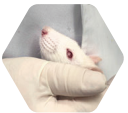


글로벌 기업과
함께 하는
신뢰받는
비임상 CRO

나손사이언스는 신약 후보물질의 기전 연구 및 약효 평가를 위한
세포 기반 분석부터 in vivo 실험까지 통합 솔루션을 제공합니다.

Core Services



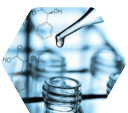
특화 모델 구축 (Specialized Models)

- 5XFAD/ α -Syn/SOD1-G93A TG
- 퇴행성/안구/통증/심혈관/면역/대사 질환 모델
- 맞춤형 모델 개발



효능 평가 (Efficacy Testing)

- 질병 특이적 세포/동물 모델 기반 약효 평가
- 타겟 기반 유효성 검증 수행



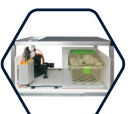
약동학/약력학 분석 (PK/PD)

- In vivo 약물동태학/약력학 분석
- 최적화된 약물 전달 시스템 제공



작용 기전 연구 (MoA)

- 바이오마커/유전자 발현 분석
- IHC 자동화 기술 기반 정량적 조직 분석



Home Cage Analysis (HCA)

- 스트레스 최소화 환경에서의 행동 분석 시스템
- 객관적·정량적 행동지표 확보

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- 글로벌 제약사와의 풍부한 연구 경험
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- IHC 자동화 기반 정량적 조직 분석 서비스 제공
- Home Cage 기반 비침습적 행동분석 가능
- 고객 맞춤형 유연한 협업 체계

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설국농장의 한국유전자종축센터는 첨단 생명공학 기술을 바탕으로
우수 형질의 종모우를 개발·생산·보급하는 **종축 전문 기업**입니다.

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- 250두 규모 '농장형 연구소'의 실증 데이터
- 전북 장수군 '저탄소한우 산업지구' 기반 저탄소 한우 산업 선도 및 글로벌 파트너십
- 농가 소득 증대를 이끄는 혁신 파트너



13년의 집념, 독보적 종모우 KGBC0001

- **2014.03** OPU(생체 난자 흡입술) 본격 활용
- **2015.03** 독자적 우수 혈통 구축 및 초대형 공란우(1,100kg급) 선발·수정란 생산
- **2016.06** 슈퍼한우 생산 성공(육량·육질 동시 개선 쾌거)
- **2025.09** 설국농장 종모우 'KGBC0001' 2025년 하반기 한우 당대검정 선발

- **생산지** 전북 장수(설국농장)
- **생년월일** 2024-09-12
- **등록번호** 235203964



혈통

부 KPN 1459

조부 KPN1216
조모 228093124

모 234405990

외조부 KPN1416
외조모 231398740

생산형질 유전능력

항목	EPD	Acc
냉도 체중 (kg)	54.8	75%
배최장근단면적 (cm ²)	10.24	70%
등지방두께 (mm)	-1.02	69%
근내지방도(점)	1.17	71%

체형 유전능력(12개월령, cm)

항목	체고	십자	체장	흉심	흉폭	고장	요각	곤폭	좌골	흉위
EPD	4.44	4.59	6.04	1.81	2.15	2.2	1.96	2.52	1.45	5.87

분할육(비율) 유전능력(%)

항목	안심	등심	채끝	목심	앞다리	우둔	설도	사태	양지	갈비
EPD	0.02	0.09	0.06	0.12	0.27	0.27	0.39	0.17	0.14	-0.23



크로넥스는 바이오 의료기기, 신약개발에 필요한 다양한 서비스를 제공합니다.

계약 위탁 연구 서비스



- 고객 맞춤형 시험 디자인
- 시험 기술 지원 일체
- 보고서 작성
- 시험 검체 샘플링

시험분석 서비스



- X-ray
- 초음파
- 생리활성 분석
- 이화학분석
- 조직 분석
- Micro-CT
- MRI

실험동물 공급



- 미니 피그
- 일반 돼지
- 비글
- 일반견(Mongrel)
- 토끼

실험동물 위탁관리



- 동물종 맞춤 사육 시설
- Animal Care 서비스

주요 비임상효능평가 분야

치과 분야



적용 분야

- 발 치: Premolar, Molar
- 급 이: 발치 후 유동식을 급이하여 동물의 섭식에 이상이 없도록 관리
- 평 가: 발치 전 후 및 시험기간에 x-ray 촬영을 실시하여 진행상태를 확인
- 조직검사: 전문기관과 협력하여 조직평가를 진행

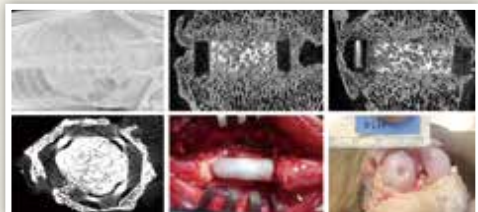
피부 분야



적용 분야

- 약물, 화장품의 피부투과 모델
- 고주파치료기, 레이저 치료기
- 패취형 약물 시험
- 생물학적 드레싱재
- 창상피복재

외과 분야



적용 분야

- 척추 이식물 효능평가
- Bone defect 골이식재 효능평가
- 연골재생 효능평가
- X-RAY, Micro-CT, Histology

제약 분야



적용 분야

- 약물 효능평가
- 약물동력학(PK) 시험

기타분야

- 알츠하이머: 질환모델, 행동평가
- 당뇨: 당뇨유발, 약물 치유효과
- 골다공증: 골다공증 질환모델



크로넥스는 고객의 작은 가치도 소중하게 생각합니다

크로넥스(주)

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TEL: 031-352-7669 / FAX: 031-357-4067

주소: 충북 청주시 흥덕구 강내면 황탄리길 110
TEL: 043-716-2300 / FAX: 043-716-2301

www.cronex.co.kr



비임상시험과 의료기기 효능평가에 최적화된 실험동물은 크로넥스 M-Pig®입니다!

고품질의 실험용 미니피그!! 합리적인 가격으로 경험해 보십시오~

크로넥스는 병성 검증 및 유전자 판별기법을 통해 선별된 고품질의 SPF(특정병원균부재) 미니피그를 생산·공급합니다. 또한 질환동물 개발 및 이종장기 연구에 적합한 동물 원종을 나고야 의정서에 준하여 제공할 수 있습니다.

◆General Biological Profile

Criterion/Parameter	Minipigs(CRONEX)	Conventional Swine
Birth weight (kg)	0.6 ~ 0.8	1.3+
2-year old weight (full maturity), kg	45 ~ 60	150 ~ 200
Life span/longevity (years)	10 ~ 15	12 ~ 15
Gestation period (pregnancy), days	111 ~ 114	~ 114
Litter size (no. of piglets)	5 ~ 9	9 ~ 11
Estrous Cycle (days)	18 ~ 24	~ 21
Sexual maturity onset	3 ~ 5	4 ~ 6

크로넥스는 최고품질의 실험동물을 생산·공급하기 위해 국내 최고 수준의 생산 설비 구축과 함께 엄격한 생산 관리 기준에 따라 안전하고 효율적으로 관리·운영되고 있습니다.



◆병성검사 목록

바이러스 검사	박테리아 검사	기생충 검사
PRRS (생식기 호흡기 증후군)	Mycoplasma hyopneumoniae	Mites
CFS (돼지열병)	Brucella	Lous
FMDV (구제역)	Erysipelothrix rhusiopathiae	Ticks
PRV (오제스키)	Haemophilus	
JEV (일본뇌염)	Lawsonia intracellularis	
PPV (파보 바이러스 감염증)		
TGEV / Circovirus		

크로넥스는 AAALAC 기준의 시설에서 모든 동물을 관리하고 있으며 각 실험에 적합한 관리 서비스를 제공하고 있습니다.

실험동물 국제관리 기준 GLP, AAALAC 적합 시설

Criteria	CRONEX	GLP, AAALAC
Temperature (°C)	24±1	18~28
Humidity (%)	50±5	40~60
Class (particles/cm³)	<10,000	<10,000
Air Exchanges (시간당 횟수)	15	10~15
Pressure	Positive	Positive
Noise (dB)	10	<60
Light Intensity (lux)	200	150~300



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[용법·용량] 첫 1개월 간 매주 1회, 2개월 간 2주에 1회, 이후 증상 완화될 때까지 매달 1회 [분류] 의료기기/비급여 [포장 단위] 1 prefilled syringe/박스

(주)한국팜비오

본사 : 서울시 서초구 논현로 83 공장 : 충북 충주시 대소원면 첨단산업9로 36 연구소 : 경기도 성남시 중원구 사기막골로 45번길
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글로벌 규제 기준에 맞춘
품질 관리 시스템



확장 가능한 생산 시스템

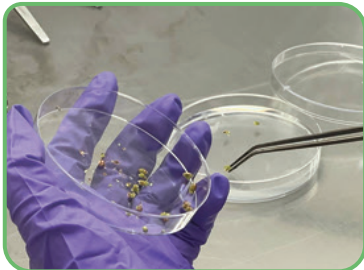
Lab-scale부터
산업화 규모까지 유연한 확장



전문성과 신뢰의 파트너십

풍부한 경험의 전문가들이
성공적인 개발을 지원

OUR PROCESS



01 세포주 개발

우수형질 스크리닝
고생산성 세포주 선별
세포주 특성 분석



02 바코드기반 세포주 관리

바코드 기반 세포주 추적관리
계대일자, 작업자 이력관리



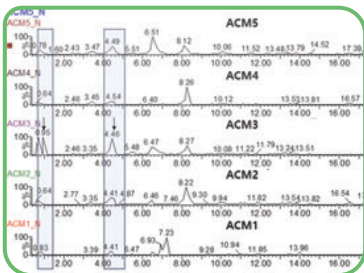
03 배양공정 개발

배지 및 배양 조건 최적화
Scale-up 및 공정 검증
배양 공정 모니터링



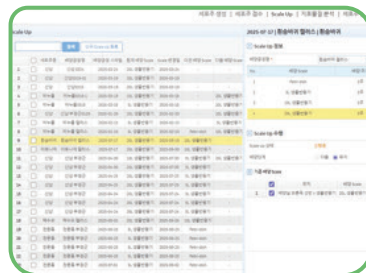
04 대량 배양

대용량 배양 운영
최적의 수용 및 일관성 확보
공정 데이터 관리



05 분석 및 품질 관리

세포주 성분프로파일링
정량데이터기반 품질 평가



06 웹기반 세포주 모니터링

자사 솔루션 NAOS 적용
실시간 배양공정 모니터링

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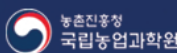
28161 충북 청주시 흥덕구 오송읍 의료단지길 116 Tel. 070-4060-7394 E-mail. 2015cocoon@gmail.com

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생명자원의 발굴·보존·분석·생산 전 과정을 아우르는
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한국발생생물학회
Korean Society of Developmental Biology

Program

Convergent Embryology: Bridging Organoids and ICT for Precision Regeneration

October 15 (Thu)

Ocean Suites Jeju Hotel, Canola Hall

Time	Title	Chair & Speaker
09:00-09:30	Opening Ceremony	President: Sang-Hwan Hyun
		Congratulatory Remarks: President of Jeju National University
	Animal Medical & ICT Convergence Talent Training Center	Chair: Prof. Eui-Jong Lee (Chungbuk National University)
09:40-10:00	Synchrotron Radiation & AI-Driven Bio-Imaging: From Micro-Structural Analysis to Potential Organoid Applications	Prof. Nam-Soon Lee (Chungbuk National University)
10:00-10:20	Public Data-Driven Analysis of Reproductive Toxicity and Genital Disease: Case Studies of Micro/Nanoplastics and Canine Pyometra	Prof. Young-Bum Son (Chonnam National University)
10:20-10:40	Coffee Break	
10:40-11:00	Development of AI Analysis Technology for Thyroid Scintigraphy	Eunseo Kang (Chungbuk National University)
11:00-11:20	LMT-Agarose based room temperature storage as a practical alternative to cryogenic transport of canine mesenchymal stem cells	Su-Hyun Song (Chonnam National University)
11:20-11:40	FGF2/BMP4-Induced Heterogeneous Extraembryonic Lineage Priming in Porcine Expanded Potential Stem Cells and Cardiac Lineage Characteristics in 3D Culture	Haneul Kim (Gyeongsang National University)
11:40-13:00	Lunch	
	Keynote Lecture I	Chair: Prof. Sung-Hwan Moon (Chung-Ang University)
13:00-13:40	Dissecting functional diversities of human sensory neurons to develop new cell and gene therapy platforms for pain disorders	Prof. Gabsang Lee (Johns Hopkins School of Medicine)
	Regular Member Symposium	Chair: Prof. Sung-Hwan Moon (Chung-Ang University)
13:40-14:05	Introduction to SW·AI Applications in Biology Research	Prof. Eui-Jong Lee (Chungbuk National University)
14:05-14:30	A primary tissue-derived zebrafish liver organoid platform resolves cellular dynamics across homeostatic, regenerative, and oncogenic states	Prof. Tae-Young Choi (Wonkwang University)
	Keynote Lecture II	Chair: Prof. Woo-Sung Kwon (Kyungpook National University)
14:30-15:10	Aware Modeling of Full-Length Transcriptomes and Its Application to Ovarian Cancer	Youngjun Piao (School of Medicine Nankai University)
	Young Scientist Symposium I	Chair: Prof. Woo-Sung Kwon (Kyungpook National University)
15:10-15:35	Development of Next-Generation Cardiac Assist Devices and Heart Failure Animal Models for Preclinical Application	Prof. Junho Moon (Jeonbuk National University)
15:35-16:00	Made to be Broken: The Architecture and Functions of Skin Fracture Lattice in Spiny Mice	Prof. Hansol Yang (KAIST)
	Special Lecture	Chair: Prof. Woo-Sung Kwon (Kyungpook National University)
16:00-17:00	Jeju Language and Culture	Dr. Soonja Kim
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Program

Convergent Embryology: Bridging Organoids and ICT for Precision Regeneration

October 16 (Fri)

Ocean Suites Jeju Hotel, Canola Hall

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	Regular Member Symposium	Chair: Prof. Tae-Joo Park (UNIST)
09:40-10:05	Product Development and Industrial Application Based on an Aquatic Animal Cell Culture Platform	Dr. Sang-Yoon Lee (CellQua)
	Young Scientist Symposium II	Chair: Prof. Tae-Joo Park (UNIST)
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Program

Convergent Embryology: Bridging Organoids and ICT for Precision Regeneration

October 16 (Fri)

Ocean Suites Jeju Hotel, Canola Hall

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Ocean Suites Jeju Hotel, Camellia Hall

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Jeju National University, Ocean Hall

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09:40-10:20	Effect of dietary supplementation of spirulina and spirulina byproduct on growth performance, feed utilization, and health in olive flounder (Paralichthys olivaceus)	Dr. Byung-Hoon Kim (Jeju National University)
10:20-11:00	Evaluation of Microalgae-Derived Extracellular Vesicles as a Drug Delivery System during Early Development of Aquatic Organisms	Dr. Woon-Yong Choi (KIOST)

Abstracts

**Convergent Embryology: Bridging Organoids and ICT
for Precision Regeneration**

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Keynote Lecture I

Dissecting functional diversities of human sensory neurons to develop new cell and gene therapy platforms for pain disorders

Prof. Gabsang Lee

Johns Hopkins School of Medicine

Pain and itch are subtypes of somatosensation that can be evoked by a wide range of chemical, thermal, and mechanical stimuli and are sensed by nociceptive (pain-sensing) and pruriceptive (itch-sensing) neurons in the dorsal root ganglion. Efforts to develop novel non-opioid analgesics have been largely unsuccessful despite the promise shown in heterologous expression systems and animal models. This is partly because these models do not recapitulate the activity of neurons in their native environment, and due to species-specific differences in ligand-receptor activity of key molecules involved in pain signaling. Additionally, pain arises from the interplay of inflammatory signals that activate and sensitize nociceptors within injured tissues. Most analgesics fail clinically due to their mono-targeted mechanisms. Human pluripotent stem cells (hPSCs) have emerged as a new cell source for pain treatment research because they produce large quantities of cells that are otherwise hard to obtain. Here, we report the generation of purifiable populations of human transient receptor potential cation channel subfamily V member 1 (TRPV1)⁺, sodium voltage-gated channel alpha subunit 9 (SCN9A)⁺, and Mas-related G protein-coupled receptor X1 (MRGPRX1)⁺ neurons from hPSCs. Our functional studies show distinct functional polymodality of the three subsets. Additionally, we have also constructed a transcriptomic continuum of developing hPSC-derived primary afferents and validated that mature hPSC-derived sensory neurons (hPSC-SNs) recapitulate key molecular signatures of human DRG neurons. Transcriptomic and functional profiling revealed that CD200^{high} hPSC-SNs closely resemble human nociceptors, expressing pain-relevant receptors and ion channels. Strikingly, ectopic transplantation of CD200^{high} hPSC-SNs into the knee joint of osteoarthritic mice reduced pain and promoted bone and cartilage repair, whereas CD200^{low} cells exhibited no benefit. Mechanistically, human and mouse proteomics suggest that CD200^{high} hPSC-SNs sequester inflammatory ligands while secreting reparative factors in joint tissues. These findings uncover a fundamental role of nociceptors in tissue repair, providing a multi-targeted, disease-modifying strategy for OA and chronic pain. Lastly, by exploiting the differentially expressed pathway regulators and membrane receptors among these three subsets, we identified voltage-gated channel subfamily Q member 2 (KCNQ2) as a novel pain target and installed a protective mutation in this gene using adeno-associated virus (AAV) delivery of CRISPR-Cas9 constructs. Results demonstrate that this missense mutation confers robust pain-resilient effects in mature hPSC-SNs in mouse and human system. In summary, we have generated a humanized platform that exploits the distinct molecular profiles of human nociceptor subtypes and developed cell and gene therapy strategies for managing chronic pain.

Keynote Lecture II

Aware Modeling of Full-Length Transcriptomes and Its Application to Ovarian Cancer

Youngjun Piao

School of Medicine Nankai University

Recent advances in long-read sequencing have enabled direct observation of full-length transcript isoforms, providing new opportunities to study alternative splicing, alternative polyadenylation, and transcript-level heterogeneity. However, accurate interpretation of full-length transcriptomes remains challenging because regulatory signals can act over long genomic distances and conventional sequence models often have difficulty capturing both local sequence motifs and broader contextual dependencies. In this study, we propose a context-aware modeling framework for full-length transcriptome analysis based on a hybrid state-space model and attention architecture. The framework is designed to integrate local regulatory patterns with long-range sequence context while maintaining computational efficiency for long biological sequences. Building on this modeling strategy, we develop an analysis platform for the characterization of alternative splicing and alternative polyadenylation, including splice-site modeling, transcript structure analysis, quantification, and visualization. We also present our application of this framework to ovarian cancer, where long-read transcriptomic data are used to investigate cancer-associated isoform diversity and transcript processing patterns. Overall, this study highlights the potential of hybrid AI models and long-read sequencing to advance transcript-level analysis and precision cancer genomics.

Keynote Lecture III

Getting competence for early embryo development in mammals: From folliculogenesis to implantation

Prof. Yong-Pil Cheon

Sungshin University

Early stage embryo development of mammals is markedly different from that of other animals, due to the embryo implantation and uterine support for continuous embryo development. During the cleavage stage, the embryo should reach the blastocyst stage, which prepared or acquired the competence for the recognition of the prepared endometrial sites and gastrulation. For such successful cleavage, the mature oocytes should acquire a suitable accumulation of various factors, including determinants and nutrients during folliculogenesis and maturation both in nucleus and cytoplasm. After ovulation, the mature oocyte is fertilized with sperm to acquire totipotency. It is known that implantation of the blastocyst is accomplished by the systemic regulation of steroid hormones, E2 and P4. During these processes, it has been suggested that the environmental factors are harnessed to embryos. It may be accomplished through different chains as compensation and ongoing development. Such adaptive development of the early embryo is one of the interesting areas of my laboratory, and the mechanisms involved have been studied with the mammalian system. In here, as the adaptive factors, steroid hormones and decidualization were introduced along with some extracellular matrix substances and genes of the embryo and endometrium. These studies will extend the understanding of early embryo development and advance in health care.

Key words: early stage embryo development, competence, adaptive development, decidualization, steroid hormone

Acknowledgement: This research was supported by grants from the National Research Foundation of Korea(NRF) funded by a Korea government (RS-2023-NR077285, (Old) NRF-2023R1A2C3007223)

Keynote Lecture IV

Contextual Artificial Neural Networks for the Rapid Classification of Gene Sequences

Maciej Huk

Wrocław University of Science and Technology

The talk presents the usage of contextual neural networks for rapid classification of gene sequences. It is concentrated on the example of protein toxicity prediction and uncovers ideas behind obtaining state-of-the-art results thousands of times faster than deep learning models analysed in the literature. Potential applications of the presented technique include not only near-real-time medical diagnostics and embedded water bio-safety sensors, but also a multitude of other tasks in which very-low-latency, energy-restricted information processing is crucial. Finally, observations of the proposed models provide important insights into the organization of information processing in living creature.

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² Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju, Korea
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¹Department of Marine Life Science, Jeju National University, Jeju, 63243, Republic of Korea

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Functional Characterization of a Novel HGSNAT Variant: Towards Understanding RP73 Pathogenesis

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Retinitis Pigmentosa (RP) represents a clinically and genetically heterogeneous group of inherited retinal dystrophies characterized by chronic photoreceptor degeneration, leading to progressive vision loss ranging from night blindness to central visual impairment. RP73 is a rare autosomal recessive nonsyndromic form of RP caused by mutations in HGSNAT, which encodes a lysosomal enzyme critical for heparan sulfate degradation. While genetic sequencing identifies numerous HGSNAT variants, their functional consequences often remain elusive, complicating definitive diagnosis and leaving the underlying pathogenic mechanisms poorly understood. Therefore, functional validation is required to bridge the gap between genetic discovery and clinical application. Using *Xenopus laevis* embryos as an in vivo model, we injected a translation-blocking morpholino oligonucleotide (MO) targeting the HGSNAT start codon into the left dorsal blastomere at the 8-cell stage to induce targeted knockdown in the eye fields. To validate variant pathogenicity, we co-injected either wild-type or VUS-carrying HGSNAT mRNA, engineered with synonymous codons to evade MO-mediated inhibition while maintaining the original amino acid sequence. Loss of HGSNAT severely disrupted the laminar organization of the retina. While co-injection of wild-type mRNA effectively restored these structural defects, the variant mRNA failed to provide similar recovery, leaving the retinal layers significantly disorganized. In conclusion, our functional characterization provides compelling in vivo evidence that the identified HGSNAT VUS are indeed pathogenic, establishing a critical foothold to elucidate how lysosomal dysfunction drives retinal dysmorphogenesis.

Retinitis Pigmentosa, *Xenopus laevis*, HGSNAT, VUS

Steroid hormones involved in Prnp expression regulation in reproductive organs

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Specific gene transcription occurs under the regulation of cis and trans factors. DNA methylation, one of the cis factors, plays a crucial role in the access of transcription regulators to specific elements and is essential for development. The Pmp gene is expressed in various cell types regardless of prion disease and exhibits sex differences in prion diseases. However, the physiological role of Pmp expression and its regulatory mechanisms have not yet been fully elucidated. To determine whether sex steroid hormones and endometrial differentiation are involved in Pmp expression, we analyzed the expression patterns of Pmp transcript in reproductive organs and DNA methylation patterns. Additionally, we analyzed the expression of Prnd and Spn in ZH3 KO mice. Real-time PCR, Western blot, and non-sulfite sequencing techniques were employed. Pmp mRNA transcript levels fluctuated cyclically during the estrous cycle, peaking during the estrous stage. Western blot analysis confirmed that PrPC protein expression exhibited the same pattern as that of mRNA. DNA methylation patterns in the promoter and enhancer region (including CpG islands) were different between stages in the examined organs. PrPC expression was also changed in uterus by pregnancy and endometrial differentiation. In ovariectomized mouse models, the expression levels of Pmp mRNA and PrPC were induced by a single injection of steroids and inhibited by the antagonists. However, the expression profiles were altered by the steroid combination. These results suggest that Pmp expression is regulated by sex steroid hormones and that DNA methylation also plays a crucial role in its regulation in reproductive organs.

Key words: Sex steroid hormones, Pmp expression, Methylation, Ovary, Uterus

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Spatiotemporal Expression and Evolutionary Mechanisms of Fragmented Hox Gene Clusters in the Leech, *Helobdella austinensis*

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Hox genes are a highly conserved family of homeodomain transcription factors necessary for determining regional identity and segmentation along the anteroposterior (AP) axis during animal development. In well-known groups such as the Deuterostomia and the Ecdysozoa, Hox genes are generally found in ordered groups on chromosomes and show spatiotemporal collinearity: the physical order of the genes matches the timing and place of their expression. By contrast, in the Lophotrochozoa and especially in annelids like leeches, recent genomic studies have shown that these ancestral gene clusters have become extremely fragmented as a result of rapid genome rearrangements. The leech *Helobdella austinensis*, which is a strong model organism in the field of evolutionary developmental biology (Evo-Devo) because of its direct development, the fact that its embryos are available all year round, and its suitability for embryonic manipulation, has 17 Hox genes that are scattered throughout the genome in the form of incomplete clusters or as single genes, including several duplications and deletions.

To determine whether this severe disruption of gene structure affects the regulation of developmental genes, the current study examined the spatial and temporal expression patterns of certain Hox genes (Scr, Lab, and Lox) in *H. austinensis* embryos. The findings show that, even with extreme genomic fragmentation and in the absence of an ordered cluster, the spatiotemporal expression patterns of these Hox genes are still conserved and are not negatively influenced by the disrupted genomic organization. Furthermore, analyzing duplicated Hox genes in this species offers valuable insights into the evolutionary fate of gene duplicates, making it possible to determine whether they acquire new functions, partition the original functions, or lose functionality. The research thus separates physical gene organization from developmental regulation and has important implications for our understanding of AP-axis evolution and segmentation in Lophotrochozoa.

Keyword: Hox, Leech, Lophotrochozoa, Segmentation, Anteroposterior (AP) axis

Morphological and Transcriptomic Dynamics of Bidirectional Regeneration in *Perionyx excavatus*

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The earthworm *Perionyx excavatus* is a model organism for studying regeneration because of its regeneration capacity. It can regenerate its entire body parts, including the complex anterior structures such as the central nervous system, heart, circulatory system, and clitellum. This study investigated the histological and molecular mechanisms underlying bidirectional regeneration of *Perionyx excavatus*. To characterize regeneration in *P. excavatus*, we examined its regeneration process using scanning electron microscopy (SEM) and histological sections. SEM observations showed sequential morphological changes from wound closure to blastema formation and the re-establishment of anterior and posterior structures. Histological sections were used to examine the corresponding internal tissue organization. To analyze molecular mechanisms of regeneration, we also performed bulk RNA-seq on head and tail parts of *P. excavatus*. We extracted total RNA from each stage, prepared sequencing libraries, and performed sequencing; we mapped the reads to the reference genome to quantify expression levels for each gene locus, enabling identification of stage-specific expression changes. Analysis of the expression levels of Yamanaka reprogramming factors (Oct4, Sox2, and Klf4) and organogenesis-related factors in both the head and tail regions showed that these key regenerative gene clusters were highly expressed during the early stage of regeneration, followed by a subsequent decrease in expression. We then identified gene clusters that show specifically high expression levels in the head and tail regions, respectively. Hemerythrin and CD109 were identified as head-specific genes, while Evx1 and tap1 were identified as tail-specific genes. To validate the *in silico* results, we performed fluorescence in situ hybridization (FISH) using RNA probes. In FISH, Yamanaka factors showed broad expression throughout the regeneration region at 5 dpa (day post-amputation), followed by expression in specific cell masses and regenerating intestinal tissue at 7 dpa, when tissue differentiation occurs. Similarly, FISH for the head-specific genes (Hemerythrin, CD109) and the tail-specific gene (Evx1) showed broad expression at 5 dpa, while by 7 dpa, expression was concentrated in specific regions. These findings suggest that spatiotemporally regulated gene expression plays an important role in tissue reorganization and differentiation during bidirectional regeneration in *P. excavatus*.

Epithelial Remodeling during Seam Resolution in Chick Cranial Neural Tube Closure

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Unlike scar formation after adult wound healing, the seam formed during embryonic tissue closure (e.g., neural tube closure, body wall closure, etc.) completely disappears. During neural tube closure, bilateral neural folds approach and fuse, generating a transient seam between the non-neural ectoderm (NNE) at the dorsal midline. This seam subsequently resolves as the bilateral epithelia become integrated into a continuous epithelial sheet. However, the cellular and molecular mechanisms underlying seam resolution remain poorly understood. In this study, we characterize the spatiotemporal dynamics of NNE seam formation and resolution and examine associated changes in cell morphology, F-actin organization, and extracellular matrix (ECM) architecture in the cranial region of chick embryos extending to the level of the first somite. Spatiotemporal analysis of the seam region revealed that epithelial fusion and subsequent seam resolution occurred as temporally distinct, sequential events. Seam resolution was first detected near the central region of the analyzed axis and subsequently expanded in both cranial and caudal directions. In seam-present regions, F-actin exhibited a strong localization along the seam, whereas this structure was no longer observed in seam-resolved regions. NNE cells adjacent to the seam were significantly more elongated than cells located far from the seam and showed a more consistent orientation perpendicular to the seam. In contrast, these spatial differences in cell shape and orientation were no longer detected in seam-resolved regions. Analysis of fibronectin and laminin further showed that their continuity along the basal surface appeared reduced in seam-present regions compared with seam-resolved regions. Together, these findings indicate that the NNE seam formed during cranial neural tube closure is a dynamic epithelial structure associated with distinct F-actin organization, localized cell morphology, and alterations in ECM architecture. The changes in these features associated with seam resolution suggest that coordinated local remodeling of epithelial cells, the actin cytoskeleton, and the surrounding ECM accompanies the integration of bilateral epithelial sheets into a continuous tissue.

Key words: neural tube closure, non-neural ectoderm, epithelial seam, F-actin, extracellular matrix

PAH metabolite 1-hydroxyphenanthrene causes oxidative stress-driven microphthalmia in *Xenopus laevis*

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants, and their hydroxylated metabolites pose elevated toxicological concern due to greater biological reactivity than the parent compounds; however, the developmental hazard of 1-hydroxyphenanthrene (1-OHPhe), the primary phenanthrene metabolite and a validated human exposure biomarker, has not been systematically characterized in a vertebrate embryo model. Using *Xenopus laevis* embryos and the Frog Embryo Teratogenesis Assay (FETAX), we found that 1-OHPhe induced concentration-dependent lethality and malformations ($LC_{50} = 15.49 \mu\text{M}$, $EC_{50} = 10.84 \mu\text{M}$, $TI = 1.43$), with microphthalmia as the most prominent phenotype alongside axial growth retardation, edema, and gut miscoiling. 1-OHPhe significantly reduced eye area and length and disorganized retinal layering, while Whole-mount in situ hybridization (WISH) and qRT-PCR at stages 32 and 41 revealed comprehensive downregulation of the ocular gene network including retinal factors *rx1*, *pax6*, and *six3* and lens markers *cryba1* and *prox1* indicating combined suppression of retinal and lens differentiation. A β -galactosidase lineage-tracing assay further showed impaired migration of eye-fated progenitor cells from the D1.1 blastomere, while HyPer biosensor imaging and TUNEL staining at stage 32 demonstrated elevated reactive oxygen species (ROS) and increased apoptosis in the developing eye field. Together, these findings support a model in which 1-OHPhe induced oxidative stress drives progenitor migration failure and apoptotic depletion of ocular cells, disrupting the ocular transcriptional network and culminating in microphthalmia. This study provides the first systematic in vivo mechanistic characterization of 1-OHPhe developmental toxicity in a vertebrate embryo, establishing hydroxylated PAH metabolites as a developmentally hazardous contaminant class warranting dedicated risk assessment.

Keywords: 1-Hydroxyphenanthrene, polycyclic aromatic hydrocarbon metabolite, *Xenopus laevis*, FETAX, teratogenicity, microphthalmia, eye development

Developmental Toxicity and Teratogenic Effects of Fine Particulate Matter (PM_{2.5}) in *Xenopus laevis* Embryos

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Particulate matter with an aerodynamic diameter of $\leq 2.5 \mu\text{m}$ (PM_{2.5}) is a major air pollutant associated with various adverse health effects. However, its effects on embryonic eye development remain insufficiently understood. In this study, we investigated the developmental toxicity of PM_{2.5}, with a focus on ocular development, using *Xenopus laevis* embryos and the frog embryo teratogenesis assay-Xenopus (FETAX). Embryos were exposed to various concentrations of PM_{2.5} and evaluated using FETAX. Whole-mount in situ hybridization and real-time qPCR were performed to assess developmental gene expression. Cell migration, reactive oxygen species (ROS), and apoptosis were also examined. Exposure to 70 $\mu\text{g/mL}$ PM_{2.5} induced developmental abnormalities, including reduced body and eye growth, edema, small eyes, gut miscoiling, and cardiac edema. Real-time qPCR showed reduced expression of eye development-related genes, including *rx1*, *pax6*, *six3*, *cryba1*, and *prox1*, as well as organ development-related genes, including *myh6*, *ldlrba1*, and *darmin*. PM_{2.5} also impaired eye-directed cell migration and was associated with increased cranial ROS levels and ocular apoptosis. Taken together, these findings demonstrate that PM_{2.5} disrupts multiple developmental processes and induces developmental and teratogenic effects in *Xenopus laevis* embryos.

Keywords : *Xenopus*, PM_{2.5}, FETAX, Teratogenicity, eye development

The Effect of PEG-QD on Improving Sperm Motility in Mouse Sperm Cryopreservation

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Mouse sperm cryopreservation is widely used for the conservation of genetic resources and the maintenance of lineages. However, the physical and chemical stress and excessive reactive oxygen species (ROS) generated during the freezing process can cause structural and functional damage to sperm, thereby reducing sperm quality after thawing. Despite advances in cryoprotectant and freezing methods, it remains challenging to completely prevent structural and functional damage to spermatozoa during cryopreservation. Carbon quantum dots (QDs) are carbon-based nanomaterials with size- and composition-dependent optical and electronic properties, as well as excellent optical properties and high photostability; depending on their composition and surface properties, they may interact with ROS. In particular, polyethylene glycol (PEG)-modified PEGylated-CQD (PEG-QD) can reduce particle aggregation and improve dispersion stability in aqueous solutions by forming a hydrophilic hydration layer. Therefore, this study aimed to evaluate the effect of PEG-QD concentration on the kinematic parameters of frozen mouse sperm and to confirm its potential as a functional additive for mouse sperm cryopreservation. After thawing the spermatozoa, their motility and kinematic characteristics were analyzed using a computer-assisted Sperm analysis (CASA) system. The results demonstrated that PEG-QD treatment significantly affected sperm motility in a concentration-dependent manner, with improvements in velocity-related parameters, including VCL and VSL, compared with the untreated control group. These findings suggest that PEG-QDs may enhance the motility of cryopreserved sperm, with potential applications in assisted reproductive technologies by improving sperm functional competence and potentially contributing to improved fertilization outcomes. Overall, the application of biocompatible PEG-QDs may offer a promising nanomaterial-based approach to support reproductive function and early developmental processes.

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Keywords: mouse spermatozoa, cryopreservation, CASA, sperm motility, quantum dots

Deep cell contribution drives tension-dependent rapid wound healing in early chick embryos

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Embryonic wounds are characterized by rapid and scarless healing. Mechanical cues, such as tissue tension and extracellular matrix stiffness, are increasingly recognized as important regulators of wound healing. However, how these mechanical factors influence embryonic wound repair remains incompletely understood. In this study, we investigate the effects of tissue tension on wound healing in early chick embryos. Wounds generated in the extraembryonic area *opaca* exhibited tension-dependent differences in closure dynamics, with markedly delayed wound closure under increased tension. To investigate the cellular mechanisms, we first examined cell proliferation and actomyosin organization during wound repair under higher and lower tension conditions, which showed no differences between the two conditions. Notably, an actomyosin cable persisted even after wound closure under the lower tension, while cells accumulated within the closed area. These wound-filling cells displayed reduced epithelial characteristics as assessed by immunohistochemistry. Consistent with this observation, cell tracking using DiI labeling and live imaging analysis suggested that cells filling the wound originate from the deeper cell layer. In situ hybridization revealed tension-dependent differences in the expression of *DACT2*, a regulator of Wnt signaling. Pharmacological modulation of Wnt signaling altered wound closure, suggesting a potential role for Wnt signaling in tension-dependent wound healing. Together, our study suggests a non-conventional wound healing mechanism in developing embryos, driven by non-epithelial deep cells and a possible contribution of Wnt signaling. By uncovering how tissue tension influences cellular behavior during embryonic wound repair, this study provides broader insights into mechanically regulated tissue repair and regeneration in epithelia.

Key words: Embryonic wound healing, Mechanical tension, Chick embryo, Actomyosin, Wnt signaling

CD47-Based Enrichment of Ventricular-Like hiPSC-Derived Cardiomyocytes for Functional and Cardiotoxicity Assessment

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Reliable functional and cardiotoxicity assessment using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) requires cell populations with reduced subtype heterogeneity and consistent electrophysiological properties. In this study, we evaluated CD47 as a surface marker for enriching ventricular-like cardiomyocytes generated through three-dimensional hiPSC differentiation. Beating spheroids were collected after 14 days and subjected to CD47-based fluorescence-activated cell sorting. Following sorting, CD47-positive cells increased to 96.28%, while 99.89% cTnT and 85.63% co-expressed CD47 and cTnT. Comparable enrichment was observed in an independent hiPSC line, supporting reproducibility across cell sources. Compared with metabolically purified cardiomyocytes, CD47-purified cells showed less variation in spontaneous contraction intervals and beating frequencies. Patch-clamp analysis of 40 cells revealed that 75% exhibited ventricular-like action potential profiles, characterized by longer APD80, greater action potential amplitude and maximum upstroke velocity, and a more negative maximum diastolic potential. Structural analysis further showed MLC2v expression, organized sarcomeric features, and increased sarcomere length relative to metabolically purified cardiomyocytes. In multielectrode array analysis, CD47-purified cardiomyocytes exhibited larger field-potential amplitude changes following remdesivir and quinidine treatment, enabling clearer detection of drug-induced electrophysiological responses. Collectively, these findings support CD47-based selection as a reproducible strategy for enriching functionally consistent ventricular-like hiPSC-CMs for electrophysiological analysis and in vitro cardiotoxicity testing. This work was supported by the Korean Fund for Regenerative Medicine (KFRM) grant funded by the Ministry of Science and ICT, the Ministry of Health & Welfare (25A0203L1) and the Commercialization Promotion Agency for R&D Outcomes (COMPA) grant funded by the Korea government Ministry of Science and ICT(MSIT)(RS-2024-00418245). This research was also supported by the BK21 FOUR funded by the MOE of Korea.

Key words: Human pluripotent stem cells, Cardiomyocytes, Ventricular-like cardiomyocytes, Fluorescence-activated cell sorting, Drug safety assessment

Chemically defined modulation of endoderm-associated signals promotes human cardiomyocyte differentiation

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Because the adult human heart has little capacity to replace lost cardiomyocytes and lacks resident cardiac stem cells, differentiation of pluripotent stem cells (PSCs) is a primary source of human cardiomyocytes. Although medium conditioned by mouse visceral endoderm-like cells supports cardiac induction, its xenogeneic origin complicates purification and limits clinical translation. We therefore developed a chemically defined small-molecule strategy to reproduce this activity. Cytokine profiling of the conditioned medium identified 12 factors involved in cardiomyocyte differentiation or heart development, and a corresponding small-molecule modulator was selected for each. Compounds were evaluated with the HES3-NKX2-5 eGFP/w reporter, which marks cardiac progenitors. During mesoderm induction, MCP-1 blockade with RS504393 produced the greatest response, with maximal NKX2-5-dependent GFP expression at 1 μ M. Sequential treatment with WAY-262611 and AHU377 during cardiac specification increased the cTnT-positive population by approximately 1.5-fold relative to controls, as measured by flow cytometry. Increases in cardiac-lineage transcripts and proteins further confirmed lineage progression. Together, these findings demonstrate a scalable and controllable small-molecule replacement for endoderm-conditioned medium and provide a human cardiomyocyte source for disease modeling and regenerative applications. This work was supported by the Korean Fund for Regenerative Medicine (KFRM) grant funded by the Ministry of Science and ICT, the Ministry of Health & Welfare (25A0203L1) and the Commercialization Promotion Agency for R&D Outcomes (COMPA) grant funded by the Korea government Ministry of Science and ICT(MSIT)(RS-2024-00418245). This research was also supported by the BK21 FOUR funded by the MOE of Korea.

Key words: pluripotent stem cells, cardiomyocyte differentiation, small molecules, cardiac progenitors, conditioned medium

Correlative Raman–Photoluminescence Mapping Reveals Perinuclear Nano–Bio Interactions of PEGylated Carbon Quantum Dots in Mouse Mesenchymal Stem Cells

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Carbon quantum dots (CQDs) possess intrinsic photoluminescence with characteristic emission features, enabling their intracellular detection without additional fluorescent labeling. Combining this optical property with label-free Raman spectroscopy provides an opportunity to simultaneously trace nanoparticle localization and characterize the surrounding cellular biochemical environment. In this study, we applied correlative photoluminescence (PL) and Raman mapping to investigate the intracellular behavior of PEGylated yellow carbon quantum dots (PEG-YQDs) and their local interactions with MC3T3-E1 pre-osteoblast stem cells. Cells were cultured on SiO₂/Si substrates to enable spatially resolved spectroscopic analysis at the single-cell level. PL mapping revealed that PEG-YQD-associated signals were preferentially enriched in the perinuclear cytoplasmic region rather than being uniformly distributed throughout the cell. Raman mapping of the corresponding regions showed that PEG-YQD-enriched areas partially overlapped with protein-rich domains and membrane-associated spectral features, suggesting preferential localization within organelle- and membrane-dense intracellular regions. Under higher exposure conditions, local Raman spectral alterations were further observed in the perinuclear membrane-rich region, indicating changes in the biochemical microenvironment surrounding PEG-YQD-enriched areas. These localized spectroscopic changes were consistent with independently observed cellular stress-related responses. Collectively, these findings demonstrate that the intrinsic photoluminescence of PEG-YQDs can serve as a direct optical marker for intracellular detection, while correlative Raman analysis provides complementary information on the surrounding molecular environment. This combined approach enables spatially resolved assessment of nano–bio interactions and may contribute to the development and evaluation of CQDs for stem-cell imaging and other biomedical applications

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Key word: Mesenchymal stem cell, Carbon quantum dots, Raman, photoluminescence, Intracellular localization

PEGylation Attenuates Yellow Carbon Quantum Dot-Induced Oxidative Stress and Cytotoxicity While Enhancing Proliferation in Mouse Embryonic Stem Cells

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Carbon quantum dots (CQDs) exhibit distinctive optical properties, including broad absorption spectra, excitation-dependent photoluminescence, and high photostability, which support their use in bioimaging and biomedical applications. However, their interactions with biological systems are strongly governed by physicochemical properties, particularly surface chemistry and exposure concentration. PEGylation is a widely used surface-modification strategy that improves colloidal stability and biocompatibility by reducing nonspecific interactions with biological components. Here, we investigated the cytotoxic effects of yellow-emitting carbon quantum dots (YQDs) and examined whether PEGylation confers similar protective effects in mouse embryonic stem cells (mESCs). YQDs markedly reduced cell viability and proliferation at low concentrations, with substantial growth inhibition occurring at 5 µg/mL. Low-dose YQD exposure altered FOXO family members and cell-cycle- and apoptosis-associated factors, as assessed by qRT-PCR and western blotting, while TUNEL analysis confirmed apoptotic cell death. YQDs also induced mitochondrial reactive oxygen species (ROS) generation. In contrast, PEGylation markedly decreased YQD-associated cytotoxicity, with PEG-YQDs requiring approximately 15–20-fold higher concentrations to produce comparable growth inhibition. PEG-YQDs induced mitochondrial ROS at substantially higher concentrations, whereas low-dose exposure reduced mitochondrial ROS and exhibited ROS-scavenging activity. Notably, low-dose PEG-YQD treatment maintained or enhanced proliferative activity and the expression of pluripotency markers, suggesting improved support for mESC self-renewal under conditions in which unmodified YQDs suppressed cell growth. At higher concentrations, PEG-YQD exposure showed a tendency to influence differentiation. Collectively, these findings indicate that PEGylation can modulate stem cell fate and contribute to the safer application of CQDs in developmental and reproductive biology.

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Key word: Mouse embryonic stem cell, cytotoxicity, ROS, Carbon quantum dots, PEGylation

Mapping the locations of *barx1*-expressing prechondrogenic condensation domains in the posterior arches during ceratobranchial cartilage formation in zebrafish

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During the evolution of vertebrate heads, the emergence of dorso-ventrally segmented facial skeletal elements in the jaw and branchial basket structures is a key feature invented in jawed fish. The segmental development begins from the two distinct prechondrogenic condensations in the dorsal and ventral regions of the respective pharyngeal arches in facial skeletal formation. Interestingly, in zebrafish, a single *barx1*-expressing prechondrogenic condensation occurs in the posterior arches, in which the ventral ceratobranchial cartilages of the branchial basket form. While the single condensation is expected to cover the ventral regions in the posterior arches for the development of the ventral cartilage, the location of the condensation domain in the arches has not been evaluated yet. Here, we mapped the location of the *barx1*-expressing condensation domain in the arches through comparative gene expression analyses. The single *barx1*-expressing condensation domain occupied the whole ventral area and expanded to the middle region of the intermediate area of the respective arches. From the dorsal regions of the condensation domain, the *sox9a*-expressing chondrocytes for the ventral ceratobranchial cartilages initially arose, with the *sox9a* expression sustained in the dorsal ends of the developed ceratobranchial cartilages. Our results suggest that the single condensation domain covers not only the ventral but also intermediate regions of the arches, consequently resulting in the dorsal areas of the ventral ceratobranchial cartilages probably originating from the intermediate regions of the arches.

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Key words : *Barx1*, Prechondrogenic condensations, Posterior arches, Ceratobranchial cartilages, Zebrafish

Identification of a Novel nkx2.3 Transgenic Reporter Specifically Visualizing Craniofacial Muscle Development in Zebrafish

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Transgenic reporters offer valuable opportunities to investigate the genetic and cellular basis of embryonic development in live organisms. While the specificity of transgenic reporters is essential for their effectiveness in developmental studies, many existing transgenic muscle reporters used for craniofacial muscle development in zebrafish also label trunk skeletal and cardiac muscles, as well as other head tissues. Here, we report the identification of a new transgenic reporter marking craniofacial muscles, Tg(nkx2.3:Alcama-GFP), in zebrafish. This transgenic reporter exhibits Alcama-GFP expression in growing muscle fibers at embryonic stages and in grown muscle fibers at larval stages. However, endogenous nkx2.3 transcripts are barely detected in growing craniofacial muscle fibers by in situ hybridization, and nkx2.3 mutants display fairly normal ventral jaw muscles adjacent to the lower jaw cartilages and extraocular muscles adjacent to the eye. Although an essential role for Nkx2.3 in craniofacial muscle formation remains to be determined, the Tg(nkx2.3:Alcama-GFP) craniofacial muscle reporter provides better opportunities for the precise analysis of craniofacial muscle development at the cellular and genomic levels.

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Key words: craniofacial muscles, nkx2.3, transgenic reporter, zebrafish

Characterization of DMRT Homologs During Embryonic and Reproductive Development in the Hermaphroditic Leech *Helobdella austinensis*

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DMRT genes are conserved transcription factors that play key roles in the regulation of sexual development across metazoans. Although DMRT genes have been well studied in gonochoristic species, their roles in hermaphroditic invertebrates remain unclear. To address this, we analyzed DMRT homologs in the hermaphroditic leech *Helobdella austinensis*, focusing on both their evolutionary relationships and their expression dynamics during development and gametogenesis. Phylogenetic reconstruction revealed three homologs (Hau-DMRT93B, Hau-DMRT99B, and Hau-DMRT-like) belong to canonical DMRT groups. During early development, Hau-DMRT93B was restricted to anterior precursor regions and later expanded to multiple developing structures, including the proboscis, ganglia, and visceral muscle. In the gametogenesis, all DMRT homologs were detected in both male and female reproductive tissues testisac, ovisac, seminal vesicle, and atrium. To better understand, we additionally examined the expression of representative germline markers (SoxE, β -catenin1, β -catenin2, FoxL2, and Piwi). These genes also exhibited bidirectional expression patterns in reproductive organs and each developing germ cells. Our findings indicate that DMRT homologs in *H. austinensis* are more closely associated with germ cell differentiation than with sex-specific determination. Furthermore, their spatial overlap with other germline-related factors suggests that DMRT genes may function as components of a coordinated regulatory system governing germ cell development.

Keywords: Dmrt, Leech, Hermaphroditic invertebrate, Embryogenesis, Gametogenesis

Spatiotemporal expression of oxytocin/vasopressin homolog in glossiphoniid leech *Helobdella austinensis*

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The oxytocin/vasopressin (OT/VP) system is a conserved neuropeptide pathway regulating osmoregulation, reproduction, and social behavior across animals. In annelids, annetocin induces egg-laying, but its role in leech embryogenesis remains unknown. This study examined annetocin (Hau-AT) and its receptor (Hau-ATR) during embryonic to juvenile stages in *Helobdella austinensis* to investigate the potential role of the OT/VP system during development. Phylogenetic analysis showed that Hau-AT clustered closely with *E. fetida* annetocin within Lophotrochozoa, and SignalP prediction identified a signal peptide typical of secreted neuropeptides. Spatiotemporal expression patterns of Hau-AT and Hau-ATR mRNAs were examined by in situ hybridization throughout embryonic and juvenile development. Hau-ATR was expressed in the teloplasm of stage 1 zygotes, localizing through the CD blastomere, teloblasts, and their descendants. In contrast, Hau-AT was undetectable through stages 1-10 but selectively expressed at stage 11 in four neurons of the subesophageal ganglion. In juveniles, Hau-ATR persisted in sucker tissues, whereas Hau-AT remained restricted to this neuronal population. These distinct spatiotemporal expression patterns suggest that the OT/VP ligand and receptor may have different roles during early embryonic and later neuronal development in leeches.

Keywords: oxytocin/vasopressin system; annetocin; *Helobdella austinensis*; embryogenesis; in situ hybridization

Pro-Adipogenic Effects of AME in 3T3-L1 Preadipocytes

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AM is a plant distributed across several regions of Asia, including Mongolia and Siberia, yet relatively little is known about its biological activity. In this study, we examined whether AM extract (AME) affects adipogenesis in 3T3-L1 preadipocytes. Treatment with AME increased lipid accumulation during differentiation in a concentration-dependent manner. This increase was accompanied by higher expression of the adipogenic markers peroxisome proliferator-activated receptor γ (PPAR γ), fatty acid-binding protein 4 (FABP4), and adiponectin. AME also elevated expression of fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC), which are involved in lipid synthesis. Together, these changes indicate that AME facilitates adipocyte differentiation and lipid accumulation by stimulating adipogenic and lipogenic gene expression. These findings identify a previously uncharacterized pro-adipogenic activity of AME and provide a basis for further studies to determine its active constituents and underlying mechanisms.

Key words: Adipocyte, Adipogenesis, Lipogenesis, Differentiation, 3T3-L1

Effect of SR Extract on White Adipocyte Differentiation at Different Stages of Adipogenesis

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SR is a medicinal plant native to Central America and has traditionally been used to treat liver disorders and rheumatism. However, little is known about its effects on adipocyte differentiation. We examined the effect of SR extract on white adipogenesis in 3T3-L1 preadipocytes and evaluated whether its activity varied by differentiation stage. SR extract increased lipid accumulation in a concentration-dependent manner. Expression of the adipogenic markers PPAR γ , FABP4, and adiponectin was also increased. Similarly, several genes involved in lipid synthesis, including FAS, SCD1, SCD2, and ACC, showed higher expression following SR treatment. To determine the stage at which SR primarily acts, cells were exposed to the extract for different periods during adipocyte differentiation. The strongest increase in adipogenesis was observed when SR was administered during the intermediate stage. These results show that SR extract enhances white adipocyte differentiation and lipid accumulation, and increases the expression of adipogenic and lipogenic genes. The effect of SR appears to depend, at least in part, on the timing of treatment during adipocyte differentiation.

Keywords : adipocyte, differentiation, adipogenesis, lipogenesis, 3T3-L1

Compound D Suppresses Adipogenesis and Improves Insulin Sensitivity via ERK/AMPK Signaling and DPP-4 Inhibition

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Compound D is a plant-derived bioactive molecule with reported anti-inflammatory, anticancer, and immunomodulatory properties. However, its effects on adipocyte differentiation and insulin sensitivity remain unclear. We investigated the effects of Compound D on adipogenesis in 3T3-L1 cells and on insulin resistance in high-fat diet (HFD)-induced obese mice. In vitro, Compound D reduced lipid accumulation and adipogenic/lipogenic gene expression, accompanied by modulation of ERK and AMPK signaling. In vivo, Compound D did not affect body weight or food intake but reduced adipocyte size, hepatic lipid accumulation, and lipogenic gene expression in white adipose tissue, while improving insulin resistance. Network pharmacology was used to explore potential T2DM-related targets. Molecular docking and molecular dynamics simulations suggested stable binding of Compound D to DPP-4, and an in vitro enzyme assay confirmed DPP-4 inhibition. These findings suggest that Compound D suppresses adipogenesis and improves insulin sensitivity, potentially through ERK/AMPK signaling and DPP-4 inhibition.

Key words : adipocyte, high-fat diet, adipogenesis, obesity, insulin sensitivity

CTE Modulates White and Brown Adipocyte Differentiation and Mitochondrial Activity

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CT is a plant found in South and Central America and has been reported to exhibit antioxidant activity. However, little is known about its effects on adipocyte differentiation and mitochondrial function. We examined the effects of CT extract (CTE) in white and brown adipocytes. In white adipocytes, CTE increased lipid accumulation during differentiation and upregulated the expression of adipogenic and lipogenic genes. In mature white adipocytes, CTE also increased PGC-1 α expression, mitochondrial DNA content, mitochondrial gene expression, and oxygen consumption. CTE also promoted brown adipocyte differentiation, with increased expression of thermogenic genes. In mature brown adipocytes, thermogenic and mitochondrial gene expression increased, while mitochondrial DNA content remained unchanged. Major compounds in CTE were identified by LC-MS, and possible molecular targets were examined using in silico analysis. These results suggest that CTE promotes differentiation of both white and brown adipocytes and enhances mitochondrial function.

Keywords: adipocyte differentiation, LC-MS, in silico analysis, Differentiation, mitochondrial function

Stage-Dependent Effects of HSN Extract on Adipocyte Differentiation in 3T3-L1 Cells

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HSN is a medicinal plant distributed across Eurasia and has traditionally been used to treat rheumatism, asthma, nervous disorders, and stomach pain. However, little is known about its role in adipocyte development. We examined the effect of HSN extract on 3T3-L1 adipocyte differentiation, with particular attention to the stage at which it acts. Treatment with HSN extract increased lipid accumulation in a concentration-dependent manner. Expression of the adipogenic markers FABP4 and adiponectin was also elevated, along with the lipogenic genes FAS and ACC. When HSN extract was applied at different stages of differentiation, its effect on adipogenesis was greater during the early and intermediate stages than during the late stage. These results show that HSN extract facilitates adipocyte differentiation and lipid accumulation and suggest that its pro-adipogenic activity occurs mainly during the early to intermediate stages of differentiation.

Keywords : adipocyte, differentiation, adipogenesis, lipogenesis, 3T3-L1

Influence of dietary inulin on growth and intestinal ecology in Pacific white shrimp (*Litopenaeus vannamei*)

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This study investigates the influence of dietary inulin on the growth and health of juvenile shrimp *Litopenaeus vannamei* over an 8-week trial, focusing on growth, health, and metabolic impacts because of diet variation. A total of 240 shrimp with an initial mean weight of 0.26 ± 0.01 g were distributed across 12 recirculating tanks and assigned diets with varying inulin concentrations: 0% (Control), 0.5% (T1), 1.0% (T2) and 1.5% (T3). Results indicated that the 1.0% inulin diet resulted in the highest specific growth rate, weight gain, and survival rate. An increase in antioxidative and immune enzyme activities suggested improved health conditions at optimal inulin levels, evidenced by the reduction of malondialdehyde content and potentiation of enzymes like superoxide dismutase and lysozyme. Microbiome analysis via 16S rRNA sequencing showed a significant shift in gut bacterial populations, with an increase in beneficial bacteria such as *Xanthomarina* and a reduction in pathogenic *Vibrio* and *Flavobacteria* in inulin-supplemented groups. Higher dietary inulin increased gut microflora diversity significantly in the T2 group. Metabolomic analysis showed distinct pathway enrichment and differential metabolite regulation, linking inulin intake to significant alterations in lipid metabolism, including pathways like bile secretion, arachidonic acid metabolism, steroid hormone biosynthesis, and ABC transporters. Furthermore, correlation and KEGG enrichment analyses revealed intricate interactions within the shrimp metabolic network, pointing to compensatory and regulatory mechanisms in response to inulin supplementation. In conclusion, dietary inulin substantially improves the health and growth of *L. vannamei*, likely by modulating gut microbiota and metabolic pathways. This study highlights the potential of inulin as a significant dietary supplement for enhancing shrimp aquaculture efficacy.

KEYWORDS: growth performance, inulin, *Litopenaeus vannamei*, metabolome, microbiota

Reproductive characteristics and F₁ progeny production of the neon damselfish (*Pomacentrus coelestis*) under captive conditions in a temperate region

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Neon damselfish (*Pomacentrus coelestis*) is a species primarily distributed in tropical and subtropical regions but has recently exhibited a rapid population expansion into the temperate waters of Northeast Asia. Even within the same species, reproductive activity can be influenced by different environmental factors depending on habitat. However, the reproductive strategies of *P. coelestis* inhabiting temperate waters remain largely unexplored. This study aimed to elucidate the reproductive strategies and characteristics of *P. coelestis* in temperate habitats by investigating the annual reproductive cycle, sex characteristics (including onset of puberty and sex ratio), and F₁ progeny production under controlled laboratory conditions. Specimens were collected monthly over a one-year period from the coastal waters of Jeju, South Korea, and annual variations in the gonadosomatic index (GSI) and gonadal histomorphological changes were evaluated. Gonadal maturity was further assessed in individuals collected during the peak spawning period (July 2025), and breeding was conducted in the controlled laboratory conditions, providing a photoperiod of 12L:12D and maintaining a water temperature of 26 °C. GSI began to increase in June and peaked in August for both females (8.7 ± 3.9) and males (6.1 ± 3.3). Fertilized eggs were successfully produced under laboratory conditions, and F₁ generation was reared by feeding rotifers enriched with *Nannochloropsis* followed by copepods. These results indicate that *P. coelestis* inhabiting the temperate waters of Jeju exhibits a distinct spawning season from June to September, with reproductive activity strongly correlated with seasonal fluctuations in water temperature and photoperiod, demonstrating a reproductive pattern significantly different from those of tropical and subtropical populations. The observed female-biased sex ratio during the spawning season and the determination of a minimum reproductive size of 3.7 cm provide new insights into the reproductive ecology of this species in temperate environments. Moreover, the successful production of F₁ progeny highlights the reproductive adaptation of *P. coelestis* to a temperate region and underscores its conservation potential. These findings establish essential fundamental data for understanding reproductive strategies of fishes in temperate ecosystems and contribute to predicting geographic distribution shifts and ecological responses of marine species under climate change.

Key words: reproductive cycle, sexual maturity, captive breeding, F₁ progeny production, temperate range expansion