factors, including photo-oxidation, thermal and chemical oxidation, biodegradation, and physical abrasion. Microplastics (MPs) are commonly considered to be plastic particles that are <5 mm in size. MPs are classified into primary and secondary MPs according to the origin of plastic waste: primary MPs are derived from personal care products and cosmetics, and secondary MPs are obtained by the breakdown of large plastics through environmental factors.

MPs are aquatic pollutants, and they can accumulate in the fish body, with potentially negative physical and physiological impacts (Miller et al. 2020). MPs can be ingested by aquatic animals accidentally through respiration and ingestion, as their small size can cause these animals to mistake them for food. MPs can accumulate in the gills by attaching to gill filaments, resulting in structural damage and dysfunction, such as ionic balance, gaseous exchange, and osmoregulation. MPs accumulation in the intestine can cause intestinal blockage, dysmotility, and inflammation, thereby reducing appetite, energy metabolism, and nutrient absorption. Additionally, MPs accumulated in these organs can pass through endothelial cells or intestinal lymphatics, enter the circulatory system, and translocate to other organs, such as the liver. MPs accumulation in tissues can have toxic effects on physiological functions, including metabolism, homeostasis and redox balance.

Key words: Microplastics, Toxicity, Bioaccumulation, Hematological parameters, Oxidative stress, Immuno-neuro toxicity

Zebrafish genetic tauopathy models to unveil the underlying mechanisms of Tau clearance and to identify modifiers *in vivo*

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Abstract: Tauopathy is a neurodegenerative disease characterized by accumulation of intracellular neurofibrillary tangles (NFTs) originating from the abnormal aggregation of hyperphosphorylated Tau protein, leading to synaptic dysfunction and neuronal cell death. Despite numerous efforts, the precise mechanisms behind tauopathy remain inconclusive, and Tau-targeting compounds and antibodies to treat the disease have been unsuccessful in clinical trials, necessitating further research to understand *in vivo* mechanisms and to identify therapeutic targets. In current study, we adopted zebrafish as an animal model for tauopathy study, known for its well-developed genetic manipulability, live imaging capabilities with high resolution, and suitability for high-contents drug screening approach for human diseases. We generated new transgenic zebrafish tauopathy model lines expressing human TAU^{P301L} under a neuron-specific promoter directly (*Tg(Elavl3:hTau*^{P301L}*-mCherry)*) or using a QF3/QUAS binary system (*Tg(Elavl3:QF3;13xQUAS-hTau*^{P301L}*-tdtomato)*). Using tauopathy-related phenotypes such as Tau protein accumulation, phosphorylation, and axonal degeneration exhibited in these transgenic zebrafish models, we identified VCP/p97, a cytoplasmic chaperone, as a potential modulator for tauopathy pathogenesis via autophagy. We also validated that a bacterial metabolite, known as a potential agent for preventing age-related diseases, ameliorated the tauopathy-related phenotypes in vivo. Versatile experimental approaches to identify potential therapeutic targets for tauopathy using Tau-overexpressing zebrafish models will be further discussed.

Keywords: Tauopathy, zebrafish transgenic model, VCP, bacterial metabolite

Liver regeneration in the zebrafish, which may contribute to the modeling of human diseases

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The zebrafish (Danio rerio) is emerging as a useful vertebrate model organism in developmental biology. Zebrafish models are critical for advancing our understanding of human pathogenesis and are a popular model for liver disease, allowing researchers to identify therapeutic targets and test novel drugs. The larval transparency of the zebrafish is a major advantage for real-time imaging in liver studies. The liver is the most regenerative organ, but this capacity is severely limited in the diseased liver, making liver transplantation the only definitive treatment for end-stage chronic liver disease. However, the scarcity of donor livers makes this therapy extremely limited. If innate liver regeneration could be enhanced in patients with chronic liver disease, it could alleviate the disease and improve quality of life. Therefore, means to enhance innate liver regeneration are considered a desirable and necessary alternative therapy. Understanding the mechanisms of liver regeneration is a prerequisite for the development of such a therapy. Here, we describe epcam mutants in zebrafish as a useful model system to study primary biliary cholangitis (PBC), formerly known as primary biliary cirrhosis. PBC is a disease that impairs the liver's ability to function. In people with PBC, the bile ducts become injured, then inflamed, and finally permanently damaged. our findings suggest that epcam positively regulates liver development and regeneration and may play a role in bile duct formation in the regenerative response. A large number of inherited and acquired diseases are associated with liver dysfunction. Zebrafish can be a valuable model system to study a rare inherited disease, Sjogren-Larsson syndrome (SLS). SLS is a rare autosomal recessive disorder characterized by severe ichthyosis, mental retardation and spasticity due to a fatty aldehyde dehydrogenase (FALDH) defect associated with mutations in the aldehyde dehydrogenase 3 family member A2 (ALDH3A2) gene. FALDH, a key component of the detoxification pathway of aldehydes generated by lipid peroxidation, and the enzymatic function of ALDH3A2 are well known, but the disease model is needed to investigate the molecular mechanisms of which fatty aldehyde or metabolic pathway in the liver primarily contributes to the pathogenesis of SLS ichthyosis symptoms.

우리나라 양식 참다랑어의 성성숙과 산란가능성

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우리나라 태평양참다랑어(*Thunnus orientalis*, 이하 참다랑어) 양식 연구는 일본이나 호주, 지중해 연안국보다 20~35년 늦은 2005년에 시작되었다. 가장 먼저 해상가두리와 육상수조 등 사육시설을 구축했고, 이어서 자연산 종자를 채포해 어미까지 양성하는 연구를 수행했다. 연구 개시 이후, 해상가두리와 육상수조에서 양성중이던 어미로부터 2015년과 2020~2021년 수정란을 생산한 바 있으나, 계획적이고 안정적인 수정란 생산은 불안정한 실정이다.

참다랑어 양식기술 개발, 특히 전주기 양식(full-cycle aquaculture)의 달성을 위해서는 양성 중인 어미로부터 안정적 수정란 생산을 위한 번식생물학적 이해가 선행되어야 한다. 그러나 참다랑어는 고가의 대형 종이며, 종래까지 우리나라에서 사육이 원활하게 이루어지지 않았기 때문에 국내 환경에서 양식되는 참다랑어의 번식생리연구를 위한 어미(시료) 확보가 어려웠다.

그러나 현재는 민간기업도 해상가두리에서 참다랑어의 축양(fattening)을 통한 양식 기반이 구축되어 있으며, 연중 출하가 가능해짐에 따라 생식소를 활용한 기초 연구가 가능해졌다. 따라서 본 연구는 참다랑어의 양식 생산성을 높이기 위해 국내에서 사육된 양식 참다랑어의 생식주기를 구명하고 번식 가능성에 대한 기초 자료 확보를 위해 수행되었다.

시료 확보는 통영 욕지도 인근 해역 해상가두리에서 상업 목적으로 양성된 5~6세어 참다랑어 70마리를 대상으로 이루어졌다. 연구기간은 2020년 4월부터 2021년 3월까지였으며, 해당 기간 동안 환경자료(수온, 광주기)를 수집하고, 암·수컷 생식소를 확보해 조직학적분석을 통한 성숙 단계를 분류하였다. 연구 결과, 성숙한 생식소를 지닌 참다랑어가 출현하는 시기는 수컷의 경우 5~8월, 암컷의 경우 6~8월 사이였다. 또한 암·수컷의 GSI와 sex steroid hormone 분석 결과, 모두 5월부터 상승하여 6월에 가장 높게 나타났으며, 이 후 낮아지는 경향을 나타냈다. 번식 가능 시기의 수온은 22~24℃의 범위였으며, 광주기는 14L:10D로 확인되었다. 상기 연구결과들을 종합하면 남해안 해상가두리에서 양성중인 참다랑어는 번식이 가능한 상태였으며, 연구 수행 이듬해 동일 해역 해상가두리의 어미로부터 산란 확인 및 난 채집에 성공하였다. 본 연구는 국내에서 최초로 수행된 참다랑어 번식생물학 연구로써 참다랑어 종자의 대량생산을 위한 기초자료로 활용될 것으로 판단된다.

Key words: 태평양참다랑어, 번식생리, 성성숙주기

Coordination of cell cycle and morphogenesis during organ formation

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Organ formation requires precise regulation of cell cycle and morphogenetic events. Using the Drosophila embryonic salivary gland (SG) as a model, we uncover the role of the SP1/KLF transcription factor Huckebein (Hkb) in coordinating cell cycle regulation and morphogenesis. The *hkb* mutant SG exhibits defects in invagination positioning and organ size due to the abnormal death of SG cells. Normal SG development involves distal-to-proximal progression of endoreplication (endocycle), whereas *hkb* mutant SG cells undergo abnormal cell division, leading to cell death. Hkb represses the expression of key cell cycle and pro-apoptotic genes in the SG. Knockdown of *cyclin E* or *cyclin-dependent kinase 1*, or overexpression of *fizzy-related* rescues most of the morphogenetic defects observed in the *hkb* mutant SG. These results indicate that Hkb plays a critical role in controlling endoreplication by regulating the transcription of key cell cycle effectors to ensure proper organ formation.

Keywords: *Drosophila*, salivary gland, Huckebein, endoreplication, endocycle, apoptosis, epithelial tube formation

심송 신진과학자 강연

Nuclear structure and chromatin dynamics during hematopoietic cell development

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The nucleus is spatially compartmentalized by chromosome and interchromatin functional components. Chromatin architecture influences gene transcription, but little is known about the role of nuclear proteins that serve as structural scaffolds. To address this topic in the context of hematopoiesis, we focused on an abundant nuclear scaffolding protein Matrin-3(MATR3), and deleted the entire gene body by CRISPR/Cas9 in human and mouse erythroid progenitor cells. Matr3 loss leads to morphological and gene expression changes characteristic of accelerated maturation, as well as broad alterations in chromatin organization similar to those accompanying differentiation. MATR3 protein interacts with CTCF and the cohesin complex, and its loss perturbs their occupancy at a subset of sites. Destabilization of CTCF and cohesin binding correlates with altered transcription and accelerated differentiation. This association is conserved during embryonic stem cell differentiation and myogenesis, demonstrating the role of this protein is general. Acute depletion of MATR3 using a targeted protein degradation platform reveals that MATR3 directly mediates differentiation through stabilizing chromatin accessibility and chromatin loop-domain interactions. Overall, our findings highlight a conserved and direct role of MATR3 in maintaining chromosomal structure and regulating cell differentiation.

KEYWORDS
differentiation, hematopoiesis, 3D genome organization, Matrin-3, inner nuclear protein, chromatin conformation, CTCF, cohesin, nuclear architecture, chromosome compartment, blood, transcription

Organoid modelling of human fetal lung development

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In the developing human lung, alveolar development requires highly systemic, spatiotemporal interactions between subsets of differentiating progenitor cells of epithelial and mesenchymal lineages. Extensive studies have been performed to understand the underlying mechanisms which organise alveolar differentiation and cell patterning in the developing alveolar niche in human, however, it remains elusive. In this study, we have generated multiomic cell atlas of human lung development that combines single-cell RNA and ATAC sequencing, high-throughput spatial transcriptomics, and single-cell imaging. The atlas identifies alveolar-fated epithelial progenitors in late-stage distal lung regions that can be modelled as self-organising organoids *in vitro*. We functionally validate spatiotemporal cell-cell interaction between the alveolar-fated progenitors and surrounding multiple mesenchymal cells, showing that Wnt signaling from differentiating alveolar fibroblasts promotes alveolar-type-2 cell identity, whereas myofibroblasts secrete the Wnt inhibitor, NOTUM, providing spatial patterning. We also identify a Wnt-NKX2.1 axis that controls alveolar cell fate determination and differentiation and allows derivation of human fetal lung-derived alveolar organoid model. The fetal-derived alveolar type 2 organoids are stable over long-term passaging, efficiently process and secrete surfactants, and can differentiate into AT1-like cells. Using this model, we reveal the underlying mechanisms of surfactant protein C maturation and an interstitial lung disease. Our single cell analysis in combination with lung organoid system revealed key aspects of human fetal lung stem cell biology, allowing mechanistic experiments to determine the cellular and molecular regulation of human alveolar development.

Kew words: Human lung development; single-cell; organoid modelling; cell-cell interaction; alveolar development; Wnt-NKX2-1; novel alveolar type 2 organoids

Microalgae biomass cultivation strategies overcoming the climatic disadvantages of Korea

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미세조류 바이오매스는 단위면적당 높은 생산성과 광합성을 통한 탄소저감 효과를 바탕으로 식품, 화장품, 의약품바이오, 연료 등의 다양한 산업 분야에서 주목받고 있다. 이에 세계적으로 많은 기업에서 미세조류 바이오매스를 생산하기 위해 여러 규모의 개방형 또는 밀폐형의 배양시설과 자연광을 이용하여 광 배양이 이루어지고 있다. 그러나 우리나라에서는 원료가 되는 미세조류 바이오매스 대부분을 수입에 의존하고 이를 국내에서 제품화하는 실정이다. 이는 우리나라에서 완전히 개방된 형태의 배양시스템을 사용할 시, 강수가 집중되는 여름철 장마기간에 강수의 유입으로 인해 미세조류 바이오매스의 생산성이 크게 감소하고 겨울철 낮은 기온과 짧은 낮 길이는 연중 바이오매스의 생산량과 성분 차이를 야기하기 때문이다. 따라서 우리나라의 환경적 조건을 극복하고 연중 안정적인 미세조류 바이오매스 생산을 위해서는 전 배양 기간동안 고농도 종자배양에 요구되는 밀폐형 배양시스템과 산업적 규모 배양에 필요한 반 개방형의 배양시스템의 혼합 사용이 제안된다. 이 연구에서는 빛 투과성을 증가시키고 배양액 순환을 개선하여 고농도 종자 배양이 가능한 밀폐형 배양기를 개발하고자 하였으며, 미세조류 세포의 광합성효율 및 밀도를 기존 상용화된 배양기와 비교하였다. 이후, 이전의 연구에서 다년간의 국내배양을 통해 입증되었던 빌딩 정보 모델링 (Building information modeling, BIM)기반의 반 개방형 배양시스템에서 연중 낮 길이차를 극복하는 인공 광시나리오를 확립하고자 하였다. 광주기, 파장 및 혼합비율을 포함하는 광시나리오는 실험실 규모에서 확립되었다.

종자 배양을 목적으로 개발된 밀폐형 배양기(ROSEMAX)에서는 기존 원통형 배양기에 비해 세포의 광합성효율이 1.14 배 그리고 배양액 내 미세조류 순수 세포 수의 비율이 1.64 배 증가하는 결과를 통해 실용가능성이 확인되었다. 미세조류 색소 생산을 위한 광시나리오는 24L:0D 의 명암주기 및 빨간색(627 nm)과 파란색(454 nm) 8:2 비율로 확립되었으며, 자연광을 이용하는 야외 배양에서는 밤에만 광시나리오를 적용할 경우, 연중 바이오매스 생산량 1.2-1.8 배 증가 및 색소 생산량 1.7-2.5 배 증가하는 효율적인 생산 결과를 확보하였다. 이 연구를 통해 개발된 밀폐형 배양시스템과 인공 광시나리오가 적용된 반 개방된 수로형 시스템이 우리나라와 유사하게 야외 배양에 불리한 지역에 연중 배양 가능한 시스템으로써 활용될 것으로 기대한다.

Key words: 미세조류, 바이오매스, 배양시스템, 생산성, 광합성 색소

Positional information during primitive streak formation in the chick embryo

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In a developing embryo, cells adopt their identity and differentiate at an adequate time to accomplish proper tissue patterning. The 'positional information' theory by Lewis Wolpert (1969) proposed that cells read a gradient of morphogen diffused from a local source while responding with discrete thresholds, which was explained metaphorically by the 'French Flag problem'. It can successfully explain how spatial patterns are generated in a tissue in many developing systems (e.g. vertebrate neural tubes, limb regeneration, several imaginal discs of fruit flies, etc. However, how cells access their positions while receiving different kinds and amounts of morphogens and how the field of positional information is scaled in variously-sized systems are much unknown. First, in this study, we explain how the cells in the pre-primitive-streak stage embryo communicate across the embryo to position the primitive streak. We show that long-range communication by traveling calcium activity generates positional information to locate the site of primitive streak formation by regulating the activity of the bone morphogenetic protein. We also show how cells interpret signals locally while receiving different amounts of inducer (cVG1) and inhibitor (BMP4) molecules during the formation of the primitive streak. We propose a model called 'neighbourhood watch' explaining that the cells sense the relative amount of signals compared to their neighboring cells. Our study shows long- and short-range communication to establish embryo polarity across the embryo. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (RS-2023-00239555).

Kew words: chick, embryo polarity, primitive streak, gastrulation, positional information

신진과학자 강연

Extending the application of tissue clearing techniques in developmental biology

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Abstract

The recent growth of tissue clearing technology supports the dissemination and generalization of three-dimensional (3D) observations of complex biological tissues. Quantitative 3D imaging of tissue volumes aimed at understanding tissue development and the organization of spatial relationships and biological circuits first requires tissue clearing. These techniques increases the transparency of intact tissues in adults or late developmental stages and enables deep imaging in various biological organisms. Successful implementation of tissue clearing methodologies requires a good grasp of sample processing, immunostaining, refractive index matching and the possibilities offered by high-resolution image analysis. In addition, since many clearing techniques have been proposed, understanding the basic principles of tissue clearing is essential for understanding their technical characteristics and selecting the appropriate one in study design. Here, we highlight how current tissue clearing techniques can revolutionize the histological analysis of pathological samples and developmental processes in biological organisms containing human and other animals. We advise on how to implement effective tissue clearing methods, high-resolution imaging strategies and analysis methods for developmental biology research.

Keyword: Tissue clearing, 3D imaging, Developmental biology, Pathological sample, Transparent tissue

Dynamic sperm translation promotes the functional capacitation of spermatozoa to acquire fertility.

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Notwithstanding unfavorable conditions for transcription and translation in mature spermatozoa due to the unencumbered by essential cytoplasmic organelles, the contradictory reports about the presence of various mRNA and protein upregulation during capacitation opened many possibilities of de novo protein synthesis in spermatozoa during capacitation. Recent advances in fluorescent noncanonical amino acid tagging system (FUNCAT) allows the spatiotemporal observation of newly synthesized proteins in cells without biological processes6-8. Herein, a strong fluorescent signal was observed in spermatozoa following the metabolic labeling using FUNCAT system during time-sequential capacitation, which was arrested by translation inhibitor chloramphenicol (CP), indicating protein translation might occur in spermatozoa during capacitation. Belated upregulation of “Golgi to plasma membrane transport”, which is associated with exocytosis/the acrosome reaction in order to penetrate and fusion with oocyte, during sperm capacitation may lead to the irregular acrosome reaction and delayed priming the penetration of oocyte. Moreover, adaption of FUNCAT and proximity ligation method in spermatozoa allows to comprehend understand that the fertility varies are derived by different spatiotemporal translation during sperm capacitation.

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Keywords: Spermatozoa, Capacitation, Translation, Fluorescent noncanonical amino acid tagging system, Fertility

Non-clinical developmental toxicity studies of hazardous materials and new drug candidates with laboratory animals

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Non-clinical toxicological studies of hazardous materials and new drug candidates using laboratory animals are essential procedures before the direct exposure to humans. Among various types of non-clinical toxicological studies, developmental and reproductive toxicity (DART) studies investigate the potential toxicological effects in the developmental and reproductive cycle, including fertility, influences during pregnancy, teratogenicity, and post-natal development, with laboratory animals. In this presentation, it aims to introduce an overview of non-clinical DART studies (especially developmental toxicity studies) of various hazardous materials and new drug candidates using various laboratory animals with some examples. Through this presentation, it will be possible to understand non-clinical developmental toxicity studies using various experimental animals and understand the applicability and future of non-clinical toxicological studies.

Keyword: developmental toxicology, hazard identification, teratogenicity, pregnancy

Implication of CpG islands-mediated dual-mode gene regulation

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Understanding global gene regulation is crucial for comprehending complex biological systems and human aging. Gene regulation involves multiple layers, including transcriptional, epigenetic, and 3-dimensional chromosomal architecture. These layers are interconnected, requiring an integrative approach for accurate understanding. Our extensive data analyses and experiments show that CpG islands (CGIs) help reconcile competing models of nuclear gene localization. CGI+ genes are found at the nuclear center, engaging in long-range interactions, while CGI− genes, associated with heterochromatin, move to the nuclear center upon activation through enhancer–promoter interactions. Comparative epigenomic analyses show that this dual-mode regulation is common in various eukaryotes, even those lacking CGIs. Vertebrates and plants exhibit genes with high-methylation promoters forming heterochromatin and context-dependent expression, while low-methylation promoters remain euchromatin. Invertebrates without DNA methylation, like fruit flies and nematodes, also show gene regulation divergence: some genes are regulated by Polycomb proteins, others by heterochromatin. This establishes gene type divergence and dual-mode regulation as fundamental features in higher eukaryotes and provides insight into the evolutionary aspects of gene expression regulation.

Keywords: Gene regulation, CpG islands, Heterochromatin, Polycomb

Expanding the knowledge about teleost intestinal immunity by exploiting intestinal organoids

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Intestine that performs functions such as nutrient absorption is a crucial organ of vertebrates. In addition, its components take part in innate and adaptive immune defences to evoke appropriate responses against food antigens and potential pathogens. Intestinal epithelium consists of differentiated cells of secretory and absorptive progenitors derived from intestinal stem cells. These differentiated cells are the first responders of the defence system and play a vital role in maintaining intestinal homeostasis. Our teams have been constantly gathering information regarding intestinal inflammation and intestinal cells of fishes. To further expand our understanding of intestinal immune system of fishes, we believe that intestinal organoids that can closely mimic the complex organization and functionality of the intestine, could be a useful toolkit. Although mammalian studies have benefited from intestinal organoids, piscine research on these lines has not been conducted yet. We intend to employ this system for infection studies as well as to explore the effect of specific dietary components. We plan to generate intestine organoids for promising and commercially important fish models such as zebrafish (*Danio rerio*), Atlantic salmon (*Salmo salar*), European seabass (*Dicentrarchus labrax*) and Nile Tilapia (*Oreochromis niloticus*). We are certain that organoid research will open the door to exciting and interesting studies that will benefit fundamental fish biology and immunology research as well as the aquaculture industry.

Keywords: Intestine, Organoids, Teleost, Immunity, Nutrition

젊은과학자 강연

Genomic prediction for genetic improvement of low-fishmeal diet adaptability in Olive flounder (*Paralichthys olivaceus*)

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For the sustainable aquaculture of olive flounder, it is important to substitute the moisture pellet (MP) with the extruded pellet (EP) and to reduce the fishmeal content in the EP. Therefore, developing new olive flounder lines adapted to various nutritional conditions under the EP feeding regime is required. In this study, we conducted a genome-wide association study (GWAS) to elucidate the genetic architecture of growth traits in olive flounder under a low-fishmeal EP feeding regime. Moreover, we constructed prediction models to estimate the genomic estimated breeding value (GEBV), which will be utilized for selecting superior broodstock to generate a genetically improved progeny population.

The feeding trial was conducted from June 2023 to October 2023. Phenotypic data were collected for 1,200 individuals, and passive integrated transponder (PIT) tags were implanted for growth tracking. The feeding trial continued for 4 months and additional phenotypic data were collected every two months. After the feeding trial, 768 individuals were randomly selected, and genomic DNA was extracted from caudal fin samples for genotyping using a 70K SNP chip designed by our laboratory. After preprocessing and quality control, we obtained 763 samples with 50,969 high-quality SNPs. For subsequent analysis, final weight (FW, g), weight gain rate (WGR, %), and specific growth rate (SGR, %/day) were used as input data. The narrow-sense heritability was low to moderate, ranging from 0.09 to 0.52. The genetic correlation among traits exhibited low to moderate positive correlations. We identified one significant SNP associated with FW on chromosome 1 based on the Bonferroni threshold from GWAS. The genomic prediction models were constructed using 10 different prediction methods, and prediction ability was estimated through ten replicates of five-fold cross-validation. As a result, the prediction model constructed using FW with GBLUP or Bayesian models showed the highest prediction ability, and these models were optimized under various scenarios. For performance equivalent to a model constructed with the entire dataset, the population size and the number of SNP markers need to be at least 400 and 5,000, respectively. Additionally, we observed a significant improvement in prediction ability compared to the basic model when selecting SNP markers in descending order based on the *p*-value obtained from the GWAS.

The findings of this study provide crucial insights into the genetic architecture and the optimal prediction model for the genomic estimated breeding value of growth traits in olive flounder. These results can ultimately be used to develop new olive flounder lines with improved growth performance under the EP feeding regime with various nutritional conditions. Moreover, these studies will pave the way for sustainable aquaculture of olive flounder and serve as a valuable reference for future research on other aquatic species.

Key words: *Paralichthys olivaceus*, Genome-wide association study, genomic selection, growth trait, low-fishmeal adaptability

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Elucidating Dopaminergic Neuron Development Using Pig Embryonic Stem Cells *In Vitro*

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Pigs with gyrencephalic brains are more similar to human brains in terms of anatomy and development compared to rodents with lissencephalic brains, making them valuable non-primate models in neuroscience. Despite these advantages, there is a lack of understanding of porcine midbrain dopamine (mDA) neuron development in vitro. We utilized a floor-plate transition-based method to derive ventral midbrain (VM) progenitors from porcine embryonic stem cells (pESCs). This protocol includes ventralization with SHH and caudalization using the GSK3 inhibitor CHIR99021 (CH) and FGF8. The absence of GSK3 inhibition during porcine VM patterning led to the upregulation of forebrain-related genes, while a high concentration of CH (3.0 μM) promoted hindbrain marker expression. Conversely, 1.5 μM CH directed progenitors toward a midbrain fate, indicated by high levels of caudal VM marker genes and EN1-positive cells by day 16. Modifying the protocol with a high concentration of SHH enhanced VM identity by increasing $FOXA2^{+}$ cells and reducing PAX6 expression. Through in vitro maturation of VM progenitors using nerve growth factors, we observed neural dendrites and confirmed robust expression of mDA neuron-specific genes in differentiated cells by day 28. Immunostaining revealed the presence of mDA neuron markers and synaptic proteins in the differentiated cells. Their functionality was validated by demonstrating action potential firing and synaptic connectivity. These stem cell-derived porcine mDA neurons are a crucial intermediary step that bridges in vitro research and in vivo applications. They enhance our understanding of Parkinson's disease, facilitate the development of new therapies, and improve the reliability and ethical considerations of preclinical studies using pig models.

Key words) *Porcine, embryonic stem cells, floor plate, midbrain dopaminergic neuron*

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Drosulfakinin signaling encodes early-life memory for adaptive social plasticity

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Drosophila establishes social clusters in groups, yet the underlying principles remain poorly understood. Here we performed a systemic analysis of social network behavior (SNB) that quantifies individual social distance (SD) in a group over time. The SNB assessment in 175 inbred strains from the Drosophila Genetics Reference Panel revealed a tight association of short SD with long developmental time, low food intake, and hypoactivity. The developmental inferiority in short-SD individuals was compensated by their group culturing. By contrast, developmental isolation silenced the beneficial effects of social interactions in adults and blunted the plasticity of SNB under physiological challenges. Transcriptome analyses showed genetic diversity for SD traits, whereas social isolation reprogrammed select genetic pathways, regardless of SD phenotypes. In particular, social deprivation suppressed the expression of the neuropeptide Drosulfakinin (*Dsk*) in three pairs of adult brain neurons. Male-specific DSK signaling to Cholecystokinin-like receptor 17D1 mediated the SNB plasticity. In fact, transgenic manipulations of the DSK signaling were sufficient to imitate the state of social experience. Given the functional conservation of mammalian *Dsk* homologs, we propose that animals have evolved a dedicated neural mechanism to encode early-life experience and transform group properties adaptively.

Keywords: Drosophila; social network behavior; early-life experience; social memory; Drosulfakinin.

Myo-inositol improves the oxidative stress and mitochondrial dysfunction in porcine embryos after parthenogenetic activation

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Myo-inositol (Myo-Ins), the most abundant form of inositol, acts as an antioxidant and facilitates embryonic development in mammals. Nevertheless, there is a paucity of research examining the effects of Myo-Ins supplementation in the *in vitro* culture medium of porcine embryos. This study aimed to investigate the impact of Myo-Ins inclusion in porcine zygotic medium (PZM3) with different concentrations (0, 5, 10, and 20 mM) for 7 days. The porcine embryos were examined on day 2 for cleavage and day 7 for blastocyst formation rates. Supplementation with Myo-Ins at concentrations of 10 and 20 mM significantly enhanced the blastocyst formation rate compared to the control group. However, no significant increase was observed in either the cleavage rate or the total cell number in comparison with the control group. Myo-Ins significantly decreased the levels of reactive oxygen species (ROS) and markedly increased the levels of glutathione (GSH) in the embryos of the 20 mM group at 4-5 cell stages on the second day, when compared to the control group. The Myo-Ins treatment causes a reduction in the apoptotic rate and apoptotic index in blastocysts compared to the control group. Myo-Ins was observed to significantly enhance antioxidant genes, including nuclear factor erythroid 2-related factor 2 (*NRF2)*, heme oxygenase (*HO-1)*, and glutamate-cysteine ligase catalytic subunit (*GCLC)* in blastocysts treated with 20 mM compared to the control group, while mitochondrial function-related genes such as solute carrier family 2 member 1 (*SLC2A1*) and ATP synthase (*ATP5F1A*), demonstrated a significant increase in the 10 and 20 mM treated groups compared to the control group. Moreover, Myo-Ins at 10 and 20 mM significantly improved mitochondrial dysfunction by enhancing mitochondrial membrane potential (MMP), increasing mitochondrial quantity at 20 mM, and notably reducing mitochondrial oxidative stress at 20 mM. Furthermore, Myo-Ins treatment at 20 mM significantly increased *HO-1* expression in the blastocysts compared to the control group. In short, Myo-Ins treatment increased the embryonic developmental potential, reduced apoptosis, decreased oxidative stress and improved the mitochondrial dysfunction through activating *NRF2* pathway.

Key words) *Myo-inositol, parthenogenesis, oxidative stress, mitochondria, embryos*

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Effect of regional characteristics of spawning and growing sites on growth of Pacific oyster, *Crassostrea gigas*

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In the animal, insulin-like growth factors (IGFs) system is known to be a major factor in the neuroendocrine control of various physiological actions such as growth and maturity. It is reported that this applies similarly to the growth of aquatic organisms, but it has not been clearly identified in bivalves. In particular, IGFs expression is directly related to the nutritional status of individuals. In this study, to confirm the effect of different growing sites, Pacific oyster were collected in Busan (BS), Tongyeong (TY), and Namhae (NH) in July 2022 and pre-grown until June 2023. The oysters were then cultured in different growing sites (Tongyeong and Geoje) for six months, with monthly sampling until December 2023 to assess growth performance, mRNA expression of Molluscan insulin-related peptide (MIP), IGF binding protein complex acid labile subunit (IGFBP_ALS), *Crassostrea gigas* Insulin receptor-related receptor (CIR), and IGF-1 and 2 protein expression in the adductor muscle. The total weight of oysters grown at the Tongyeong site (BS: 53.08 ± 1.60 g, TY: 57.78 ± 1.93 g, NH: 41.72 ± 1.68 g) was significantly higher than at the Geoje site (BS: 47.87 ± 1.69 g, TY: 48.63 ± 1.47 g, NH: 47.99 ± 2.88 g), except for the NH ($P > 0.05$). Additionally, the soft tissue weight, condition index (CI), and soft tissue weight rate were also significantly higher at the Tongyeong site ($P < 0.05$). The mRNA expressions of IGFs and the protein expressions of IGF-1 and 2 showed high level at the Tongyeong site in most sampled months ($P > 0.05$). However, in taste sensory analysis using an electronic tongue, the results were opposite. Oysters from the Geoje site had higher scores for umami. In a previous study, Pacific oysters with higher CI levels showed higher expression of IGF-1. This was similar to the result of relatively high IGFs expressions in this study because the CI level in the Tongyeong site experimental group was higher than that in the Geoje site experimental group. Therefore, there was no clear difference in regional characteristics according to the spawning site, while distinct regional characteristics were evident according to the growing site.

Key words: Pacific oyster, Regional characteristics, Growth, Insulin-like growth factors, Taste sensory anlysis

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Identification of the functional role of Dorsal switch protein 1 (DSP1) in Drosophila

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Drosophila DSP1 (Dorsal Switch Protein), a mammalian homolog of HMGB1, is well studied as a co repressor of Dorsal. DSP1 has HMG-box domain and it acts as a transcriptional regulator in Drosophila melanogaster. DSP1 roles in embryonic development, it regulates dorsal-ventral patterning by controlling the gene expression. Additionally, DSP1 is involved in cellular systems like cell fate determination and tissue differentiation during early embryogenesis. HMGB1, homolog of DSP1, is studied as factor for neuronal disease. However, while function of DSP1 in embryonic development is well-studied, roles in adult brain is not identified. In this study, we investigated the neuronal function of DSP1 in Drosophila by using UAS-Gal4 system with DSP1-overexpressed and downregulated flies. We regulated DSP1 expression in neurons and glia cells using Elav-Gal4 and Repo-Gal4. We found that lifespan and locomotion were reduced in DSP1 overexpressed flies. On the contrary, when DSP1 downregulated, both of lifespan and locomotion were increased. Furthermore, DSP1 overexpression caused neuromuscular junction (NMJ) defect in larva, reduction of eye size and Dopaminergic neurons in adult flies. This study suggests that DSP1 is involved in neuronal toxicity, DSP1 might be new therapeutic target in neurodegenerative disease.

Keywords: Neurodegeneration, DSP1, Drosophila

The PI3K/PDK1/AKT Signaling Pathway During Capacitation: Application as a Biomarker for Reproductive Toxicity

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Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/phosphoinositide-dependent protein kinase (PDK1)/protein kinase B (AKT) signaling pathway is considered the central integrator of various signals, regulating cell growth, proliferation, and apoptosis. In particular, due to its involvement in numerous diseases and tumorigenesis, drugs targeting proteins in this pathway are continuously being developed. In addition, this signaling is related to many stages of male reproduction, including spermatogenesis, regulation of sperm autophagy, acrosome reaction, and capacitation. Despite this knowledge, the role of PI3K/PDK1/AKT signaling pathway-related proteins during capacitation remains unclear. Therefore, we evaluated the expression of PI3K/PDK1/AKT-related proteins depending on the incubation time during capacitation and analyzed their correlation with sperm function. Furthermore, we investigated the effects of treatment with compounds being developed as therapeutics, focusing on the PI3K/PDK1/AKT signaling pathway in spermatozoa. As a result, correlation analysis showed that changes in PI3K/PDK1/AKT signaling pathway-related proteins during capacitation were correlated with sperm function. Moreover, aberrant PI3K/PDK1/AKT signaling caused by treatment with therapeutic agents inhibited sperm motility, capacitation status, and acrosome reaction. Consequently, our results indicated that alterations in PI3K/PDK1/AKT-related proteins during capacitation are associated with sperm function, and aberrant protein expression in these signaling pathways had detrimental effects on male reproduction. This study may improve our understanding of the role of PI3K/PDK1/AKT signaling in spermatozoa during capacitation. Additionally, these findings suggest that the PI3K/PDK1/AKT signaling pathway-related proteins might be a potential biomarker for assessing the reproductive toxicity of therapeutic agents such as anticancer drugs or antivirals.

Keywords: PI3K/PDK1/AKT signaling pathway, spermatozoa, capacitation, male reproductive toxicity, biomarker

The transcript levels of clock genes on Japanese eel (*Anguilla japonica*) in response to photoperiod and moonlight

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Cryptochrome (Cry) and Period (Per) are clock genes that regulate circadian rhythms by photo signal. Clock genes regulate physiological circadian rhythms such as sleep, hormone secretion, and temperature changes, and affect reproduction by activating the hypothalamic-pituitary-gonadal (HPG) axis in response to photo signals. In fish, clock gene expression is primarily regulated in the retina. This study was conducted to investigate the correlation of clock genes with reproductive characteristics in the eel (Anguilla japonica). For this purpose, the expression patterns of clock genes (Cry, Per) were examined under different photoperiodic conditions.

The experimental fish were acclimatized to 12 h light: 12 h dark (12L:12D) for one week in fresh water before the experiment, and then divided into the following experimental groups. 1) 12 h light: 12 h dark (12L:12D) and 3 days of 24 h dark (24D) and 24 h light (24L), 2) 1week of short photoperiod (SP; 9L:15D) and long photoperiod (LP; 15L:9D), and 3) 2 weeks of new moon (NM) and full moon (FM). Sampling was performed every 4 hours for 24 hours, and retinas were extracted for Real-Time PCR (qPCR) to analyze the expression of clock genes.

Clock genes in the retina decreased in expression during photophase and increased during scotophase. Per1, Per3, and Cry3 were upregulated before sunrise. Per2 increased in expression after sunrise and Cry2 and Cry4 increased in expression before sunset. Per3 and Cry4 expression in the retina did not oscillate in the long photoperiod (LP) condition, but did oscillate in the short photoperiod (SP) condition. Cry4 oscillations of the retina were not detectable in the full moon condition (FM) and a constant oscillation was detected in the newmoon (NM) condition.

These results suggest that the eel's retina can recognize clock gene photoperiod and moonlight changes. Furthermore, the expression characteristics of clock genes may be closely related to the behavioral and reproductive characteristics of eels, and further studies are needed.

Key Word: Clock gene, Cryptochrome (Cry), Period (Per), Photoperiod, Moonlight

Evaluation of cardiotoxicity using human cardiomyocytes

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Drug-induced cardiotoxicity is a critical issue in drug development, often causing severe cardiac side effects. Traditional models are limited in predicting human cardiac responses due to species-specific differences. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) offer a physiologically relevant alternative for assessing cardiotoxicity. Here, we used hiPSC-CMs as a cell model to evaluate the cardiotoxicity of remdesivir, an antiviral drug for SARS-CoV-2. Remdesivir showed significant antiviral activity in hiPSC-CMs compared to Vero E6 cells; however, it also induced moderate cardiotoxicity in these cells. To gain further insight into the drug-induced arrhythmogenic risk, we assessed QT interval prolongation and automaticity of remdesivir-treated hiPSC-CMs using a multi-electrode array (MEA). The data indicated a potential risk of QT prolongation when remdesivir is used at concentrations higher than the estimated peak plasma concentration. In conclusion, this study suggests the potential of hiPSC-CMs in enhancing the predictability of human cardiac responses in drug safety assessments. This work was supported by National Research Foundation of Korea (NRF) funded by the Korea government (MSIT) (2022R1A2C1006622) and Korea Institute of Marine Science & Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries, Korea (RS-2023-00235057).

Keywords: Drug-induced cardiotoxicity, human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs), Multi-electrode array (MEA)

포스터 초록

Transcriptional Disruption Induced by ulp2Δ is Mitigated through an Adaptive Mechanism via in vitro Evolutionary Processes

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DNA replication and repair are vital for preserving genome stability, and their regulation involves a complex interplay of post-translational modifications. In Saccharomyces cerevisiae, the SUMOylation pathway, involving Small Ubiquitin-like Modifier (SUMO) proteins, plays a crucial role in modulating protein functions associated with DNA metabolism. SUMO is covalently attached to the lysine residues of target substrates across all eukaryotes. The key enzymes involved in SUMOylation include the SUMO E1, E2, and E3 enzymes. Equally important is the reverse process facilitated by desumoylation enzymes such as Ulps in yeast and the homologous SENPs in mammals. Sumoylated proteins are also directed to the degradation pathway mediated by SUMO-targeted ubiquitin ligase (STUbL). In Saccharomyces cerevisiae, the STUbL complex, composed of Slx5 and Slx8, specifically targets poly-sumoylated proteins for degradation. Slx5, a component of this complex, contains SUMO-interacting motifs (SIMs) that are essential for its interaction with SUMOylated substrates. Deletion of SLX5 (*slx5Δ*) disrupts these processes, leading to growth defects, including temperature sensitivity. After 300 generations of laboratory evolution, *slx5Δ* strains exhibited restored growth, accompanied by a point mutation in *ULP2*, as revealed by whole-genome sequencing (WGS). This mutation in *ULP2* appears to be integral to the adaptive mechanisms compensating for defects arising from the loss of the STUbL complex. This suggests a functional interaction between the STUbL complex and Ulp2 in the regulation of SUMOylated proteins. Previous research has associated the Ulp2 desumoylation enzyme with the regulation of ribosomal DNA (rDNA). We aim to elucidate the mechanisms underlying the suppression of the slow growth phenotype in *slx5Δ* cells by the *ulp2Q838** mutation. This suppression highlights a compensatory pathway that mitigates the need for Slx5 in maintaining proteostasis and cell cycle progression. Understanding this compensatory mechanism provides significant insights into the coordination of SUMOylation and ubiquitination pathways in genome stability. Moreover, it reveals potential targets for genetic or pharmacological interventions in situations where proteostasis is compromised, such as in certain genetic disorders and cancers. This study contributes to our broader understanding of post-translational modifications in cellular regulation and emphasizes the importance of finely tuned interactions between SUMO proteases and ubiquitin ligases in maintaining cellular homeostasis.

Key words: SUMO, Slx5, Slx8, Ulp2, Dcp2

In vitro evolution of *Saccharomyces cerevisiae* in high- temperature and low-acidic condition

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Abstract

All living things have a willpower and ability to overcome stresses in the harsh surrounding conditions as the generation goes on to the extent that the condition is not that much severly life threatening.

Saccharomyces cerevisiae, which is known to grow best in the range of 20~32°C and pH4~6, is the best model to research how it overcome high temperature or low-acidic condition in that cerevisiae can grow about 6.9 generations a day.

Here, we conducted *in vitro* evolution (adaptive laboratory evolution) and analyzed what kind of change in genome has made it possible to overcome these conditons.

Tho2-mediated escort of Nrd1 regulates the expression of aging-related genes

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The relationship between aging and RNA biogenesis and trafficking is attracting growing interest, yet the precise mechanisms are unknown. The THO complex is crucial for mRNA cotranscriptional maturation and export. Herein, we report that the THO complex is closely linked to the regulation of lifespan. Deficiencies in Hpr1 and Tho2, components of the THO complex, reduced replicative lifespan (RLS) and are linked to a novel Sir2-independent RLS control pathway. Although transcript sequestration in *hpr1Δ* or *tho2Δ* mutants was countered by exosome component Rrp6, loss of this failed to mitigate RLS defects in *hpr1Δ*. However, RLS impairment in *hpr1Δ* or *tho2Δ* was counteracted by the additional expression of Nrd1-specific mutants that interacted with Rrp6. This effect relied on the interaction of Nrd1, a transcriptional regulator of aging-related genes, including ribosome biogenesis or RNA metabolism genes, with RNA polymerase II. Nrd1 overexpression reduced RLS in a Tho2-dependent pathway. Intriguingly, Tho2 deletion mirrored Nrd1 overexpression effects by inducing arbitrary Nrd1 chromatin binding. Furthermore, our genome-wide ChIP-seq analysis revealed an increase in the recruitment of Nrd1 to translation-associated genes, known to be related to aging, upon Tho2 loss. Taken together, these findings underscore the importance of Tho2-mediated Nrd1 escorting in the regulation of lifespan pathway through transcriptional regulation of aging-related genes.

Keywords: Nrd1; THO complex; aging-related genes; replicative lifespan; transcription.

Histone H3 lysine 9 tri-methylation is associated with pterygium

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Abstract

Pterygium, abnormal growths of conjunctival tissue onto the cornea, are common ocular surface conditions with a high risk of recurrence after surgery and potential ophthalmic complications. The exact cause of pterygium remains unclear, and the triggers are still unknown. In this study, we investigate the epigenetic profiles of patients with pterygium, focusing on histone H3 lysine 4 (H3K4) and lysine 9 (H3K9) trimethylation (me3). While H3K4me3 levels showed no significant genome-wide change, they were significantly altered in genes related to development and ocular diseases. Conversely, H3K9me3 levels were markedly elevated genome-wide, particularly at the promoters of 82 genes involved in developmental pathways. Furthermore, we identify six genes, *ANK2*, *AOAH*, *CBLN2*, *CDH8*, *CNTNAP4*, and *DPP6*, with decreased gene expression correlated with substantially increased H3K9me3, suggesting their potential as biomarkers for pterygium. This study represents the first report linking histone modification to pterygium progression, providing valuable insights into therapeutic strategies and potential drug targets.

Key words: Pterygium, Histone modification, Methylation, Chromatin immunoprecipitation (ChIP)

Auto-sumoylation of the Ubc9 E2 SUMO-conjugating Enzyme Extends Cellular Lifespan

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Calorie restriction (CR) provides anti-aging benefits through diverse processes, such as reduced metabolism and growth and increased mitochondrial activity. Although controversy still exists regarding CR-mediated lifespan effects, many researchers are seeking interventions that mimic the effects of CR. Yeast has proven to be a useful model system for aging studies, including CR effects. We report here that yeast adapted through in vitro evolution to the severe cellular stress caused by loss of the Ulp2 SUMO-specific protease exhibit both enhanced growth rates and replicative lifespan, and they have altered gene expression profiles similar to those observed in CR. Notably, in certain evolved ulp2Δ lines, a dramatic increase in the auto-sumoylation of Ubc9 E2 SUMO-conjugating enzyme results in altered regulation of multiple targets involved in energy metabolism and translation at both transcriptional and post-translational levels. This increase is essential for the survival of aged cells and CR-mediated lifespan extension. Thus, we suggest that high Ubc9 auto-sumoylation exerts potent anti-aging effects by promoting efficient energy metabolism-driven improvements in cell replication abilities. This potential could be therapeutically explored for the development of novel CR-mimetic strategies.

Exploring the Impact of Ubc9 Autosumoylation in Yeast Sumoylation Pathways

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Small ubiquitin-related modifier (SUMO) plays a crucial role in post-translational protein modification, impacting cell cycle, growth, and transcription. SUMO is deconjugated by two proteases, ulp1 and ulp2. Our previous research revealed that yeast with a deletion of ulp2 overcame growth defects over generations, highlighting changes in the enzyme Ubc9, critical to the sumoylation pathway. Through autosumoylation, Ubc9, an E2 ligase in the SUMO pathway, is known to positively affect the synthesis of sumo chains. Subsequent investigation revealed that Ubc9's autosumoylation is inhibited by a mutation that changes lysine residues to arginine. We used chromatin immunoprecipitation with SUMO-specific antibodies to investigate the effect of this mutation on gene sumoylation. Subsequently, we used qPCR to identify notable variations in sumoylation at different gene promoters. The genes impacted by the Ubc9-2KR mutation were identified by subsequent whole-genome ChIP-sequencing based on sumoylation levels. This study provides insight into the regulatory mechanisms of sumoylation and its effects on gene expression, highlighting the critical role of Ubc9 autosumoylation in the sumoylation pathway.

Investigating the role of FGF8 in porcine oocyte maturation and embryonic development

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Fibroblast growth factor 8 (FGF8), a pivotal cytokine in embryogenesis, is well-documented for its influence on murine oocyte maturation; however, its role in porcine oocytes remains to be elucidated. This study aimed to delineate the effects of FGF8 supplementation during in vitro maturation (IVM) on several key parameters, including nuclear maturation, intracellular glutathione (GSH) levels, reactive oxygen species (ROS) levels, cumulus expansion, and parthenogenetic activation (PA). Initially, immunohistochemistry (IHC) was employed to localize FGF8 and its receptor FGFR4 throughout porcine follicular development. FGF8 was detected in both somatic cells and oocytes at all follicular stages, whereas FGFR4 localization was confined to oocytes. Subsequently, porcine oocytes were matured in vitro with FGF8 concentrations of 0, 1, 10, and 100 ng/mL. Following 42 hours of IVM, the group supplemented with 100 ng/mL FGF8 exhibited significantly ($p < 0.05$) higher nuclear maturation rates compared to the control. Additionally, the 100 ng/mL FGF8 group demonstrated significantly ($p < 0.05$) elevated GSH levels, indicative of enhanced cytoplasmic maturation, and significantly ($p < 0.05$) reduced ROS levels compared to the control. Furthermore, supplementation with 100 ng/mL FGF8 significantly ($p < 0.05$) promoted cumulus expansion relative to the control, facilitating oocyte maturation. In PA, the blastocyst rates were significantly ($p < 0.05$) higher in the group treated with 100 ng/mL FGF8 compared to the control group. These findings suggest that FGF8 enhances both nuclear and cytoplasmic maturation of porcine oocytes, thereby improving embryonic development. Future research will focus on elucidating the mechanisms by which FGF8 enhances porcine oocyte maturation and embryonic development through comprehensive analysis of signaling pathways and mRNA expression profiles.

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NUCB1 is required for proper insulin signaling to control longevity in *Drosophila*

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NUCB1, also known as Nucleobindin-1 or Nesfatin-1, has been identified as a satiety molecule in various organisms, including mammals. However, the function of NUCB1 involved in aging has not been investigated. In this study, we found that NUCB1 knockdown flies show an extended life span and metabolic defects such as increased circulating carbohydrates, and starvation resistance. Drosophila is an excellent model for studying aging and age-related diseases because flies have a relatively short lifespan, with a typical life cycle of a few weeks. The aging process in Drosophila is intricate and depends on various genetic, cellular, and environmental factors Several critical pathways, including the Insulin/Insulin-like Growth Factor (IGF) signaling pathway, mechanistic Target of Rapamycin (mTOR) pathway, Sirtuin pathway, and Dietary Restriction (DR), have been identified as playing crucial roles in regulating aging in Drosophila. The messenger RNA expression levels of Drosophila insulin-like peptides (Dilps) were reduced in NUCB1 knockdown flies. Furthermore, the level of phosphorylated AKT, a downstream component of insulin signaling, was decreased in NUCB1 knockdown flies than in the control flies. Also, the nuclear localization of FOXO and its target gene expressions such as d4E-BP and InR were elevated. These findings suggest that NUCB1 controls life span by regulating insulin signaling in Drosophila.

Keywards: *Dilps*, *Drosophila melanogaster*, insulin signaling, longevity, NUCB1.

Tumor necrosis factor-inducible gene 6 protein promotes liver regeneration by enhancing lipid uptake in mice with partial hepatectomy

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Although liver transplantation (LT) is regarded as the only effective therapeutic option for end-stage liver diseases, it has been limited by the supply of donor organs. To address the shortage of donor, living donor-LT is being performed as an alternative to whole-organ LT. However, it also has several risks including possible complications in both donors and recipients, and specifically about 30% of donors are reported to suffer serious postoperative complications. Thus, liver regeneration without complication is necessary to both donors and recipients. Mesenchymal stem cells (MSCs)-derived secretome emerges as a promising therapy for liver disease. Tumor necrosis factor-inducible gene 6 protein (TSG-6), a cytokine released from MSCs, was shown to have hepato-protective effect in chronically damaged liver. Herein, we investigated the regenerative effect of TSG-6 and its underlying mechanism in mice with partial hepatectomy (PH). 8-wks-old male C57BL/6J mice receiving 2/3 PH were intraperitoneally injected with either PBS (CON group) as a vehicle or 50ng of recombinant TSG-6 (TSG-6 group) at the time of surgery, and then sacrificed at 0, 12, 24, 48 or 72h after PH to collect serum and liver. Enlarged and pale-colored liver with lipid accumulation started to be evident at 24h and 48h in the TSG-6 group and the CON group, respectively. TSG-6 treatment remarkably facilitated hepatocytes proliferation by upregulating cell cycle-related genes in PH-liver compared with PBS treatment. Hepatic functions, such as glycogen storage, albumin synthesis and detoxification, and serum levels of ALT/AST were also restored significantly earlier in the TSG-6 group than the CON group. Immunoprecipitation with TSG-6 coupled with LC-MS/MS showed that asialoglycoprotein receptor 1 (ASGR1) interacted with TSG-6 as a binding receptor in hepatocytes. ASGR1 being abundant in hepatocytes is known to decrease in proliferating hepatocytes. The TSG-6 group had significantly reduced levels of ASGR1 and proprotein convertase subtilisin/kexin type 9 (PCSK9) which is a ASGR1 downstream effector, 24h earlier compared with the CON group. Lipid uptake-related genes, *Cd36*, and *Ldlr*, which are degraded by PCSK9, were significantly elevated 24h earlier in the TSG-6 group compared with the CON group. Enhanced hepatic triglyceride level supported increased lipid uptake in the TSG-6 group. In addition, significant upregulation of ATP production in the TSG-6 group indicated that increased amount of lipid in the remnant liver of TSG-6-treated mice might be used in ATP production. These findings demonstrate that TSG-6 enhances lipid uptake by regulating ASGR1-PCSK9 signaling to provide energy needed in hepatocyte proliferation, and improves the hepatic regenerative capacity, suggesting TSG-6 as an effective therapeutic agent for liver regeneration in PH liver.

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Kewords: Liver regeneration, Partial hepatectomy, Tumor necrosis factor-inducible gene 6 protein, Asialoglycoprotein

TSG-6 alleviates alcohol-related liver disease by inhibiting CD44 cleavage in hepatic stellate cells

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Alcohol-related liver disease (ALD) is global prevalent chronic liver disease caused by excessive and/or habitual alcohol consumption. However, the effective and practical treatment against ALD has not been developed. Previously, we have demonstrated that tumor necrosis factor-inducible gene 6 protein (TSG-6), one of cytokines released from mesenchymal stem cells, reduces liver fibrosis and induces successful liver repair in mice with chronically damaged liver. TSG-6 was also shown to attenuate activation of hepatic stellate cells (HSCs), a main player in liver fibrosis, by interacting with cluster of differentiation 44 (CD44). CD44 is cleaved and releases its intracellular domain (ICD) fragment, which translocate into the nuclear and turn on fibrotic genes, contributing to liver fibrosis. However, it remains unclear whether and how TSG-6 has protective function in ALD. Hence, we investigated therapeutic effect of TSG-6 and its underlying mechanism in mice with ALD. 7-week-old male C57BL/6 mice were fed isocaloric pair diet (the CON group) or 5% (v/v) ethanol-containing Lieber-DeCarli liquid diet (EtOH group) for 12 weeks. At 9 weeks after the liquid diet, 1.5 μg/kg of TSG-6 (EtOH + TSG-6) or saline (EtOH + Veh) as vehicle was intraperitoneally injected every other day along with feeding liquid diet for 3 additional weeks. These experimental mice were sacrificed 12 weeks after the diet feeding to collect blood and liver samples. Human primary HSCs at 70% confluence were serum starved overnight and cultured in medium containing 2% FBS with 20ng/ml of TSG-6 or saline for 6hr, 12hr and 24hr. Chronic alcohol feeding increased liver weight/body weight (LW/BW) ratio and caused severe liver damage including fat accumulation, hepatocyte death, inflammation and fibrosis. However, the EtOH + TSG-6 group had significantly lower LW/BW ratio and less alcohol-induced liver damage than the EtOH +Veh group had. Moreover, TSG-6 treatment remarkably reduced liver fibrosis in EtOH treated mice. The both levels of full-length CD44 and ICD significantly increased in the EtOH + Veh group compared with the EtOH + TSG-6 group. The nuclear translocation of CD44ICD was also significantly reduced in TSG-6-treated alcohol-fed mice. Immunostaining for CD44 showed that CD44-positive HSCs-looking cells were more apparent in the EtOH + Veh group than the TSG-6 treated group. In addition, TSG-6 treatment significantly alleviated expression of CD44ICD and suppressed its nuclear accumulation in human primary HSCs, leading to HSC inactivation. These findings demonstrate that TSG-6 attenuates alcohol-induced liver damage and fibrosis, by blocking cleavage of CD44 to CD44ICD, and suggest that TSG-6 has therapeutic potential for ALD with fibrosis.

Keywords: Alcohol-related liver disease, Liver fibrosis, Tumor necrosis factor-inducible gene 6 protein, Cluster of differentiation 44

CREBBP and EP300 are gatekeepers to transforming the immune landscape during small-cell lung cancer development

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The heterogeneous landscape of genomic alterations in cancer is a major barrier to discovering pathways to malignancy, often precluding crucial insight into the development of preventive and therapeutic strategies. Here, functional characterization of 19 recurrently mutated genes in small-cell lung cancer (SCLC) using genetically engineered preneoplastic cells reveals that CREBBP and EP300 exert the strongest suppressor effects on both proliferation and immune evasion. A CREBBP/EP300-driven transcriptional program is sufficient and required for the expression of MHC-I presentation pathway components and other cell surface markers essential for T cells, natural killer cells, and macrophages, as well as for the expression of IL1RN, a key regulator of IL1 signaling. Alterations in this transcriptional program result in the depletion of cytotoxic immune cells, as well as the infiltration of monocytes with characteristics of myeloid-derived suppressor cells. This study thus provides a functional map of the SCLC tumor suppressor landscape, featuring CREBBP/EP300 as critical gatekeepers to the neoplastic transformation of the immune microenvironment. Understanding these mechanisms is vital for developing new therapeutic strategies to restore effective immune surveillance and combat tumor progression in SCLC. The implications of these findings extend beyond SCLC, offering broader insights into the mechanisms of immune evasion in cancer and potentially guiding future research and therapeutic approaches across various malignancies.

Key words: SCLC, CREBBP, EP300

Effect of endosomal LGR4 signaling on osteoclast differentiation

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LGR4 (Leucine-rich repeat-containing G-protein coupled receptor 4, also known as GPR48) is a membrane receptor and known as a negative regulator of RANK signaling cascade during osteoclast differentiation. Although, membrane receptor binding to its ligand enters the cells as an internalized endosome for a very short time, its role has not been elucidated to activate various pathways.
In this study, we confirmed the difference between membrane-bound LGR4 signaling and internalized LGR4, and whether the LGR4 signaling cascade gives RANKL signal in internalized endosomes as a positive regulator during RANKL-induced osteoclastogenesis.
Herein, we showed that LGR4 is endocytosed to endosome after binding to RANKL in osteoclast precursor cells RAW 264.7s. The internalized LGR4 activates LGR4-RANKL signaling in the early endosome. When RANKL is bound to LGR4, it is endocytosed and located in the Rab5 positive ealry endosome. In LGR4-down regulated RAW 264.7 cells, it was analyzed that the early endosome signal increased and the inhibitory phosphorylation of GSK-3beta decreased. Raw 264.7 cells treated with Dynasore (Dynamin inhibitor) confirmed the same reduction in inhibitory P-GSK3B as LGR4 CKO(Conditional Knock-out) cells. With similar results, it was confirmed that the reduced inhibitory P-GSK3B was recovered when DRG2 KO mice were treated with Dynasore, which is similar to the results of DRG2 WT mice. As a result of confirming NFATC1 nuclear translocation by RANKL treatment in LGR4 CKO Raw 264.7 cells and DRG2 KO mouse, nuclear translocation of NFATC1 increased in both groups. In addition, decreased bone density and increaesd TRAP activity in DRG2 KO mice which is known to be increased the early endosome duration were investigated.
Taken together, we conclude that internalized LGR4 plays an important role in the modulation of osteoclastogenesis via LGR4-RANKL signaling in endosomes.
Keyword : LGR4, RANKL, DRG2, Osteoporosis, Osetoclast

Crebbp drive expression of IL1RN regulating pro-tumorigenic microenvironment in Small Cell Lung Cancer.

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Small cell lung cancer (SCLC) frequently exhibits Crebbp mutations, and recent studies underscore the critical role of the tumor microenvironment in cancer progression. However, research on the tumor microenvironment in SCLC remains limited. IL-1β, a major proinflammatory cytokine, has been shown to promote tumor progression in other cancers, such as breast cancer, by affecting immune cell infiltration and cytokine signaling. We noted a consistent decrease in expression of Il1rn encoding interleukin 1 receptor antagonist (IL1RN). We found that lentiviral restoration of IL1RN in Crebbp-mutant preSCs inhibited growth of subcutaneous tumor in B6 mice. Likewise, treatment of anakinra reduced the ability of Crebbp-mutant preSCs to form subcutaneous tumor. Furthremore, the tumorigenic capacity of Crebbp-mutant preSCs was significantly reduced in the flanks of Il1β $^{-/-}$ mice than did in those of Il1β $^{+/+}$ mice, and ablation of the receptor IL1R1 drastically reduced tumor development in Il1r1$^{-/-}$ RPR2 mice compared to Il1r1$^{+/+}$ RPR2 mice. Our study identifies CREBBP's impact on the tumor microenvironment, revealing that it regulates IL1RN expression, and we also show the progression of SCLC can be attenuated by anakinra(recombinant human IL1RN). Theses findings highlight the role of IL-1b signaling in modulating tumor microenvironment and provide new therapeutic targets

Key words: “Crebbp”, “IL1RN”, “SCLC”

Establishment of an embryo model to study wound healing mechanism

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The wound healing process in adult skin results in an accumulation of extracellular matrix and altered tissue patterns, which are called ‘scar’. The main goal of wound repair studies is rapid wound closure without scar formation. However, it is difficult to figure out which factors affect different cellular events of wound repair due to the complicated and mixed action of different cell types (e.g. fibroblasts, immune cells, and endothelial cells) and factors (transcription factors and secreting molecules). In this study, we aim to establish an embryo-based wound healing research model using early chick embryos. Unlike adult systems, the developing embryos show remarkably rapid wound healing without scar formation. The chick embryo has several advantages as a wound healing research model: it can be cultured in vitro with minimal effort and has simple cell layers as well as easy access to multiple developmental stages. Using embryological methods, we develop methods in which we can generate artificial circular wounds with predictable sizes by using modified glass microneedles in different regions of the blastoderm. Based on morphological changes, we subdivide the wound healing process into four phases. Additionally, by using whole-mount in situ hybridization, we show some evidence that similar gene sets that are expressed in embryonic edges of epibolic movement are shared during embryonic wound healing, suggesting a possible similar mechanism between developmental tissue closure and the wound healing process.

Kew words: chick, wound healing, embryonic wound, tissue closure

BCAT1 promotes protein translation and mitochondrial respiration in small-cell lung cancer

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Small cell lung cancer (SCLC) is a tumor characterized by rapid onset and a high propensity for metastasis. In a previously study demonstrated that the development of SCLC driven by *Mycl* depends on enhanced ribosome biogenesis and protein translation, and is sensitive to inhibition of RNA Polymerase I. we found that comparative profiling of precancerous cells and cells transformed by *Mycl* revealed *branched chain aminotransferase 1* (*Bcat1*) as one of the most up-regulated genes in the transformed cells. We confirm that BCAT1 protein levels are elevated in mouse SCLC cells relative to precancerous cells, and that BCAT1 is also expressed in a subset of human SCLC cells Interestingly, and we present data that suggest that BCAT1 increase global protein translation. These novel findings suggest that the elevated expression of BCAT1 supports the growth of SCLC, potentially by promoting protein translation. Therefore, our findings are significant in supporting the concept of targeting BCAT1 as a novel and particularly valuable therapeutic strategy for SCLC, offering new avenues for treatment and research aimed at improving patient outcomes and understanding SCLC biology more deeply.

Key words: BCAT1, Mycl, SCLC

Protective Effects of N-acetylcysteine, Parthenolide, and 3-Methyladenine Against BBP-Induced Male Germ Cell Toxicity

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Abstract

Benzyl butyl phthalate (BBP) is a commonly used phthalate ester with potential health risks, including various diseases. Whereas extensive research has focused on its effects on the reproductive system, its direct molecular impacts are less understood. This study investigated BBP's toxicological effects on GC-1 spermatogonia cells, focusing on cell proliferation, reactive oxygen species (ROS) generation, apoptosis, autophagy, and key kinase signaling pathways. BBP exhibited concentration-dependent toxicity with an IC_{50} of 53.9 μM. At 50 μM, BBP significantly increased ROS production and apoptosis. Western blot analysis revealed BBP-induced activation of both intrinsic and extrinsic apoptotic pathways proteins. BBP also triggered autophagy, indicated by the increased presence of autophagic vesicles and upregulation of ATG5 and ATG7, alongside decreased phosphor-p62 levels. Furthermore, BBP disrupted the PI3K-AKT-mTOR pathway, reducing the phosphorylation of PI3K, AKT, and mTOR, while increasing ERK and p38 MAPK phosphorylation and decreasing JNK phosphorylation. A triple combinatorial treatment with parthenolide, N-acetylcysteine, and 3-methyladenine was explored to mitigate BBP-induced toxicity. This combination treatment substantially restored cell proliferation and the expression level of apoptosis, autophagy, and proliferation regulatory proteins. These findings provide fundamental insights into BBP-induced male germ cell toxicity and suggest potential therapeutic strategies for mitigating phthalate-induced cellular damage.

Key Words: Benzyl butyl phthalate, GC-1 spg cells, Reactive oxygen species, Apoptosis, Mitigation strategy

Acknowledgment

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Combination Treatment Strategies to Alleviate Toxicity Induced by Phthalate Esters Mixture in Male Germ Cells

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Abstract

Phthalate esters (PAEs) are ubiquitous environmental contaminants with potential health hazards. Due to various types of PAEs, humans are exposed to multiple PAEs in mixed forms, leading to significant health concerns. The impact of diverse phthalate exposure on male germ cells remains unclear and requires further investigation. This study examined the cytotoxic effects of a mixture of PAEs (MP) on GC-1 spermatogonia (spg) cells. MP inhibited GC-1 spg cell proliferation in a concentration-dependent manner, with an IC_{50} value of 16.9 μg/mL. At 25 μg/mL, MP significantly induced reactive oxygen species generation and apoptosis. MP activated both intrinsic and extrinsic apoptotic pathways, shown by increased levels of BAX, cytochrome c, cleaved-caspase 9, FAS, cleaved-caspase 8, and cleaved-PARP. MP exposure also substantially activated autophagy, evidenced by increased red-to-green fluorescence intensity ratio, Beclin, ATG7, and LC3 II/I expression, and decreased p62 phosphorylation. Additionally, MP inhibited the phosphorylation of key kinases in the PI3K/AKT/mTOR signaling pathway, essential for cell proliferation and survival. Treatment of parthenolide, N-acetylcysteine, and 3-methyladenine reduced cell proliferation inhibition, decreased apoptosis and autophagy markers, and increased phosphor-mTOR levels, suggesting a protective synergistic effect. These findings offer new insights into MP-induced male germ cell toxicity and highlight the potential therapeutic efficacy of the triple inhibitor combination in mitigating male germ cell toxicity from diverse PAEs exposure.

Key Words: Mixture of PAEs, GC-1 spg cells, Apoptosis, Autophagy, alleviation strategy

Acknowledgment

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (NRF-2018R01A6A1A03025159), and by an NRF grant funded by the Ministry of Science and ICT (RS-2024-00347342).

Xenopus as an alternative model to study CHIP (Clonal Hematopoiesis of Indeterminate Potential)

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Clonal hematopoiesis of indeterminate potential (CHIP) is a condition that contains a subpopulation of mutated hematopoietic stem cells (HSCs) or blood progenitor cells without hematologic malignancy. At the beginning of the CHIP condition, only a few parts of HSCs or progenitors are mutated with exposure to risk factors. As time passed, CHIP contributes to giving rise to hematologic diseases such as acute myelogenous leukemia (AML) and atherothrombotic risk. *Xenopus laevis* has been used as an animal model system for developmental biology. *Xenopus* embryos have a short period for development and are easy to manipulate genetic characteristics. A subset of cells in developing embryos can be targeted for generating mutations according to the cell fate map, similar to the case of CHIP. In this study, we used *Xenopus laevis* as an alternative model system to study CHIP and leukemia. Using the CRISPR system, mutation on genes related to hematologic malignancies such as Tet2 and DDX41 was induced in *Xenopus* embryos. In this procedure, we injected CRISPR into only a subset of the blood-lineaged cells to obtain a CHIP-like developmental process. We obtained embryonic blood cells and confirmed blood cell phenotype with Giemsa-Wright staining and quantitative PCR using blood cell markers discovered through *Xenopus* blood single-cell sequencing. We found out that abnormal blood phenotypes were detected in some embryos, and further studies are undergoing.

Keyword : blood cell, CHIP, leukemia, *Xenopus laevis*, animal model system

The effect of YAP signaling on tenogenic differentiation

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Hippo signaling is one of the powerful pathways that regulates numerous biological processes, and it is involved in various physiological functions such as cellular proliferation, organ formation, tissue regeneration, and wound healing. According to previous research, Yes-associated protein (YAP), a downstream effector of the Hippo pathway, is known to significantly impact the tenogenic differentiation of mesenchymal stem cells. Through other research, we were able to set the conditions for tenogenic differentiation of BMSCs (Bone Marrow Mesenchymal Stem Cells). To determine whether this tenogenic differentiation is influenced by YAP, we treated Verteporfin, which is a drug that inhibits the nuclear localization of YAP/TAZ. We could also confirm that tenogenic differentiation did not proceed properly under Verteporfin's condition. During the tenogenic differentiation of BMSC, mature tenocyte marker COL1A1 is significantly decreased in the Verteporfin-treated condition compared with CTL. However, in the case of expression levels of tenocyte progenitor markers like SCX and TNMD did not decrease but instead showed an increase when treated with Verteporfin. Additionally, when comparing the expression levels of FAT Atypical Cadherin (FAT), which is an upstream regulator of YAP/TAZ signaling. Especially, FAT2 expression level is significantly high compared with other FAT family genes. So, we could suppose that FAT2 has crucial roles in the tenogenic differentiation process. We performed a cross-check of BMSC data with Xenopus laevis embryo. In drug treatment conditions, the tenocyte progenitor marker TNMD is deceased in YAP inhibition status and has increased in YAP activation status. Also, when we induce YAP activation by knocking out the FAT family at Xenopus laevis embryo, it shows a decrease in TNMD expression level. Based on these results, we thought YAP signaling may play a crucial role in switching between tenocyte progenitor cells and mature tenocytes. Also, by studying the impact of YAP signaling on tenogenesis, we expect to reveal that YAP could play a key role in tendon regeneration research.

Keyword : YAP, Tenogenic differentiation, FAT, BMSC, *Xenopus laevis*

Identification of novel chondrogenic gene using *Xenopus* model system

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Introduction : The aging population has led to a growing prevalence of elderly individuals suffering from joint-related ailments such as arthritis. Consequently, the demand for appropriate treatment and strategies has become imperative. Currently, the precise causes and mechanisms underlying arthritis remain elusive, resulting in a focus primarily on palliative pain relief through analgesics and anti-inflammatory agents, as fundamental therapeutic interventions are lacking.

Inflammatory responses trigger a detrimental cycle in cartilage tissues, whereby various matrix degradation byproducts released during inflammation exacerbate the secretion of cartilage matrix-degrading enzymes, leading to deterioration. This process involves the interaction of cartilage matrix fragments with surrounding synovial and immune cells via integrins. Previous research has validated enhanced cartilage formation and regenerative potential through the inhibition of integrin signaling mediated by the ITGBL1 gene.

Method : Comparing the gene expression profiles from RNA-seq between 5-day and 10-day, and 5-day and 15-day intervals for each sample, genes exhibiting consistent alterations in expression were identified. To validate the cartilage developmental potential of the candidate gene cluster, a dual approach involving the Xenopus model and micromass culture system employing MSCs was employed. This combination facilitated a comprehensive assessment of the identified genes' contribution to cartilage formation.

Results : in situ hybridization and CRISPR/Cas12a(Cpf1) system reveals cartilage expression pattern and cartilage disruption phenotype of candidate genes. Also, siRNA treated micromass culture presented difference of cartilage development compare to control BMSC.

Conclusion : In this study, a specific group of genes exhibiting increased expression levels during the cartilage formation enhancement process induced by ITGBL1 overexpression is identified. Through the Validation of the Candidate Gene Cluster, we aim to unearth novel genes associated with cartilage development. This exploration holds the potential to identify targets for recovering cartilage damage caused by arthritis.

Key words : Chondrogenesis, CRISPR/Cas12a, Osteoarthritis

Evaluation of developmental toxicity and teratogenicity of 1-Hydroxypyrene (1-hp) using the frog embryo teratogenesis assay-*Xenopus* (FETAX)

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1-hydroxypyrene is an indicator of exposure to polycyclic aromatic hydrocarbons (PAHs) that are one of the atmospheric pollutants. It is known that high concentrations of 1-hydroxypyrene can be found in urine of outdoor workers exposed to air pollution, causing various diseases such as skin cancer, lung cancer, renal cell carcinoma. There are many studies on respiratory diseases and cancers caused by 1-hydroxypyrene, but there is a lack of research on the eye. In this study, we investigated the adverse effects of 1-hydroxypyrene on organogenesis including the eyes using a frog embryo teratogenesis assay-*Xenopus* (FETAX). For morphological analysis, Xenopus laevis embryos were exposed to 1-hydroxypyrene at various concentrations, and for genetic analysis, embryos at the affected concentrations were subjected to real-time qPCR and whole-mount *in situ* hybridization (WISH). Embryos exposed to 10 μM developed malformations including reduced length, edema, small eye, gut miscoiling, and cardiac edema. Genetic analysis revealed that 1-hydroxypyrene reduced the expression of eye-related marker genes such as *rx1*, *pax6*, and *six3* as well as various organ markers genes such as *myh6*, *darmin*, and *ldlrap1*. In conclusion, our study shows that 1-hydroxypyrene affects gene expression during *Xenopus* embryonic development, leading to developmental toxicity and teratogenicity.

Key words : Xenopus, 1-hydroxypyrene, FETAX, Teratogenicity, eye development

Functional study of Lysine specific demethylase 5C(KDM5C) – Pecam1 interaction during *Xenopus laevis* embryogenesis

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Lysine-specific demethylase 5c (also known as KDM5C) is a member of the KDM family and is specific for di- and tri-demethylation of lysine 4 residues of histone 3 (H3K4 me2/3). Kdm5c is known to be involved in the development of sex chromosome-linked retardation (XLMR) and cancers as histone demethylase. In particular, mutations in Kdm5c slow down brain development, causing abnormal social behavior and nervous system abnormalities. While the studies of Kdm5c as histone demethylase have been firmly established, the function of Kdm5c targeting non-histone proteins is unexplored. Based on this, to study the developmental role of KDM5C and present a therapeutic perspective for cancer, a deeper understanding of the target genes that are non-histone proteins and interact with Kdm5c is required. In this study, we identified Platelet endothelial cell adhesion molecule (also known as Pecam1) as a new binding partner of Kdm5c and investigated their relationship using *Xenopus laevis* embryos. Kdm5c and Pecam1 were found to bind and interact with each other. Additionally, loss function study using morpholino oligonucleotide showed that *Pecam1* knockdown resulted in a small head and cartilage and reduced eye size, consistent with phenotype of *kdm5c* morphants. Kdm5c and Pecam1 were overexpressed to examine their location through immunofluorescence, and confocal data showed that Kdm5c overexpression shifted Pecam1 from around the nucleus to nuclear envelope. Our results revealed that Kdm5c directly binds with Pecam1 and they may cooperate during *Xenopus* embryogenesis. Further study is required to investigate functional mechanism of their interaction in detail.

Key words: Kdm5c, Pecam1, protein interaction, Xenopus laevis

KDM4A is important for eye and brain development in *Xenopus laevis* embryos

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Histone methylation is one of the important post-translational modifications that increases or decreases the transcription of genes by modifying the morphology of nucleosomes without affecting DNA directly. KDM4A is a histone demethylase and is known to regulate transcription by demethylating the di- and tri-methyl groups of histone 3 lysine 9 and histone 3 lysine 36. However, the physiological role of KDM4A is not well known, so we investigated its developmental role using *Xenopus laevis* and identified TRiC/CCT as a novel binding protein of KDM4A. The group II chaperonin TRiC/CCT in eukaryotes assists in the folding of important structural and regulatory proteins. TRiC consists of eight subunits, each of which plays a specific role in TRiC/CCT assembly, allosteric regulation, protein recognition, and folding. In this study, we elucidated that KDM4A is maternal gene and is expressed in brain, branchial arches, and in the lens and retinal regions of the developing eye. TRiC/CCT is also expressed in brain, neural crest, and eye, consistent with the expression pattern of KDM4A. Loss-of-function analysis using morpholino oligonucleotides showed that malformed eyes and reduced-sized head in KDM4A morphant embryos. CO-IP analysis indicated that, KDM4A interacts with subunits of TRiC/CCT. Treatment of *Xenopus* embryos with TRiC/CCT inhibitor decreased endogenous expression amount of KDM4A in a dose-dependent manner. In addition, the neural tube of *Xenopus* embryos was less closed as the concentration of TRiC/CCT inhibitors increased. In conclusion, this study provides the possibility that KDM4A is important for eye and brain development in *Xenopus laevis* embryos not only by participating in histone demethylation but also by interacting with TRiC/CCT, which is involved in protein folding.

Key words : *Xenopus laevis*, KDM4A, TRiC/CCT, eye, brain

The roles of RIP1 and RIP3 during vertebrate embryonic development

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Receptor-interacting Serine/Threonine kinase 1 (RIP1) also known as adaptor kinase is involved in a variety of signaling pathways such as NF-κB activation, apoptosis and necroptosis which is referred to as RIPK1/RIPK3 mediated necrosis. During FADD and caspase-8 deficient conditions, TNF-mediated apoptosis is inhibited and RIP1 associates with RIP3 to form a necrosome. Mixed lineage kinase domain-like protein (MLKL) interacts with this complex and activates mitochondrial phosphatase PGAM5. Activated PGAM5 in turn dephosphorylates mitochondrial fission factor (Drp1) and results in cell necroptosis. In the current study, we have investigated the developmental functions of RIP1 and RIP3 employing *Xenopus laevis* embryos. RT-PCR analysis revealed the maternal nature of *rip1* during *X.laevis* embryogenesis with downregulated expression of RIP1 during gastrulation, but this low level of expression was recovered during neurula stage of embryonic development. Our whole mount *in-situ* hybridization studies indicated the specific expression of *rip1* and *rip3* in central nervous system and somites of developing *Xenopus* embryos. The knockdown of *RIP1* and *RIP3* using antisense morpholino resulted in shortened body axis and small size head as compared to the control embryos. Surprisingly the overexpression of RIP1 in the *X.laevis* embryos showed remarkably different phenotype i.e., cell dissociation. On the contrary, no such events were observed during RIP3 overexpression. In addition, we treated RIP1 overexpressed embryos with inhibitors of different factors involved in the regulation of apoptosis and necroptosis. Cell dissociation induced by RIP1 overexpression in *X.laevis* embryos was rescued by necrostatin-1 but not by any other inhibitor. In conclusion, our results suggest the novel roles of RIP1 and RIP3 during vertebrate embryogenesis and give a possible perception of a new function of RIP1 in necroptosis independent of RIP3.

Key words : *Xenopus laevis*, RIP1, RIP3, Necroptosis

Physiological function of *kdm1a* on antero-posterior axis development during *Xenopus* embryogenesis

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Lysine specific demethylase 1A (KDM1A or LSD1) is the first discovered enzyme that removes methyl group from the histone H3 protein, specifically both histone 3 lysine 4 and histone 3 lysine 9 mono-, di-methylation (H3K4me1/2, H3K9me1/2) and it is known to regulate both gene activation and suppression via modulating H3K4 and H3K9 methylation status. Even though LSD1 was first known to regulates histone methylation, it was revealed to modulates not only histone proteins but also non-histone protein methylation such as P53, SOX2 and FOXA1. In this study, we focused on the developmental role of lsd1 and its non-histone target proteins using *Xenopus* embryos. Morpholino oligonucleotides (MO) mediated lsd1 knockdown in *Xenopus* embryos induced bent axis which represents the defect of convergent extension movements. Moreover, *lsd1* morphants were rescued by injecting exogenous wild type dishevelled (*dvl*) implies *lsd1* is closely related to the Wnt signaling pathways. To investigate non-histone target protein of LSD1, we performed Mass Spec analysis following LSD1 immunoprecipitation and we found that some of candidates were physically interacted with the LSD1 indeed and its spatial expression overlaps with *lsd1*. Taken together, our results indicated the significance of this epigenetic regulators during embryonic development together with identification of its regulatory mechanism.

Key Words: *Xenopus*, KDM1A, Development, Wnt

Morphological and Histological Study of Whole brain and Ventral Nerve Cord Regeneration in the earthworm, *Perionyx excavatus*

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The earthworm*, Perionyx excavatus* is capable of bidirectional regeneration of head and tail and considered as a new model system due to its properties such as, complete regeneration with reconstructing of central nervous system and circular system. In this study, we investigated the structure of brain and ventral nerve cord in *P. excavatus* and investigated the regenerating process of each organ through H&E staining, immunostaining and micro-CT imaging. The brain is bilobed mass lying above pharynx in the 3 to 4 segments. At 1 days post extraction of brain, some cells migrated and filled the removed site. At 3 to 5 days, a small ganglion was observed at the extracted site. After 7days, the ganglion exhibited growth and brain was completely regenerated. The ventral nerve cord runs in ventral midline to end of tail and possesses segmental ganglion. Our results revealed that tissues occupied the excision site within 1 and 3 days post extraction similar to brain regeneration. From the 3 dpe, nerve fibers were observed to emerge from both sides of the excision site. On the 5dpe, the nerve fibers were completely connected and after 7 days, the newly regenerated nerve fibers were distinct and, the regeneration of VNC was complete. This study provides fundamental data for understanding differentiation in regeneration.

Keywords : *Perionyx excavates*, regeneration, H& E staining, immunostaining, micro-CT imaging

The knockdown of CG12299, microRNA-967 target, alleviates Tau pathology in a *Drosophila* model of Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disorder marked by the hyperphosphorylation of Tau, resulting in neuronal dysfunction. To identify enhancers of Tau-related pathology in AD, we conducted a microRNA sponge line screening using *Drosophila* model. Our findings revealed that the miR-967 sponge line exacerbated phenotypes associated with human Tau (hTau), while miR-967 alone ameliorated these hTau-induced phenotypes. Among the target genes of miR-967, the knockdown of CG12299, an orthologue of a human transcription repressor gene BCL6B, exhibited a rescue effect similar to that of miR-967. Our results demonstrate that *CG12299* knockdown rescued pathological symptoms in the fly hTau AD model, including reduced locomotion, impaired neuromuscular junctions and decreased lifespan. Notably, *CG12299* knockdown reduced both total Tau levels and hTau phosphorylation, implicating its role in hTau protein degradation. This study suggests that the knockdown of *CG12299*, a target of *miR-967*, alleviates pathological symptoms in a *Drosophila* hTau AD model by promoting hTau protein degradation, underscoring its potential as a therapeutic target for AD.

Interleukin-7-induced inner cell mass growth promotes pluripotency of porcine embryonic stem cells through oxidative phosphorylation

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Interleukin-7 (IL-7) plays a crucial role in cellular development, proliferation, and viability. While its significance in porcine oocyte maturation has been elucidated, its influence on embryonic development remains to be fully characterized. Here, we aimed to elucidate whether IL-7 supplementation increases inner cell mass (ICM) formation in blastocysts, thereby enhancing the efficacy of porcine embryonic stem cell (pESC) derivation. We initially evaluated the effects of IL-7 and fetal bovine serum (FBS) supplementation *during in vitro* culture (IVC) following parthenogenetic activation (PA) on porcine blastocyst formation and ICM development. The synergistic application of IL-7 and FBS yielded a significantly ($p < 0.05$) higher proportion of hatched blastocysts compared to the FBS group. Furthermore, our findings demonstrated that IL-7 and FBS supplementation during IVC significantly modulated the expression of the ICM marker SOX2+ cells, altered the ICM ratio, and regulated phosphorylated AKT (pAKT) expression. Subsequently, we assessed pESC establishment efficiency utilizing exclusively hatched blastocysts from each group. The group treated with IL-7 + FBS showed a significantly ($p < 0.05$) higher proportion of colony-forming blastocysts compared to the FBS group. The pESCs obtained from blastocysts treated with IL-7 (IL-7-pESCs) showed a significantly ($p < 0.05$) increased expression of key pluripotency factors (*OCT4*, *SOX2*, and *NANOG*) and genes related to proliferation (*LIN28A*, *C-MYC*, *ETV4*, and *ETV5*). Interestingly, transcriptomic analysis revealed that IL-7-pESCs exhibited significantly enhanced oxidative phosphorylation activity, a finding that was further validated by qPCR results. In conclusion, our findings demonstrate that IL-7 supplementation enhances the ratio of ICM during porcine embryonic development *in vitro*. As a result, this leads to pESCs with increased pluripotency, which is mediated through enhanced oxidative phosphorylation. These insights may contribute to the establishment of a robust core pluripotency, which could potentially expand the research applications of pESCs.

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Key words) *in vitro* culture, porcine embryos, interleukin-7, porcine embryonic stem cells, oxidative phosphorylation

Estrogen regulates *PRNP* gene expression during estrus cycle

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Prnp is expressed in most of the cell types and suspected that the expression of the prion protein gene (*Prnp*) is highly regulated during development and plays an important role. It is also known that there is gender difference in outbreak of prion disease. However, the tissue specific or hormonal specific regulation is not much unmasked. To understand the expression regulation of *Prnp* by estrogen, the expression profiles and methylation patterns in upstream region of translation starting site of *Prnp* genes were examined in estrogen target organs, ovaries and uterus with various models. The organs were collected according to the estrus cycle. The tendency of *Prnp* mRNA expression was identified by treating E2 (2ug/kg body weight), P4 (40ug/kg body weight), and ICI 182,780(1mg/kg body weight), RU486(8mg/kg body weight) in ovariectomized mice. Real-time PCR and Western blotting methods were employed to analyze the expression levels. The degree of epigenetic modification and methylation of the genomic region of interest was evaluated. The expression levels of *Prnp* mRNA was changed by the estrous stages. Based on the preliminary studies it is suggested that the expression *Prnp* in estrogen target tissues is under estrogen.

Key words: Estrogen, PRNP, Methylation, Prion Protein, CpG island.

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Expression of Steroidogenic enzymes in the mouse uterus during decidualization

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Systemic sex steroid hormones control the decidualization during pregnancy. It is also suggested that local steroidogenesis exist during implantation at decidua. Steroidogenic enzymes carry out steroid metabolism. So far, the expression of several steroidogenic enzymes has been identified in the mouse and human uterus. However, the steroid metabolic mechanisms that promote stromal differentiation and endothelial proliferation during decidualization are not fully understood. Using Real-time PCR, Western blot, and LC-MS/MS analyses, we identified the expression profiles of steroidogenic enzymes during the decidualization process in vitro decidualization model and in the pregnant uteri. The expression levels of Cyp11a1, Hsd11b1, and Hsd11b2 specific mRNAs were significantly higher than that of Cyp19a1 mRNA in the decidua at gestation day 7. In vitro decidualization model the expression levels of Cyp19a1 gradually decreased by decidual induction. On the other hand, the expression levels of Hsd17b2 were increased by decidual induction. By evaluating the mechanisms of steroid hormone metabolism during the decidualization process, we demonstrate that steroid hormones can be synthesized and metabolized locally in the uterus, and these studies will help identify the causes of several endometrial diseases associated with steroid metabolism abnormalities and disturbing by exogenous substances.

Keywords: Decidualization, Steroid metabolism, Cyp19a1, Hsd17b2, Sult1e1

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A-31

Identifying a spliced beta-catenin during decidualization

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Abstract

Catenin beta-1 is a multifunctional protein that plays critical roles in cell adhesion and signaling. Although it is first identified as a component of a cell adhesion complex, it is now recognized as a central component of the developmentally important Wnt pathway. However much of the interest in β-catenin has focused on its function as a coactivator of transcription. Constitutive binding of β-catenin to LEF/TCF contributes to the development of many types of human cancer, and much effort has been devoted to the identification of mechanisms regulating the cellular concentration and localization of β-catenin. So far, the spliced forms of β-catenin is reported in several species, but is not well studied in mouse. In this study, we designed to identify the spliced β-catenin, to profile the expression patterns of a spliced β-catenin during pregnancy and identify the possible interacting molecules in decidual cells. The expected spliced form in mouse is 3 or 4. b-catenin was localized in epithelial cells in undifferentiated uterus but its expression was extended to the decidua. This work will be supporting the extension of the knowledge in uterine stromal cell proliferation and differentiation.

Keyword: beta-catenin, decidualization, prion, alternative splicing

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Roles of Spliced Yes-associated protein (YAP) during Decidualization

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The decidualization is the process in which functional and morphological changes within the endometrium. It forms decidual lining into the blastocyst implants and is a necessary process for a successful pregnancy. So far, eight alternative splicing forms of YAP have been identified in humans and seven alternative splicing forms have been identified in mice. Although Yes-associated protein (YAP), an effector of the Hippo signaling pathway, is suggested as a required molecule for the decidualization, the molecular mechanisms are not much unmasked. To evaluate the molecular mechanisms of YAP in endometrial differentiation, alternative spliced YAP was studies. Immunoprecipitation, Western blotting, LC-MS/MS analysis were employed to evaluate the expression profiles in the pregnant uteri from day 1 to day 7 of gestation. To determine which of the spliced YAP worked during stromal differentiation, deglycosylating methods was employed. Four distinct bands (70 kDa, 60 kDa, 55 kDa, 30 kDa) were detected during pregnancy. Their expression were dramtically increased in the area of decidual differentiation and showed peak at day 7 of gestation. At day 7 of gestation, the expression levels were significantly highest at the implantation sites than those of the inter-implantation sites especially the band corresponding to 55 kDa. Additional studies are needed and it will be identify the specific isoform of YAP that plays a role in decidualization and be helpful to overcome problems in diseases concerned with decidualization failure.

Keywords: Decidualization, YAP1, Hippo pathway

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DAZL protein during decidualization in mice endometrial stromal cell

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Abstract

Decidualization is a process that results in functional and histological changes in endometrium, which induced by steroid hormone in preparation for pregnancy. These include decidual differentiation of endometrial stromal cells (ESCs), the presence of uterine natural killer cells, and a dramatic angiogenesis. In some mammalian species, decidualization is triggered by attachment of embryo but others are not. It has been suggested that decidualization is a factor for embryo invasion and can be cause of miscarriage. The misregulation of gene expression during gametogenesis contributes to 12%–15% of infertility in couples worldwide. The *Daz* (Deleted in AZoospermia) gene family encodes potential RNA binding proteins that are expressed in prenatal and postnatal germ cells of males and females. The genes of the *Daz* family encode proteins with a highly conserved RNA-binding motif and a unique *Daz* repeat. The protein encoded by this gene is localized to the nucleus and cytoplasm of some cells such as fetal germ cells and to the cytoplasm of developing oocytes. Evidence to date suggests a potential role for members of the DAZL protein family as a regulator of mRNA translation. Studies have shown that increased expression of *Dazl* is associated with induction of *in vitro* differentiation. So far, the expression and functions of *Dazl* gene are not known in pregnant uterus. The expression regulation of the *Dazl* was examined in this study. In the expression profiles of mRNA and protein showed the control of estrogen. Although future studies are needed to evaluate the expression regulation mechanism and the possible roles in pregnancy, the results suggested that *Dazl* has some role in decidualization.

Key words: Dazl, Decidualization, Stromal cell, estrogen

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Anxa1 is required for ciliogenesis during vertebrate development

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Annexin1 (Anxa1) belongs to the annexins superfamily of structurally related proteins that binds to phospholipids membranes in a Ca^{+2}-dependent manner using their core domain. Anxa1 is cytosolic and is also found in association with membranes of the cytoskeleton. It was initially discovered as a mediator of anti-inflammatory functions of glucocorticoids but is also involved in many different physiological processes such as cell growth, differentiation, and intracellular vesicle trafficking. Anxa1 is well studied for inflammations but the developmental roles of Anxa1 are poorly understood and need further investigations. In the present study, we have focused on the roles of Anxa1 in vertebrate development particularly its significance in ciliogenesis using *Xenopus leavis* and human retinal human retinal pigment cell (RPE1). Our RT-PCR results indicated that *anxa1* is expressed during early embryonic stages ranging from single cell to tadpole stage of development. Moreover, mRNA expression of *anxa1* is observed in both muticiliated cells of epithelial and cement gland at tadpole stage of development. The knockdown of *anxa1* mediated by anti-sense morpholino led to the shortening of anterior-posterior axis as well as defective ciliogenesis in motile cilia during *Xenopus* embryogenesis. In addition, we observed that ANXA1 also influenced the primary cilia of human RPE1-cells and the knockdown of *ANXA1* using siRNA resulted in a reduction in the number of cells having primary cilia. Furthermore, we found that *ANXA1* knockdown caused the shrinkage of intraflagellar transport protein 20 (IFT20) which is essential for vesicle trafficking during ciliogenesis. In conclusion, we suggest that Anxa1 is crucial for ciliogenesis during vertebrate development and further in-depth analyses are required to uncover the molecular mechanism involved in the regulation of ciliogenesis by Anxa1.

Key words : Annexin A1, ciliogenesis, multiciliated cells, *Xenopus laevis*, RPE1

Defining the Notch Center: Spatiotemporal Expression Patterns of Notch Signaling in the Embryonic Development of the Freshwater Leech *Helobdella austinensis*

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Intercellular communication is highly conserved and crucial for the development of multicellular organisms. The Notch signaling pathway, a highly evolutionarily conserved mechanism, plays an essential role in the development and homeostasis of various tissues and organs across many multicellular organisms. In this study, we examined the Notch signaling pathway in the freshwater leech, *Helobdella austinensis*, an annelid model particularly useful for studying lophotrochozoan segmentation. With the recent completion of the whole genome sequencing of this species, we conducted a detailed study of Notch pathway components. We analyzed the expression patterns of the Notch receptor (*Hau-Notch*) and its ligands, Serrate (*Hau-ser*) and Delta (*Hau-delta*), as well as potential Notch target genes, including *Hau-hey* and *Hau-hes2*, using whole mount in situ hybridization in leech embryos at embryonic stages 8-11. At stage 8, the receptor *Hau-Notch* and ligands *Hau-ser* and *Hau-delta* show co-localized expression in the anterior part of the germinal plate and an additional anterior circle of cells surrounding the germinal plate, indicating the presumptive mouth region. Conversely, *Hau-hey* and *Hau-hes2* are expressed along the germinal band. By stage 10, all Notch signaling molecules are expressed along the adhesion region, ventral nerve cord, and visceral muscle. At stage 11, the Notch receptor and ligands are expressed in the posterior sucker and ventral nerve cord, while *Hau-hey* and *Hau-hes2* are limited to the posterior sucker, not the ventral nerve cord. Furthermore, phylogenetic analysis revealed a close relationship among Notch components in lophotrochozoans, ecdysozoans, and deuterostomes, suggesting minimal divergence among these phyla. Overall, this study indicates that the Notch signaling pathway is a conserved mechanism in metazoan development, highlighting its spatiotemporal expression during leech development.

Keywords: Notch signaling, Conserved pathway, Embryonic development, Lophotrochozoa, Leech

PIEZO in leech development: Spatiotemporal and Asymmetric expression pattern of the *Piezo1* gene in the leech, *Helobdella austinensis*

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Piezo-type mechanosensitive ion channel component 1 (*Piezo1*) is a member of the mechanotransduction ion channel family. Recent studies have highlighted the significance of piezo channels in cellular mechanotransduction processes within vertebrate models. However, data on lophotrochozoans remain scarce. Our previous research identified two piezo genes in the genome of the freshwater leech, *Helobdella robusta*. In this study, we focused on the sibling species, *Helobdella austinensis*, a representative model organism of the lophotrochozoan superphylum. Utilizing in situ hybridization, we revealed an asymmetric expression pattern of *Piezo1* in the dorsal region during early developmental stages, which later converged towards the center of the dorsal region, aligning with the predicted area of salivary gland development in later stages. Additionally, *Piezo1* expression was observed in the adult salivary glands of *H. austinensis*. Furthermore, phylogenetic analysis revealed conserved piezo domains among lophotrochozoans, ecdysozoans, and deuterostomes. Overall, our findings suggest that *Piezo1* is a conserved gene implicated in the development of salivary glands in metazoans.

Keywords: Piezo, Leech, Salivary gland, Mechanotransduction, Lophotrochozoans.

Developmental function of O-GlcNAcylation in salivary gland morphogenesis

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In this study, the developmental role of O-GlcNAcylation, a post-translational modification of intracellular proteins and reported to be involved in various cellular processes including cell cycle progression, signaling, transcription, and stress response, was investigated during salivary gland formation using a range of experimental tools. Immunohistochemical examination of O-GlcNAc Transferase (OGT) and O-GlcNAc during different stages of embryonic and postnatal salivary gland development indicated the potential involvement of O-GlcNAcylation in salivary gland (specifically acinar cell) differentiation. To define its precise roles in acinar cell differentiation, we inhibited OGT using a small OGT inhibitor called OSMI-1 during *in vitro* cultivation of embryonic salivary glands at E14 for designated period. Following OGT inhibition, we assessed morphological and molecular alterations using histology, immunohistochemistry, and real-time quantitative polymerase chain reaction. The inhibition of OGT led to a delay in terminal bud development and differentiation of acinar cells. Additionally, the predominant localization of ER stress markers (GRP78, HRD1, and IRE1) in the developing buds indicated the induction of ER stress after OSMI-1 treatment, which subsequently resulted in increased apoptosis. Moreover, the treatment with OSMI-1 significantly affected the localization patterns of signaling molecules associated with acinar cell differentiation, including E-cadherin, Vimentin, and Mist1. Overall, our findings provide evidences that O-GlcNAcylation, mediated by OGT, plays a critical role in the development and differentiation of salivary glands.

Keywords: O-GlcNAc Transferase, OSMI-1, Acinar cell, ER stress

Inhibition of proteasome facilitates regenerative dentin formation

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Regeneration of dentin following dental pulp exposure is a significant challenge in the clinical dentistry. In this study, we evaluated the role of MG132, a proteasome inhibitor that binds to the active site of β-subunits of the 20S proteasome, during dentin regeneration. After exposing the dental pulp, MG132 was locally delivered and covered with MTA. After 5 days of local drug delivery, we examined the morphological alteration and cellular physiology such as modulation of differentiation, with histology and immunohistochemistry. Immunohistochemical analysis revealed elevated localization patters of reparative dentin related proteins including, Nestin, Tgfβ-1, Runx2, osteocalcin, and osteopontin compared to the control suggesting reparative activity. Furthermore, MG132 treated specimens showed well-defined dentin-bridge formation after 42 days of local drug delivery when compared with the control. Micro-CT images further confirmed the dentin-bridge structure in MG132 treated specimens when compared to the control. These findings suggest that MG132 promotes dentin regeneration by modulating the expression of key markers involved in reparative dentinogenesis. Further elucidation of MG132's mechanisms could lead to novel therapeutic strategies for enhancing dental pulp regeneration.

Keywords: Proteasome inhibitor, Reparative dentin formation

Enhanced O-GlcNAcylation facilitates regenerative dentin formation

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O-GlcNAcylation is the posttranslational modification of the proteins catalyzed by two enzymes; OGT and OGA. O-GlcNAc modification of protein on serine or threonine residue is crucial for tissue specification, cell viability, and embryonic development. Moreover, the hyper O-GlcNAcylation of the cellular proteins has shown preventive effects during inflammation in the heart and vascular dysfunction. However, its role during hard tissue, especially dentin formation and regeneration, has not been explored yet. In this study, the pharmacological elevation of O-GlcNAcylation by OGA inhibitor drug following up pulp exposer in mouse molars resulted in reparative dentin formation via inflammation prevention. Altered morphological changes and cellular physiology were examined with histology and immunohistochemistry. After OGA inhibitor local delivery in the mice pulpal cavity, histology and cellular physiology, such as modulation of differentiation and inflammation, were examined using histology and immunohistochemistry. OGA-inhibited specimens showed altered localization patterns of Nestin, NF-κB, MPO, Osteopontin, Runx2, TGF-β1, and TNF-∂ after 3 and 5 days. Furthermore, OGA inhibited specimens showed facilitated dentin-bridge formation after 42 days of local drug delivery compared to the control. Micro-CT images further confirmed the dentin-bridge structure in the OGA-inhibited specimens compared to the control. From these results, we concluded that hyper O-GlcNAcylation with OGA inhibition would facilitate reparative dentin formation via inflammation prevention and activation of signalling regulations. Therefore, the elevated O-GlcNAcylation of the cellular proteins might be crucial for the prevention of inflammation and patterned dentin regeneration formation.

Keywords: O-GlcNAcylation, OGA, Pulp cavity, Inflammation, Reparative dentin formation

Identification of signaling networks for collagen biosynthesis by extracellular proline treatment

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The role of proline (Pro) in collagen biosynthesis and cellular metabolism has been revealed in many previous reports. Pro is one of the major substrates for collagen biosynthesis and required to form collagen molecule. Interestingly, there were reports on drastic increase of collagen biosynthesis with extracellular Pro in the cells cultured in glutamine (Gln)-free medium, meanwhile extracellular Pro had significant, but less impact on collagen biosynthesis in fibroblasts when cultured in the presence of Gln. These results would suggest that treatment of Pro would determine the rate of collagen biosynthesis with Gln. Based on these results, to identify and establish the underlying molecular mechanisms of collagen synthesis using extracellular Pro, we treated Pro with a range of drug delivery systems into preosteoblastic MC3T3-E1 and fibroblast cells, and conducted the genome wide screening. The candidate molecules were identified and confirmed the precise expression patterns by RT-qPCR and WB. To sum it up, we identified the *de novo* signaling pathways of the collagen biosynthesis with extracellular Pro treatment. These putative signaling networks would be plausible answers to understand the collagen biosynthesis for regenerating the connective tissues such as skin, muscle, and bone.

Keywords: Collagen biosynthesis, Proline, Drug delivery system, Genome wide screening, Signaling network

Local treatment of antioxidant facilitates alveolar bone regeneration from periodontitis

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The periodontium develops from dental follicular tissue and is differentiated into periodontal ligament, cementum, and alveolar bone for bearing and supporting the tooth. Most of pathophysiological cases in periodontium are resulted into the loss of tooth. Among those, periodontitis is the main causes of tooth loss in dental field and required to be developed with proper medication. In this study, we evaluated the regenerative function of N-Acetyl cysteine (NAC), a well-known reactive oxygen species (ROS) scavenger, in bone healing processes of alveolar bone. The local delivery of NAC was employed after the tooth loss from the induction of periodontitis. The detailed histomorphological changes were examined using HE and MTC stainings after 1 week treatment. In addition, the precise localization patterns of various cell physiology and signaling molecules including 8-Hydroxy-2'-deoxyguanosine, CD31, IL1-β, KI67, MPO, Osteocalcin, and RUNX2 were examined. Micro-CT images confirmed the facilitated bone tissue formation in the NAC treated specimens compared with control. Overall, ROS scavenging would facilitate the bone formation through modulation of inflammation and signaling network in tooth loss root socket.

Keywords: Periodontitis, Bone regeneration, ROS, Signaling network, Inflammation

Inhibition of Prickle2 modulates dentin development and regeneration

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Prickle planar cell polarity protein 2 (Prickle 2) was known to encode a homolog of Drosophila prickle, but its exact function has not been elucidated so far. After the laser microdissection and genome-wide screening of developing primary enamel knot (PEK) tissue, we found the critical expression pattern of Prickle2 in the PEK using RT-qPCR and in-situ hybridization. From the precise expression pattern of Prickle2 in the PEK, we did examine the developmental function of Prickle2 in tooth morphogenesis using in vitro organ cultivation and renal capsule transplantation methods with a gain and loss of function study. After knocking down Prickle2 with siRNA treatment at E13.5 for 2 days, the obvious altered histogenesis and cellular physiology, such as proliferation, cell adhesion, and apoptosis, were examined. Furthermore, renal transplantation at 3 weeks revealed significant alterations in histology with a thicker pre-dentin layer and morphology in tooth crown formation with altered expression of signaling molecules including Bmp, Shh, Dspp, and NESTIN. In contrast, the gain-of-function study using overexpression vector delivery showed the opposite results from the loss-of-function study. For evaluation of its developmental modulation of Prickle2 in dentin regeneration, we employed the pulp exposure animal model and treated siRNA against Prickle2 in the exposed pulp cavity of the first upper molar at 8 weeks for 6 weeks. The siRNA treatment facilitated the formation of dentinal bridge when compared with the control. Overall, our results suggest that Prickle2, which is expressed in the enamel knot, would regulate the PEK-related signaling molecules, and modulation of Prickle2 expression would be a plausible answer for dentin regeneration.

Keyword: Dentinogenesis, Primary Enamel Knot, Signaling regulation, Odontoblast.

Development of neurofibromatosis model in *Xenopus*

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The development of genetic disease in model organisms is essential for understanding the complex mechanisms underlying these conditions and for developing effective treatments. Compared to the cell-based system, animal models offer valuable insights into genetic disease due to their phenotypes presented in multiple organs and their roles in development. These models also enable researchers to monitor the effect of potential therapies and their tentative toxicity.

African clawed frog *Xenopus* is advantageous in disease modeling due to its easily manipulable, large embryos and rapid early development. However, because of its long generation time, it was unsuitable to perform conventional F2-based genetics. The application of CRISPR/Cas technology in *Xenopus* allows the study of the F0-genetics, but SpCas9, the most widely used CRISPR/Cas system, still encounters limitations of their activity related to temperature sensitivity, which presents challenges for its effective use in *Xenopus laevis*. A recently reported engineered variant Cas12a-Ultra claimed it overcomes these hurdles, enhancing endonuclease activity and reducing temperature dependence. We tested that it also has high efficacy in *X. laevis* embryos raised at low temperatures (20~22 °C).

Here, we present the *X. laevis* model of neurofibromatosis type I, a rare monogenic disorder characterized by intricate tumors comprised of axonal processes in humans, using the CRISPR/Cas12a-Ultra system. In this model, embryos with NF1 inactivation presented reduced head size, eye abnormalities, and potential congenital heart defects. Notably, irregular masses along the spinal cord emerge between stages 49-50, possibly originating from the brain periphery. We characterized their molecular signatures using single-cell analysis. Furthermore, we also confirmed that Selumetinib, an FDA-approved MEK inhibitor used for Neurofibromatosis type I could rescue the phenotypes that we observed under NF1 inactivation. Taken together, our *Xenopus* model of neurofibromatosis would be a useful model to decipher the disease mechanisms in multiple organs and to screen new therapeutic agents.

Roles of microglia subtypes during *Xenopus* spinal cord regeneration

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Mammals exhibit limited tissue regeneration capabilities, especially within the central nervous system (CNS), where spinal cord injuries (SCIs) result in irreversible loss of motor and sensory function below the injury site. In contrast, species such as zebrafish and axolotl exhibit remarkable regenerative abilities, capable of fully regrowing a functional body. African clawed frog, *Xenopus* tadpoles can also regenerate their whole tail after the amputation, including the spinal cord. Notably, lineage tracing has uncovered regeneration originating from the original tail. However, the mechanisms controlling the balance between self-renewal proliferation and differentiation during regeneration are still poorly understood.

Microglia, the resident macrophages of the CNS, are a vital component, constituting approximately 10% of total CNS cells. They play a crucial role in maintaining CNS homeostasis through continuous interactions with neuronal and non-neuronal cells. In both clinical and experimental settings of CNS injuries, microglia are the primary cell types that initiate neuroinflammatory reactions. Notably, population change was observed during spinal cord regeneration, suggesting a crucial role of microglia in the regenerative response. However, their precise involvement in CNS regeneration remains poorly understood. The study of detailed subtypes responsible for the regeneration is not fully known.

Here, we reanalyzed time series single-cell transcriptome data after tail amputation in Xenopus to identify microglia subtypes during spinal cord regeneration. We found two different microglia subtypes with different population changes depending on regeneration time points. Using an in situ hybridization chain reaction (HCR) and the marker genes identified in single-cell analysis, we also confirmed the population changes of each subtype of microglia. With this method, we found subtype 1 microglia increased in the regenerated tail while subtype 2 microglia only showed a higher population in the non-regenerated tail. Understanding the change in microglia dynamics holds promise for explaining their contributions to spinal cord regeneration beyond their classical immune functions.

Acute toxic effects and mechanism of hull in-water cleaning wastewater on embryonic flounder (*Paralichthys olivaceus*)

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Antifouling paints, which contain biocidal compounds, are applied to boat and ship hulls to prevent or minimize the attachment of fouling organisms. Despite the potential toxicity risk associated with these pollutants, there is a limited number of studies investigating and monitoring the toxic effects on embryonic fish using hull in-water cleaning (IWC) wastewater collected from ship. In this study, hull IWC wastewater from ship was collected from a ship in 2022 and toxic effect assessments were conducted on fertilized embryonic Olive flounder (*Paralichtys olivaceus*). After dividing the IWC wastewater into untreated wastewater and filtered wastewater, fertilized embryos were exposed to various dilution factors (10-, 100-, and 1000-fold dilutions). Chemical analysis of the IWC wastewater revealed high proportions of Cu, Fe, and Zn. There was no significant difference in the mortality of embryonic flounder exposed to untreated wastewater compared to filtered wastewater. However, malformations in morphogenesis, including pericardial edema, dorsal curvature, tail fin fold defects, and developmental delays, were observed in fertilized embryos following exposure to IWC from ship. To understand the molecular biology of malformation, eight genes related to (heart formation (*nkx2.5*, NK2 NK2 homeobox 5*; SOX6,* SRY-box–containing gene 6; *robo1*, roundabout receptor1), bone malformation (*bmp4*, bone morphogenetic protein 4), fin malformation *(plod2*, procollagen-lysine 2-oxo-glutarate 5-dioxygenase 2, *furin*, furin, paired basic amino acid cleaving enzyme; *wnt3a,* Wnt family member 3a), and tumors (*TP73*, tumor protein p73) were evaluated using qRT-PCR. To clarify the potential toxic effects of IWC wastewater, we also conducted RNA-seq (high-throughput sequencing) on embryonic flounder exposed to hull IWC wastewater. In embryonic flounder exposed to IWC wastewater, genes related to nervous system development, cell development, muscle development, and animal organ development pathways were significantly differentially expressed. This study provided crucial evidence of the risks associated with IWC wastewater when exposed to marine organisms. Taken together, these results may inform strategies to improve hull-cleaning wastewater pollution management to better protect coastal ecosystems.

Key Words: in-water cleaning wastewater, toxicity, embryo, development, malformation

Toxic effects of three alternative booster antifouling biocide on embryonic flounder (*Paralichthys olivaceus*): An approach to transcriptome and morphogenesis

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The use of alternative biocides for antifouling application has increased since the restriction on the use of organotin compounds. However, there is the limited information of those biocides on the developmental toxicity to nan-target marine organism. The present study determined the developmental toxic effects of the alternative antifouling compounds including Diuron, Irgarol 1051 and Sea-nine 211 on the early developmental stages of flounder (*Paralichthys olivaceus*). At 48h after exposure, frequency percentage of mortality was <10% in all the exposure group of Irgarol 1051 and Diuron. But embryos were shown 100% of mortality in the exposure group of 100 ug/L for sea-nine 211. Overall, three biocides produced a largely overlapping suite of defects, marked by the well-known effects including caudal fin fold defects, dorsal curvature, and pericardial edema. Embryos exposed to Irgarol 1051 and Sea-nine 211 were observed pericardial edema except for embryos exposed to Diuron. Those biocides may be ranked in the following order from highest malformation and mortalities; Sea nine 211 > Irgarol 1051 > Diuron. We used high-throughput sequencing (RNA-seq) to characterize the developmental toxic effects from oil exposure. Genes associated with proteolysis involved in cellular protein catabolic pathway process, and intracellular signal transduction were down-regulated in flounder embryos treated with three biocides. Genes associated with microtubule cytoskeleton and regulation of cell morphogenesis were up-regulated at embryos exposed to Irgarol 1051 and Sea-nine 211, while they were down-regulated in embryos exposed to Diuron. Embryos exposed to Diuron and Sea-nine 211 showed up-regulation in genes relating to the GTPase activator activity. Different gene expression profiles were also observed in embryos exposed to biocides with different chemical compositions. Overall, our study provides a better understanding of the underlying molecular toxic mechanisms via RNA-seq and DEGs in embryonic flounder.

Key Words: toxicity, embryo, malformation, antifouling compound, Diuron, Irgarol 1051, Sea-nine

The structure of the reproductive system and fertilization site in female *Octopus vulgaris* (Cephalopoda: Octopodidae)

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Internal fertilization in various species involves different reproductive strategies, such as the storage of sperm in the female reproductive system after copulation until fertilization. This sperm storage extends sperm lifespan, enhancing fertilization and reproduction rates. Among cephalopods, the female reproductive system of octopods, which is made up of an ovary, a pair of oviducal glands, and proximal and distal oviducts, is the simplest. In Octopodidae, research on the female reproductive system has mainly focused on gonadal development, although recent studies have investigated the function and structure of the oviducal gland. However, in octopods, studies on the microanatomical structure of the female reproductive system are rare. Thus, this study aims to provide a detailed microanatomical description of the female reproductive system of *Octopus vulgaris*, an internally fertilized cephalopod, and to identify its fertilization site. Samples were collected using octopus pots in the Yeosu, on southern coast of South Korea, from May 2022 to July 2023. A total of 373 specimens (mean total weight 431.3±238.8 g) were used for the analyses (about 25 specimens per month). Specimens were prepared for light microscopy and were stained with Mayer's hematoxylin-eosin (H-E) stain. Gonadal development was categorized into the following five stages: inactive stage, early active stage, late active and mature stage, ripe stage, spent and degenerative stage. The female reproductive system of *O. vulgaris* consists of the ovary, common oviduct, proximal oviduct, oviducal gland, and distal oviduct, and histologically, the structure of the female reproductive system is similar to that previously reported of other octopods. During all ovarian development stages, sperm were not observed in the ovary, common oviduct, or proximal oviduct. However, sperm were not observed in the spermathecae of the distal oviduct and oviducal gland during the ovarian inactive stage, but were observed from the ovarian early active stage to the spent and degenerative stage. From the ovarian early active stage to the ripe stage, sperm were densely packed in the spermathecae of the oviducal gland and were embedded on the free surface of the epithelial layer. However, in the ovarian spent and degenerative stage, sperm were separated from each epithelial layer of the spermathecae and central cavity, before being released into the lumen. These finding show that in the reproductive system of female octopuses, sperm are stored in the spermathecae of the oviducal gland for a certain period (early active stage-ripe stage), and fertilization take place within the central cavity of the oviducal gland.

Key words: octopus, reproductive system, fertilization, microanatomy, sperm storage

Ultrastructure on the spermatogenesis and sperm of the squid, *Todarodes pacificus* (Cephalopoda: Ommastrephidae)

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Spermatogenesis and the ultrastructure of sperm in aquatic animals exhibit species-specific characteristics, they can be used to understand the reproductive ecology and as complementary phylogenetic-taxonomic characters. In cephalopods, as well as crustaceans and some teleosts, males transfer sperm to females in the form of spermatophores. Spermatophores have been reported in various cephalopods, including squids such as *Todarodes pacificus* and *Loligo pealii*, and octopods like *Octopus dofleini* and *O. vulgaris*. This study aims to describe the spermatogenesis and the ultrastructure of mature sperm within the spermatophore of the squid, *T. pacificus*, to provide basic reproductive information and phylogenetic-taxonomic information for the species. Samples were collected from May to October 2020 in the northern East coast of Gangwon-do, Korea (ML 186.2 ± 25.4 mm, n=23). To observe spermatogenesis and mature sperm of *T. pacificus*, the testis and spermatophores were extracted. The extracted tissues were fixed in 2.5% glutaraldehyde (0.1 M phosphate buffer, pH 7.4) and prepared for observation using light microscopy (H-E stain), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Spermatogenesis in *T. pacificus* was classified into spermatogonia, spermatocytes, spermatids, and sperm according to cell size, staining, characteristics of the nucleus and organelles, and electron density. Spermatogonia were about 7 μm with a nucleus about 6 μm, and the cells were connected by cytoplasmic bridges. Spermatocytes were divided into primary spermatocytes in which synaptonemal complexes appear and secondary spermatocytes, with more condensed heterochromatin. Spermatids were divided into three stages during spermiogenesis. In early spermatids, the nucleoplasm was granular type with a proacrosomal vesicle forming at the upper part of the nucleus and a flagellum observed at the lower part. In mid spermatids, the nucleoplasm was fibrous type and mitochondrial spurs were present in the mid piece. Late spermatids had an elongated nucleus with fine granular type. The acrosome was about 0.5 μm in length and contained granules with high electron density inside. Mature sperm observed in the spermatophore had a blunt cone-shaped acrosome approximately 0.7 μm at the upper end, and the nucleus was an elongated rectangle about 4.5 μm in major length with electron-dense star-shaped structures. The mid piece formed a flagellum with a 9+2 microtubule structure. Along the long axis of the flagellum, a mitochondrial spur containing numerous mitochondria and electron-dense glycogen granules at the lower part was present. The ultrastructure of the sperm is related to the morphological characteristics of the oocyte, such as membrane structure. For this, detailed studies on the ultrastructure, sperm lifespan and motility, and fertilization methods in *T. pacificus* will be needed.

Key words: *Todarodes pacificus*, spermatogenesis, spermatophore, sperm, ultrastructure

Application of artificial insemination technology for selective breeding program of fleshy prawn, *Fenneropenaeus chinensis*

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The fleshy prawn, *Fenneropenaeus chinensis* was the commercially important species in shrimp farming industry of Korea during the 1990s, but the industry had collapsed due to severe losses to an epidemic of White Spot Syndrome Virus (WSSV) since 2003. Two decades later, ongoing studies are working on producing genetically improved stocks of fleshy prawn with improved growth performance and disease resistance, systematic selective breeding and technological innovation on farming practices. In the present study, we demonstrated the artificial insemination (AI) technology for selective breeding of this species. Fleshy prawns were induced in wild broodstock collected off Cheonsu bay. AI was performed two days after molting on these females when the cuticle and thelycum was still soft and flexible. A male with fully developed spermatophores were selected by appearance of large and white ejaculatory duct and the spermatophore was manually ejected by applying gentle pressure to the base of the outer corner of the spermatophore until it slips out of the genital pore. The ejected spermatophore was placed into the thelycum of female. The AI process should be completed in less than 1 minute to minimize stress to the female. The copulated females were transferred to rearing tank. Out of 51 attempts, three were successful with hatching rates less than 65.4 %. Further research will be conducted to improve retention of spermatophores, induction of maturation and spawning performance through the technology of AI under controlled conditions.

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Key words: fleshy prawn, artificial insemination, reproduction, selective breeding

Gonadal development and spawning season of the long arm octopus, *Octopus minor* (Cephalopoda: Octopodidae)

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Because of climate change and habitat destruction, amount of fishery resources is continuously decreasing. For conserve and management of fishery resources, research on reproductive ecology is essential. The long arm octopus, *Octopus minor*, is marine benthos, belonging to class Cephalopoda, order Octopoda, family Octopodidae and important fishery resource in China, Japan and Korea. In this study, we analyzed sex ratio, gonadosomatic index (GSI), hayashi index (HI), monthly gonadal development stage, spawning season to provide basic data for understand reproductive ecology of *O. minor*. From May 2022 to August 2023, 699 individuals of *O. minor* (mean total length 616.9 ± 170.0 mm) were collected from coast of Jeollanam-do, south sea of South Korea, using octopus pots. Reproductive organs were extracted and fixed in 10% neutral formalin. Specimens were followed by paraffin sectioning method 4-6 μm serial sections produced and stained with Mayer's hematoxylin-0.5% eosin (H-E) stain. Sex ratio was calculated as the ratio of males to females. The GSI was calculated as the reproductive organs weight divided by body weight. The HI was calculated as a percentage by dividing the weight of the reproductive appendages by the reproductive organs weight. Gonadal development stage was divided into 5 stages (inactive stage, early active stage, late active and mature stage, ripe stage, spent and degenerative stage). Spawning season was analyzed by results of GSI, HI and monthly gonadal development stage. The sex ratio was 1:1.03 (n=343:356), but proportion of males higher in total weight ≤ 20 g group and total weight ≥ 170 g group. The GSI showed highest value in June 2022, 2023 and rapidly decreased in July 2022, 2023, for both males and females. The HI showed highest value in July 2022, 2023 and rapidly decreased in males, while in females less than 20.0 all year around except August 2023. Testicular development stages showed inactive stage (July to August 2022), early active stage (May 2022), late active and mature stage (September to December 2022, August 2023), ripe stage (June 2022), spent and degenerative stage (January to July 2023). Testicular development pattern was analyzed group-synchronous type. Ovarian development stages showed early active stage (August to October 2022, August 2023), late active and mature stage (November 2022 to May 2023), ripe stage (June 2022, 2023), spent and degenerative stage (July 2023), inactive stage was not found. Ovarian development pattern was analyzed synchronous type. As a results gonad of *O. minor* showed seasonal changes, belonging to summer breeders and spawning season of *O. minor* was analyzed during June to July.

Key words: *Octopus minor*, Gonadal development, Reproductive cycle

Microanatomical structure of male reproductive organ in the snow crab *Chionoecetes opilio* (Malacostraca: Oregoniidae)

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Animal fertilization is divided into external fertilization and internal fertilization, depending on the location of fertilization. In general, aquatic animals use external fertilization, but some species that use internal fertilization transfer sperm to females with suspension. However, crustaceans, cephalopods and some teleost transfer sperm to female with spermatophore. Specific organ develops for this formation. In the snow crab, *Chionoecetes opilio*, sperm pass through vas deferens to form the spermatophore. Research on *C. opilio* were reported on size and composition characteristics, maturation, gonad maturation and spawning. But, research on the anatomical and histological structure of reproductive organs are rare. Thus, this study aimed to describe the microanatomical structure of *C. opilio*, reproductive organs and provide basic information on reproductive biology. The samples (CW 82.9 mm, TW 212.5 g, n=61) were collected in Uljin, Gyeongsangbuk-do, Korea. Dissected reproductive organs were fixed in 10% neutral formalin. Paraffin sectioning method was executed with thickness of 4-6 μm. Specimens were stained with Mayer's hematoxylin-0.5% eosin (H-E) stain, Masson's trichrome stain, alcian blue-periodic acid and Schiff's solution (AB-PAS, pH 2.5) reaction, and aldehyde fuchsin-alcian blue (AF-AB, pH 2.5) reaction. The male reproductive organ was ‘H’ shape and consisted of testis and vas deferens. According to location, vas deferens could be divided into anterior vas deferens (AVD), median vas deferens (MVD), and posterior vas deferens (PVD). Testis was white thin tube connected to the AVD. The AVD was similar to the testis, the MVD was thicker than the AVD, and the PVD was branched. The testis was tubular type. And simple epithelium composed of columnar epithelial cells observed at the end. In the testis, basophilic spermatogonia, spermatocytes, spermatids, and sperm were observed. The vas deferens was consisted of a connective tissue and epithelium. The epithelium was a simple layer consisted of epithelial cells and secretory cells. The AVD and MVD were composed of cuboidal epithelial cells, and the PVD was composed of columnar epithelial cells. Secretory cells showed differences in stainability depending on location. Secretory cells in the AVD and MVD were observed as vacuole in H-E stain, Masson’s trichrome stain, AB-PAS (pH 2.5) reaction, and AF-AB (pH 2.5) reaction. Secretory cells in the PVD were seen as vacuole in H-E stain and Masson’s trichrome stain, but reacted blue in AB-PAS (pH 2.5) reaction and AF-AB (pH 2.5) reaction. The seminal fluid, granular material and spermatophore were observed in lumen of vas deferens. The seminal fluid was light purple and granular material was dark purple in AB-PAS (pH 2.5) reaction. The AVD to PVD, the proportion of seminal fluid and granular material decreased and the proportion of spermatophores increased.

Key words: *Chionoecetes opilio*, reproductive organ, histology

Reliable Gender Identification Using Gender-Specific Transcript Expression Value In *Paralichthys olivaceus*

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The advancement of technology for gender identification in aquaculture has primarily focused on genetic, histological, and hematological methods, leveraging morphological or genetic dimorphism. Olive flounder holds significant importance in South Korea's aquaculture industry. Notably, female olive flounder exhibit a growth rate exceeding 30% compared to males, leading to a preference for selectively breeding females in aquaculture settings. Consequently, there's a pressing need to develop technology capable of industrially identifying female olive flounder. This study endeavors to develop a gender identification technology applicable before the period of gender differentiation in olive flounder, utilizing the expression rates of female-male specific gene transcripts. Female-related transcripts scrutinized encompass ovarian type aromatase (cyp19a), forkhead Box L2 (foxl2), and zona pelucida 3 and 4 (zp3 and zp4). Conversely, male-related transcripts include cholesterol side-chain cleavage (cyp11a), 11β-hydrexylase (cyp11b), and gonadal soma-derived factor (gsdf). Observations indicate that in females, the expression of cyp19a surpasses that of cyp11a. Utilizing the relative expression rates of specific genes, the determination of male or female is computed. For females, an individual is classified as female if the relative percentage of female-specific genes is 79.08% or higher, whereas if it is 5.5% or lower, the individual is classified as male. The research findings demonstrate the feasibility of gender identification in halibut before the period of gender differentiation using the expression ratios of female-male specific gene transcripts. Consequently, these transcripts could serve as valuable indicators for the advancement of Olive flounder gender identification technology.

Key words: gender identification, gender specific genes, olive flounder

Estrogenic effect of various plant extracts on the hepatocytes of eel (*Anguilla japonica*)

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Phytoestrogens are plant-derived compounds that can have physiological effects similar to those of the female hormone estrogen and have been studied in many areas. Hepatocytes of oviparous fish, including eels (*Anguilla japonica*), are known to be sensitive to estrogen and form vitellogenin (VTG). Therefore, fish hepatocytes can be used as a tool to test the estrogenic activity of plant extracts. In this study, eel hepatocytes were used to evaluate the estrogenic activity of several plant extracts and to compare the level of their activity with each other.

The eel hepatocytes were cultured in L-15 medium containing FBS and antibiotics, and the plant extracts used in the experiment were extracted from 10 different plants, including turmeric, guava, soybean, garlic, flax seed, alfalfa, onion peel, Schisandra, kudzu and astragalus which are known to contain many useful constituents. Following treatment with varying concentrations of each plant extract, the cells were incubated for 24 hours. The impact on cell viability was then estimated using the CCK-8 test. The expression of VTG gene in the hepatocytes was also measured by qRT-PCR.

The results showed that the plant extracts significantly increased the mRNA expression of the VTG gene in eel hepatocytes. In particular, treatment with Schisandra, astragalus and guava extracts induced higher mRNA expression of the VTG gene and had a positive effect on cell viability. The findings indicate that the plant extracts examined in this study have the potential to be employed as estrogen substitutes, and that fish hepatocytes could serve as an effective means of evaluating the estrogenic activity of plant extracts.

Keywords: Phytoestrogen, estrogen, hepatocytes, plant extracts, vitellogenin gene

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PACRG is expressed on the left side of the brain vesicle in the ascidian *Halocynthia* embryos

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The larvae of ascidian, which display a chordate ground body plan, exhibit several structures that are left-right asymmetric, including the brain (sensory) vesicle. In the brain vesicle of ascidian larvae, the ocellus and otolith pigment cells, which are thought to detect light and gravity respectively, are located on the right side, while the coronet cells, which are presumed to be dopaminergic, are located on the left side. It is speculated that the Nodal signaling is involved in the formation of these left-right asymmetric structures, but the details are not yet understood. In this study, we report the PACRG (Parkin Co-Regulated Gene), which is specifically expressed on the left side of the brain vesicle, discovered during the search for genes expressed in the central nervous system of the ascidian *Halocynthia roretzi*. Expression of the PACRG begins on the left side of the brain vesicle in late tailbud embryos. The location of PACRG expression is estimated to overlap with the area stained by the coronet cell-specific antibody. Therefore, it is suggested that PACRG might be involved in the formation and function of the left-side structures of the brain vesicle, including coronet cells, in *Halocynthia* larvae.

Key words: Ascidian, PACRG, Coronet cell, Left-right asymmetry.

참문어(*Octopus sinensis*) 초기 발생 단계에서 차별적으로 발현되는 유전자의 동정

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두족류의 발달과 초기 발생 단계에서 부화 및 생존과 관련된 유전자에 대한 연구는 많이 이루어졌다. 그러나 동아시아 참문어(*Octopus sinensis*) 유생의 발생 단계와 유전자 발현에 대한 연구는 부족한 실정이다. 이에 본 연구에서는 RNA 시퀀싱(RNA-seq) 방법을 사용하여 *O. sinensis*의 배아 발달 및 부화와 관련된 유전자를 도출하고, 발생단계별로 차등 발현되는 유전자를 검증하였다. 부화 후 0일령 및 25일령 *O. sinensis* 유생으로부터 RNA 샘플을 사용하여 cDNA 라이브러리를 구축하고, 데이터베이스화 하여 통계학적으로 유의미한 차등발현유전자(DEGs)를 조사하였다. 또한, 실시간정량 중합효소 연쇄반응(RT-qPCR) 방법을 사용하여 배아 발달단계별 RNA-seq DEGs 분석에 대한 데이터를 보완하였다. 총 12,597개의 전사체에 대하여 주석 처리되었고, 0일령과 25일령의 *O. sinensis* 유생 간에 5,468개의 DEGs가 식별되었으며, 이 중 2,715개의 전사체는 25일령 유생에서 상향 조절되었고, 2,753개의 전사체는 하향 조절되었다. 대표적인 주요 DEGs는 *O. sinensis* 유생의 부화 후 발달 단계에서 막수송, 지질생합성, monoxygenase활성, 지질수송, 신경펩티드 신호전달, 전사조절 및 단백질-시스테인 S-palmitoyltransferase 활성과 관련이 있었다. RT-qPCR 분석을 통해 SLC5A3A, ABCC12 및 NPC1 유전자 발현 수준은 수정후 20일령 및 30일령 배아에서 10일령 배아보다 유의하게 높았다($p < 0.05$). 본 연구는 *O. sinensis* 유생의 배아 발달, 부화 및 생존을 이해하기 위한 전사체 활용의 대표적인 사례가 되며, 참문어 인공종자생산 효율을 향상시키는 데 도움이 될 것으로 판단된다.

Key words: 두족류, *Octopus sinensis*, 차등발현, 배아, 유전자발현

The effect of polyploidy on gonadal development and growth differences

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In the aquaculture industry, technologies have been developed to maximize growth and avoid poor flesh quality by limiting reproductive capacity, which induces energy expenditure during maturation and spawning. Triploidy induction has been used to increase aquaculture productivity, mainly in salmonids. Because triploidy induction in fish has only been applied to specific species, there is little research on the growth effects and reproductive biological mechanisms of triploid induction in species important to the aquaculture industry. In this study, the following investigations were conducted on olive flounder, an important fish species in the Korean aquaculture industry: 1) triploidy induction in olive flounder fertilized eggs by low temperature treatment; 2) growth differences between triploids and diploids; and 3) differences in changes in reproductive endocrine axis between triploids and diploids. Comparison of year-round changes in germ cell development between triploid and diploid olive flounder showed that female and male olive flounder diploids could observe normal germ cell development stages within the spawning season in April and May, but triploids could not observe germ cell development throughout the year. There was no significant difference in body growth between diploids and triploids. In addition, changes in aromatase mRNA expression between female olive flounder diploids and triploids showed that diploids had the highest expression level in February, just before the spawning season ($P< 0.005$).

In conclusion, we confirmed that the production of triploid olive flounder inhibits germ cell development and the expression of aromatase mRNA released from the gonads. In addition, we found that reducing energy expenditure during germ cell development does not promote body growth in olive flounder. Further research is needed to understand the endocrine relationship between reproduction and growth in fish, which remains unclear.

Key words: diploid, triploid, olive flounder, gonadal developme

Direct effect of salinity change and SPE treatment on the expression of reproduction and/or salinity-related genes in the pituitary cells of eel (*Anguilla japonica*)

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Artificial sexual maturation of eel (*Anguilla japonica*) involves rearing in seawater and injecting salmon pituitary extract (SPE). The salinity of seawater and components of SPE influence hormonal activities of the eel pituitary, leading to gonad development. This study investigated the direct effects of salinity change and SPE treatment on the eel pituitary gland using primary cell cultures. Pituitary cells were cultured into four experimental groups: control culture (control), SPE-treated culture (SPE), NaCl-treated culture (NaCl) and NaCl+SPE treated culture (NaCl+SPE). We investigated the expression of genes presumably related to reproduction and/or salinity, including luteinizing hormone (LHβ), follicle stimulating hormone (FSHβ), progesterone receptor-like (pgrl), prolactin (PRL), dopamine receptor D4 (drd4), neuropeptide B/W receptor 2 (NPBWR2) and relaxin family peptide receptor (rxfp3-2b). Gene expression analysis revealed significant upregulation of LHβ in SPE and NaCl+SPE groups compared to control and NaCl ($P<0.05$). FSHβ expression did not show any significant changes. PRL showed a significant decrease in the NaCl group ($P<0.05$). Pgrl, NPBWR2, drd4 and rxfp3-2b displayed the highest expression in the control group, with downregulation observed in all treatment groups (NaCl, SPE, and NaCl+SPE) ($P<0.05$). This study demonstrated the direct effects of salinity changes and SPE treatment on the eel pituitary. Results from this study also suggest that salinity change is necessary but work together with SPE to induce reproductive process, and that LHβ, pgrl, PRL, drd4, NPBWR2 and rxfp3-2b genes are obviously associated with reproduction and salinity changes in eels.

Key Words: Eel, Pituitary, Primary cell culture, Sexual maturation, Gene expression

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북방전복 (*Haliotis discus hannai*) 건강도 평가를 위한 생체지표유전자 연구

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연근해 자원 증강의 목적 및 양식 산업의 발달 등에 의해 종자의 중요성이 증가하고 있다. 또한 종자시장의 글로벌화 및 품종보호 관련 국제 협약에 따라 세계적으로 강화되는 신품종 보호 추세에 대응할 필요성이 증대되었다. 이에 따라 신품종 개발 등 종자산업 강화 필요성이 대두되며 신품종 육성관련 연구개발에 대한 투자를 강화하고 있다. 지구온난화 등에 따른 기후변화는 수온, 염분, 해수면 등의 환경을 지속적으로 변화시켜 수산생물질병 발생을 증가시키는 중요한 요인이 되며 질병에 감염된 자연수계의 수산생물 또는 물에 포함된 병원체가 양식장내로 유입되어 해당 생물에 질병을 전파될 가능성이 높다. 이와 반대로 질병에 감염된 양식생물 및 오염된 사육수가 자연수계로 배출됨에 따라 자연수계 수산생물에 질병을 전파할 수 있는 가능성이 있다. 인공으로 생산된 패류의 종묘는 유전적 다양성의 감소에 따른 면역 약화 등의 이유로 질병의 전파 및 확산 매개체로 활동할 수 있다. 질적으로 문제인 종묘의 방류종묘로서 사용 시 새로운 가입 군에 질병을 전파하는 1차적인 영향 이외에도 병원체의 2차 저장소 (reservoir)로 작용할 수 있기 때문에 양식 환경에 지속적으로 질병원을 제공하는 문제가 발생할 수 있다. 분자생물학적 방법을 통한 유전체 연구는 생물체의 유전정보를 분석하고 활용하는데 있어 새로운 도구와 방법론을 제공함으로써 생물 산업의 기술 패러다임 자체를 변화시키고 있다. 환경요인의 변화 등에 따른 생물개체의 건강도를 평가하는데 있어서 분자생물학적인 연구방법을 통하여 발굴 및 개발한 생체지표유전자(biomarker gene)를 이용한 평가는 매우 중요하다. 전복은 연체동물문의 복족류에 속하는 Haliotidae과 Haliotis속에 속하는 대형 바다 달팽이입니다. Haliotidae과에는 Haliotis라는 속이 하나뿐이며, 이 속 하나에 여러 종의 전복이 들어 있는 것으로 알려져 있습니다. 본 연구는 북방전복을 대상으로 종자 및 성체의 건강도를 판단하기 위한 생체지표유전자를 발굴 및 개발하기 위한 연구이다. 연구의 방법은 NGS RNAseq 분석을 통한 차별적 발현 유전자를 조사하였다. 연구 결과는 생체지표유전자로의 활용에 유용할 것으로 기대된다.

Key words: 북방전복, 건강도, 생체지표유전자

Role of PI3K/PDK1/AKT pathway in Sperm Motility and Motion Kinematics

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Infertility has become a global health issue, affecting approximately 10% of couples of reproductive ages. Among many factors contributing to male infertility, asthenozoospermia, characterized by reduced or absent sperm motility, is one of the most prevalent. Sperm motility is essential for successful fertilization, enabling sperm to traverse the female reproductive tract and penetrate the zona pellucida. Recent studies have highlighted the importance of signaling pathways, particularly the PI3K/PDK1/AKT pathway, in regulating sperm functions, including motility. This study investigates the correlation between sperm motility and phosphoinositide 3-kinase (PI3K)/phosphoinositide dependent protein kinase-1 (PDK1)/protein kinase B (AKT) pathway by analyzing the expression levels of proteins related to PI3K/PDK1/AKT pathway in 100 boars. Our results indicated a positive correlation between AKT phosphorylation (Thr308: r = 0.2315, Ser473: r = 0.2513) and progressive motility (PRG). Phosphatase and tensin homolog (PTEN), known for inhibiting the PI3K/PDK1/AKT pathway, showed positive correlations with various motility parameters such as PRG (r = 0.3166), average path velocity (VAP, r = 0.2419), straight-line velocity (VSL, r = 0.3905), and straightness (STR, r = 0.2376. Additionally, p-PTEN correlated with VAP (r = 0.2942) and curvilinear velocity (VCL, r = 0.2432). Taken together, it suggested that the PI3K/PDK1/AKT pathway plays a significant role in regulating sperm motility and motion kinematics during capacitation. Especially, p-AKT, PTEN, and p-PTEN may be crucial for sperm motility and motion kinematics. These findings contribute to a deeper understanding of the molecular mechanisms underlying sperm motility and offer basic information for future research on male fertility.

Keywords: Infertility, Sperm motility, PI3K/PDK1/AKT signaling pathway, correlation

Enhancing Porcine Oocyte Maturation through Epigenetic Modification of Melatonin Receptor 1A Using CRISPR/dCas9-Tet1 Technology

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Oocyte maturation quality significantly impacts embryo viability, fertilization, and subsequent embryonic development in *in vitro* embryo production (IVEP). Melatonin has been shown to benefit follicular growth and oocyte maturation in domestic animals, improving fertilization and embryonic competence. In pigs, melatonin receptors mediate follicular maturation through AKT and ERK pathways, promoting nuclear maturation and cumulus expansion. This study aimed to enhance *in vitro* oocyte maturation (IVM) by increasing melatonin receptor expression through epigenetic modification. We employed CRISPR/dCas9-Tet1 to induce demethylation of the MTNR1A gene in porcine oocytes. Cumulus-oocyte complexes (COCs) were transfected with sgRNA and dCas9 in maturation media. After 18 hours of incubation, the treated group showed a significant increase in COC diameter compared to the control (500.7 μm vs. 430.6 μm, $p < 0.0001$) and a higher proportion of grade 3 and 4 cumulus cell expansion (48.17% vs. 37.49%, $p < 0.05$). The CRISPR/dCas9-Tet1 system efficiently demethylated endogenous MTNR1A in cumulus cells. Following in vitro fertilization (IVF) with fresh boar spermatozoa, the treated group demonstrated significantly higher cleavage rates (80.18% vs. 63.37%, $p < 0.01$) and blastocyst formation rates (24.00% vs. 15.20%, $p < 0.05$). The total cell number, an indicator of blastocyst quality, was also significantly higher in the treated group (49.67 vs. 43.69, $p < 0.05$). Gene expression analysis revealed upregulation of several genes related to the AKT and ERK pathways in the treated group, correlating with improved cumulus cell expansion. Notably, the CRISPR/dCas9-Tet1-mediated demethylation of MTNR1A also enhanced the maturation of oocytes exposed to oxidative stress. These findings suggest that epigenetic modification of MTNR1A using the CRISPR/dCas9-Tet1 system could improve IVM outcomes for both healthy and stressed oocytes, potentially increasing the success rate of porcine IVEP protocols.

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* **Keywords**: CRISPR/dCas9-Tet1, Porcine oocyte maturation, MTNR1A demethylation, In vitro embryo production, Epigenetic modification

Regulation of ROS in porcine semen cryopreservation by adding MnTBAP

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Cryopreservation of germ cells is an important technique for managing infectious disease transmission, preserving genetic diversity, and aiding population management in various species. Spermatozoa undergo diverse stress including rearrangement, destabilization, and calcium influx of the sperm plasma membrane during extremely changing temperatures. ROS, including oxygen metabolites such as H_2O_2, $O_2^{\bullet-}$, and $^{\bullet}OH$ are generated during the regular metabolic processes of aerobic cells and exhibit high reactivity with carbohydrates, lipids, proteins, and nucleic acids. In particular, damaged spermatozoa induce an imbalance in the redox signaling pathway, exposing cells to oxidative stress due to abnormal ROS production. Antioxidants help scavenge excess ROS to maintain the proper range required for cellular metabolism. Manganese (III) meso-tetrakis (4-benzoic acid) porphyrin (MnTBAP) is a newly synthesized antioxidant with positive effects on sperm morphology and capacitation in humans, rams, and stallions. In this study, porcine semen was treated with 0, 50, 100, and 150 μM of MnTBAP based on Tris-egg-yolk extender and frozen to determine whether MnTBAP can assist the quality of sperm during cryopreservation. First, motility was estimated using the computer-assisted sperm analysis system, and the 100 μM treatment group (66.8%) showed the highest motility compared to that of the control group (51.1%) ($P < 0.01$). Second, fluorescence staining was applied to examine the viability and acrosome integrity of the 100 μM treated group (62.4% and 86.4%), which showed significant differences compared to the control group (41.7% and 77.9%) ($P < 0.01$). The mitochondrial membrane potential and DNA fragmentation rate, measured with rhodamine 123/propidium iodide and the Sperm-sus-Halomax kit, tended to be higher in the treated group, but the differences were not statistically significant. Third, analysis using the FACSLyric demonstrated that the 100 μM group had a more normal lipid arrangement in the plasma membrane and lower levels of apoptosis and ROS ($P < 0.01$). We assessed the expression of genes relevant to antioxidant effectiveness using real-time RT-qPCR. Our results show that the mRNA expression of ROMO1, SOD1, and catalase, which are involved in ROS regulation, as well as SMCP, which is related to mitochondrial activity, was significantly elevated. Additionally, the BAX to Bcl-2 ratio, important for apoptosis regulation, was notably lower in the 100 μM group, indicating a reduced level of apoptosis ($P < 0.05$). Finally, the straw was dissolved and treated to matured denuded oocytes to investigate the effect on fertilization and embryo development in vitro. As a result, the cleavage rate was 77.6% vs 84.1% and the blastocyst rate was 9.7% vs 11.4% (control vs 100 μM) ($P < 0.05$). In conclusion, these results suggest that MnTBAP enhances sperm quality by effectively regulating ROS generation during freeze-thawing, which in turn improves fertilization capacity and embryo development. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean Government (Ministry of Science and ICT) (RS-2023-00250165).

Key words: MnTBAP, porcine semen, cryopreservation, ROS regulation, capacitation

Anti-Mullerian hormone and antral follicle count as predictors for optimal selection of Hanwoo donor cows in superstimulated oocyte collection

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This study explored the correlation between Anti-Müllerian Hormone (AMH) levels and reproductive efficiency in native Korean cattle (Hanwoo) as potential oocyte donors in Ovum Pick-Up (OPU) programs. This research explored the correlation between AMH levels and various factors, including the quantity of follicles, retrieved oocytes, and the proportion of transferable embryos. A total of 42 Hanwoo cows were included in this study. The amount of oocytes used an ultrasound scanner. The implantable embryos produced from the retrieved oocytes were quantified. The results show a significant positive correlation between AMH levels and the numbers of antral follicles (R^2=0.5785, p< 0.0001), retrieved oocytes (R^2=0.6857, p< 0.0001) and transferable embryos (R^2=0.4049, p< 0.0001), indicating that higher AMH levels correspond to increased numbers of antral follicles and retrieved oocytes. However, the correlation between AMH levels and the proportion of transferable embryos was not statistically significant (R^2= 0.1476, p= 0.5225). In conclusion, AMH levels were significantly correlated with the number of antral follicles and retrieved oocytes, demonstrating their potential as indicators for selecting oocyte donors for Hanwoo cattle. Although the relationship with the proportion of transferable embryos was not statistically significant, this study offers valuable insights for the improvement of the efficiency of oocyte donation plans in Hanwoo cattle by considering the AMH levels.

Keywords: Anti-Müllerian Hormone, Antral follicles count, Ovum Pick-Up, Hanwoo donor, in vitro embryo production

Medroxyprogesteron acetate가 소 정자 운동성, Calcium 및 ROS 증가에 미치는 영향

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합성 progestin으로 알려진 medroxyprogesteron acetate(MPA)는 배란조절기작을 통한 배란 억제효과를 유도하여 피임제로 많이 이용되고 있으며 정자의 운동성을 조절하는 것으로 알려져 있는 물질이다. MPA는 외부 calcium 유입기작에 의하여 정자를 자극하는 인자로 보고되었으나, pico M (pM) 수준의 매우 낮은 농도에서 chemotaxis를 유도하는 것으로 보고되어 농도에 따른 생리적 기작이 서로 다른 것으로 알려져 있다. 그러므로 본 연구에서는 MPA 농도가 소 정자 운동성, calcium 및 ROS증가에 미치는 영향을 조사하였다. MPA농도에 따른 정자 운동성을 조사하면, 1~10 pM 처리에서는 정자 운동성에 대한 변화가 관찰되지 않았으며 50~500nM에서 운동성 증가가 관찰되었다. 1~10μM MPA를 처리하였을 때, 소 정자 운동성은 매우 증가하였으며 1~2시간 장기간 노출과정에서 정자 생존율은 75%에서 1~3%로 낮아지는 것으로 관찰되었다. 동결정자에 대한 MPA처리는 정자 운동성을 지연시간 없이 바로 증가하는 것으로 관찰되었으며, Calcium 농도 또한 동일하게 증가하는 것으로 관찰되었다. 또한 50~500nM MPA에 의하여 ROS의 반응도는 증가현상이 관찰되지 않았으나, 1~10μM MPA를 처리하였을 때, 유의적으로 ROS 생산이 증가하는 것을 알 수 있었다. 그러므로 비교적 낮은 농도(nM)에서도 MPA는 칼슘의 조절인자로 작용함을 알 수 있었으며 높은 농도(μM)의 MPA는 ROS의 증가를 유도하여 정자의 운동성을 극대화를 유도한다는 것을 알 수 있었다. 이러한 사실은 progesteron이 정자에 미치는 영향을 유추할 수 있으며 자성 생식도관에서 progesteron이 정자 운동성과 생존율에 미치는 영향을 판단할 수 있는 자료라고 판단된다.

Key words: medroxyprogestoron acetate, calcium, reactive oxygen species, sperm

원심분리 방법에 의한 Resiquimod 정자 처리 방법이 소 인공수정 후대 송아지 성비에 미치는 영향

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Resiquimod(R848)는 강력한 항 바이러스 활성을 가진 imidazoquinoline 계열의 합성물질로 TLR7/8 ligand로 작용하여 면역세포를 활성화하는 물질로 최근 포유류 정자의 성분리 연구에 사용되었다. 다. R848을 활용한 정자 성분리 기법은 포유류 정자에 관하여 쥐, 소, 염소의 정자에서 원하는 성을 가진 후대를 생산하기에 경제적인 방법으로 알려져 있으나 비교적 장시간 배양시간이 필요함에 따라 소 인공수정에 활용하는 것은 효율성이 떨어진다. 그러므로 본 연구에서는 단순한 원심분리 기법이 약 45분간 탄산가스 배양기내 처리하는 방법을 대처할 수 있는 방법인지 검증하기 위하여 정자 성분리 연구를 실시하였다. 한우 암컷 16두를 선정하고 발정동기화를 유도하여 인공수정기법을 이용하였다. 한우 동결정액을 현장에서 융해한 후 개체 별 동결정액을 성분리 처리한 후 정액을 분획하여 인공수정을 실시하였다. 정자 성 분리 방법은 최종농도가 R848 0.5μM이 되도록 Triladyl 희석액을 이용하여 조정하였으며 37℃에서 45초간 융해된 정액을 기본배양액과 혼합하여 R848 0.5μM이 되도록 정액을 처리하였다. 동일한 미디어 0.5ml 위에 1ml 주사기를 이용하여 융해 및 희석된 정액을 천천히 올려두었다. 일부 구조를 변경한 Hematocrit 원심분리기를 이용하여 600rpm(34G)에서 4분간 처리하여 상층액 1/3은 Y 정액으로, 하층액 1/3은 X정액으로 구별하여 인공수정에 사용하였다. X 정액을 활용한 인공수정군에서는 66.7%가 암컷 송아지로 생산되었고, Y 정액을 이용한 인공수정군에서는 80%가 수컷 송아지로 생산되었다. R848 성 분리 정자를 이용할 때, 수태율은 61.1%로 대조군에 비하여 낮게 나타났다. 이는 일반적인 소 인공수정 기법에 사용되는 정자 수(18×10^6개)에 비하여 비교적 적은 정자 수(6×10^6개)를 사용된 인공수정의 결과로 추정되었다. R848처리와 원심분리방법에 의하여 소 동결 정자의 수태능력이 감소되지는 않는다고 추정되며 성분리 방법 최적화를 위하여 더 많은 연구가 필요하다고 판단된다.

Key words: resiquimod, sexing, artifical insemination, bovine semen

Nesfatin-1 ameliorates pathological abnormalities in Drosophila hTau model of Alzheimer's disease

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In human Alzheimer's disease (AD), the aggregation of tau protein is considered a significant hallmark, along with amyloid-beta. Abnormal phosphorylation-induced Tau tangles result in microtubule instability and neuronal toxicity, directly contributing to neuronal dysfunction and cell death. Nesfatin-1 is a neuropeptide primarily known for regulating appetite and energy homeostasis. However, the function of Nesfatin-1 in a neuroprotective role has not been investigated. In this study, we aimed to investigate the effects of Nesfatin-1 on human Tau protein using *Drosophila* as an *in vivo* model. Our findings demonstrate that Nesfatin-1 effectively mitigates the pathological phenotypes observed in *Drosophila* human *tau* overexpression models. Nesfatin-1 overexpression alleviates eye neurodegeneration and climbing phenotypes. Also, phosphorylated Tau levels are decreased and the reduced lifespan of human Tau is rescued upon Nesfatin-1 overexpression. Overall, this research explores the potential therapeutic applications of Nesfatin-1 protein in ameliorating the pathological features associated with Alzheimer's disease (AD).

Keywords: Alzheimer's disease, human Tau, Nesfatin-1, neurodegeneration, *Drosophila*

The 43rd Annual Meeting of the Korean Society of Developmental Biology

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특허, 실용신안, 상표 및 디자인 출원 및 등록
- 출원서 제출을 비롯한 특허청 관련 업무 대리
- 거절이유 통지 대응 및 등록 절차 대리 등

선행기술조사 및 출원 전 등록가능성 검토
- 등록가능성 검토 및 IP 확보 방향성 제시

특허 동향 조사 및 분석 사업
- 고객 Needs에 부합하는 특허 분석 사업
- 상장 심사를 위한 사전 특허 분석 작업

지식재산권 분쟁, 소송 및 계약 업무
- 각종 지식재산권 관련 분쟁, 소송 업무 대리
- 각종 국문/영문 계약서 작성 및 리뷰

기술 수요기업 발굴 및 기술이전 절차 수행
- 기술 수요자-공급자 발굴 및 연결 지원
- 각종 국문/영문 계약서 작성 및 리뷰

지식 재산권 관리, 자문 및 교육
- IP 관련 다양한 주제에 맞춰 방문 교육 및 강연
- IP 팀 보유가 어려운 기업의 관리 및 자문 업무

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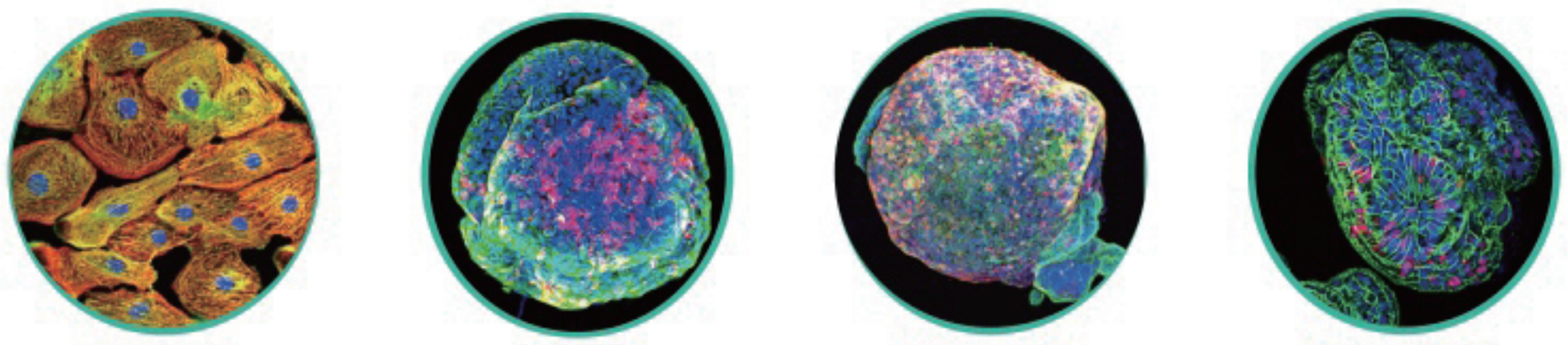

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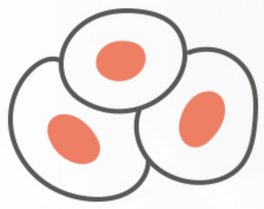
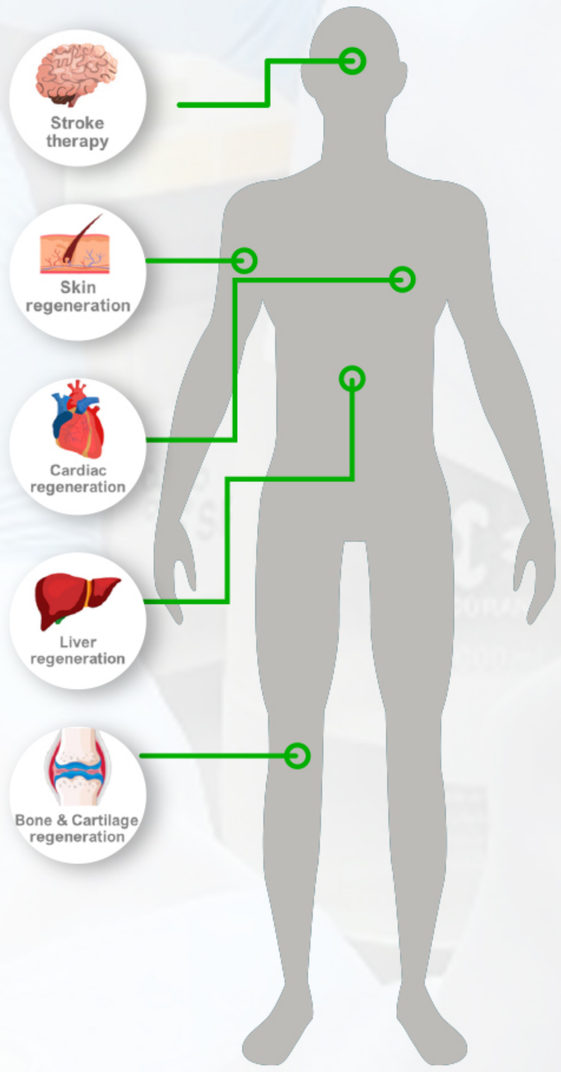


Therapeutic applications



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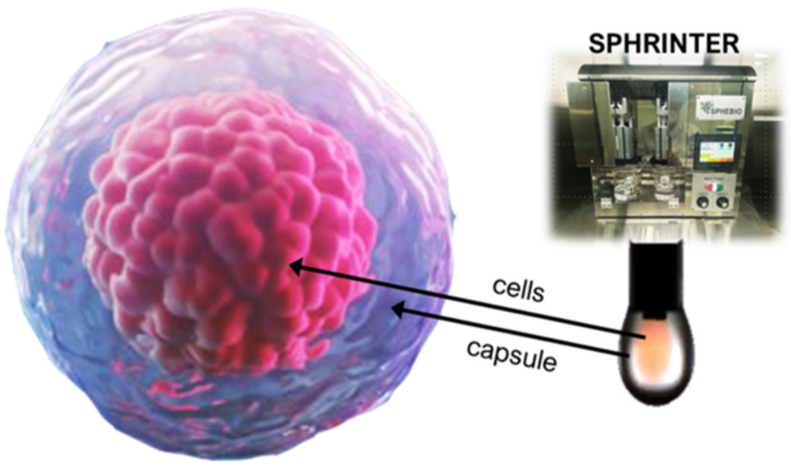

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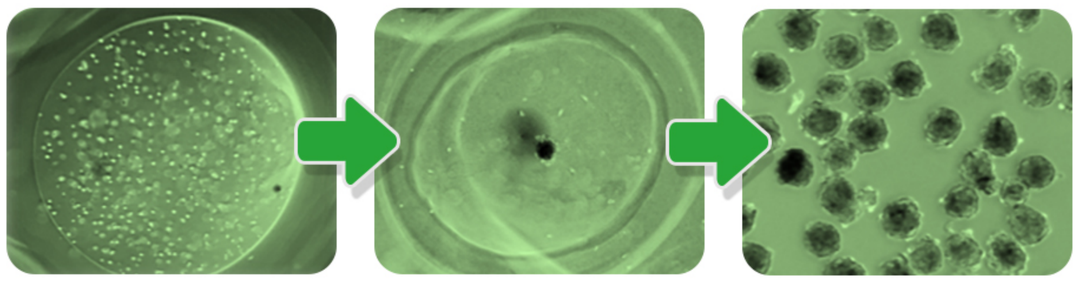

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Reference 1. 퓨레곤펜주 허가사항. 식품의약품안전처

Selected Safety Information

[제품명] 퓨레곤펜주(폴리트로핀베타,재조합난포자극호르몬) **[효능•효과]** 1. 여성: 다음과 같은 임상적 상황에서 여성의 불임증 치료 - Clomiphene citrate으로 치료되지 않은 여성의 무배란증(다낭성 난소 증후군 PCOS 포함) - 보조생식프로그램(즉 in vitro fertilization/embryo transfer : IVF/ET, gamete intra-fallopian transfer :GIFT, intracytoplasmic sperm injection : ICSI) 실시중 다수의 난포를 성숙시키기 위한 조절된 난소과자극(controlled ovarian hyperstimulation) 2. 남성: 저성선자극호르몬성 성선부전에 의한 정자형성의 결핍증 **[용법•용량] 일반적 지침:** 이 약의 투여는 불임 질환의 치료에 경험이 있는 의사의 감독 하에 실시해야 한다. 첫 투여는 직접적인 의학적 감독 하에 수행되어야 한다. 이 약은 조절된 양이 정확하게 투여되는 정밀한 의약품 주입용 기구인 펜 주입기에 장착하여 사용하는 것으로서, 임상시험 결과 통상적으로 사용되는 주사기와 비교할 때 평균 18% 많은 양이 투여되는 것으로 나타났다. 그러므로 한 치료주기 내에서 주사기에서 펜 주입기로, 펜 주입기에서 주사기로 교체할 때에는 특별한 주의를 요한다. 특히 주사기에서 펜으로 교체할 때에는 고용량이 투여되는 것을 방지하기 위해서 용량 조절이 필요할 수 있다. **용량: 1. 여성:** 외인성 성선자극호르몬(gonadotrophins)에 대한 난소의 반응은 개인 간(inter-), 개인적(intra-individuals)으로 큰 차이가 있으므로 일정한 용량 스케줄을 잡는 것이 불가능하다. 그러므로 용량은 난소 반응에 따라 개별적으로 조절되어야 하며 이때 난포 성숙에 대한 초음파 촬영이 필요하다. – 무배란: 연속적 투여(sequential treatment scheme)는 매일 이 약 50IU의 투여 개시가 추천되며 이 개시 용량을 적어도 7일 동안 유지한다. 난소 반응이 없으면 난포 성장과/혹은 혈청 에스트라디올 농도로 적절한 효력 반응이 나타날 때까지 1일 용량을 점차적으로 증가시킨다. - 보조생식술(ART)에서 조절된 난소 과자극: 여러 가지 자극 프로토콜이 사용된다. 적어도 첫 4일 동안 150-225IU의 개시 용량이 추천된다. 그 후의 용량은 난소의 반응 정도에 따라 개별적으로 조절될 수 있다. 더 긴 투여 기간이 필요할 수 있음에도 불구하고, 임상시험에서는 6-12일 동안 75-375IU의 유지 용량으로 충분함을 보여주었다. **2. 남성:** 이 약은 hCG와 병용하여 주당 450IU의 용량을 투여하되 가급적이면 150IU 단위의 용량으로 3회로 나누어 투여해야 한다. 이 약과 hCG의 병용투여는 정자 형성의 개선을 어느 정도 기대할 수 있을 때까지 최소 3-4 개월까지 계속해야 한다. 반응을 평가하기 위해, 이 약의 투여를 시작한 4-6개월 후에 정액 분석을 실시하는 것이 권장된다. **용법** 본 제품은 펜 주입기(의약품 주입용 기구)에 끼워서 사용토록 고안되었고, 피하주사를 해야 한다. 투여 부위의 지방성 위축(lipoatrophy)을 방지하기 위해 동일 부위에 반복 투여하지 않도록 한다. 의사에 의해서 적절한 교육을 받은 환자 또는 보호자는 펜 주입기를 사용하여 이 약을 주사할 수 있다. 이 약의 자가 투여는 적극적이고 훈련이 잘 되었으며 전문가의 충고에 잘 따르는 환자만이 시행해야 한다. (자세한 사항은 허가사항 용법, 용량 항 참조) **[경고]** 뱀질 알코올은 조숙아에게서 치명적인 가쁜 호흡증상과 연관이 있는 것으로 보고되었다. **[금기] 다음 환자에는 투여하지 말 것- 남성 및 여성** 1) 이 약의 주성분이나 부형제에 과민성이 있는 환자 2) 난소, 유방, 자궁, 고환, 뇌하수체 또는 시상하부에 종양이 있는 환자 3) 원발성 성선부전 환자 – 여성 1) 임신 2) 원인이 밝혀지지 않은 질출혈을 보이는 환자 3) 다낭성 난소증후군(PCOS)과 무관한 난소낭종이나 비대된 난소가 있는 경우 4) 임신이 부적합한 생식기관 기형 환자 5) 임신이 부적합한 자궁 섬유양 종양(fibroid tumours) 환자 **[신중투여] 다음 환자에는 신중히 투여할 것** (1) 이 약은 미량의 스트렙토마이신 및/또는 네오마이신이 포함될 수 있다. 이러한 항생제는 감수성이 있는 환자에서 과민성 반응을 유발시킬 수 있다. (2) 치료를 시작하기 전에 부부에게 불임증이 있는지 적절히 진단되어야 한다. 특히 환자들은 갑상샘 저하증, 부신피질 기능부전, 고프로락틴혈증, 뇌하수체 및 시상하부에 종양이 있는지 확인되어야 하며, 질환에 대한 적절한 치료가 이루어져야 한다. **[약물유해반응]** 임상시험에서 이 약을 투여받은 모든 환자군의 3%는 근육 내 주사하거나 피하주사할 때 투여 부위에 국소적 반응이 나타날 수 있으며 대부분이 사실상 경증이고 일시적이었다. 이 약을 투여받은 모든 환자군의 약 0.2%에서 전신적 과민성 반응이 때때로 관찰되었다. **[일반적 주의]** 1) 이 약을 포함한 모든 성선자극호르몬 제제 요법에 대하여 다태 임신과 출산이 보고되었다. 2) 보조생식술(ART) 후 선천 기형의 발생률이 자연 임신보다 약간 높을 수 있다. 3) 이 약을 포함한 성선자극호르몬 치료에서 혈전색전증이 보고되었다. 이 이상반응은 난소과자극증후군과 연관되거나 연관이 없는 사람 모두에게서 보고되었다. 4) 다양한 불임 치료 방법을 시도한 여성에게서 난소와 기타 생식계 종양(악성 및 양성)이 보고되었다. 성선자극호르몬 요법이 불임 여성의 이러한 종양을 증가시키는지 여부는 확인되지 않았다. 5) 이 약을 사용한 치료를 시작하기 전에 임신이 금기인 의학적 조건인지 먼저 평가되어야 한다. 6) 남성에게서 내인성 FSH의 수치의 상승은 원발성 고환부전을 나타낸다. 이런 환자들은 이 약 및 hCG 요법에 반응하지 않는다. **[상호작용]** 1) 이 약과 clomiphene citrate의 병용 투여는 난포 반응을 증가시킬 수 있다. 2) 성선자극분비호르몬 효능약(GnRH agonist)으로 유도된 뇌하수체 탈감작 후에는 적절한 난포 반응을 얻기 위해이 약의 고용량이 필요할 수도 있다. 3) 적합성 자료가 없으므로 이 약은 다른 의약품과 혼합해서는 안 된다. **[임부, 수유부] 1) 임부** 임신 중에 이 약을 사용해서는 안 된다. 임신 중 이 약에 우연히 노출되었을 경우에 대한 임상 자료가 충분치 않아 최기형성 효과를 배제할 수 없다. **2) 수유부** 임상 또는 동물 시험에서 이 약이 유즙으로 분비되었다는 자료는 없다. 이 약은 분자량이 높으므로 인체 내에서 유즙으로 분비되는 것처럼 보이지 않으나, 만약 유즙으로 분비된다면 태아의 위장관에서 분해된다. 이 약은 유즙 생성에 영향을 줄 수 있다. **[소아]** 이 약을 소아에게 투여하는 것은 적절하지 않다. 작성일자: 2021년 5월 17일 ※ **퓨레곤펜주를 처방하시기 전에 제품설명서를 참조하시기 바랍니다.**

KR-PUR-110014 12/2024

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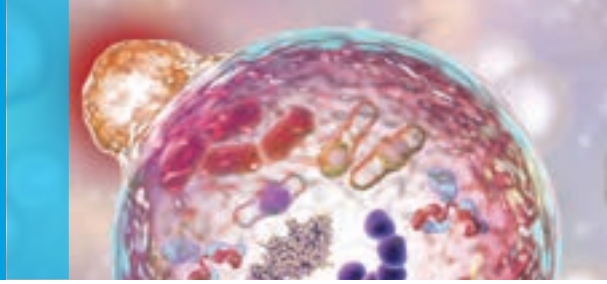

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