Model Animal Research Center of Nanjing University
MOE key laboratory of model animal for disease study
National resource center for mutant mice

ANNUAL REPORT 2022

Director's Words

We are in a rapidly changing era that influences various aspects of our life and work. However, one thing does not, and also will not, change at MARC is our passion for excellence in science. MARC has been devoted to biomedical research using animal models to make discoveries for a healthy life. Over the last decade, we've become stronger in developing genetically-modified animals for modeling human diseases. Through introducing state-of-art genome editing technologies, we not only accelerate generation of genetically-modified animals, but also start to develop more precise and complex models for studying human diseases. As a member of the International Mouse Phenotyping Consortium (IMPC), we've also established a state-of-art platform for mouse phenomics, which help us to get better understanding of gene functions in a post-genomic era. To improve our abilities for in-depth functional studies, we've expanded other facilities at MARC, including the imaging core, metabolomics core, and flow cytometry core. With these state-of-art core facilities, MARC scientists have tackled some longstanding scientific questions, and made several important discoveries this year. Looking forward to the year ahead, I believe that more discoveries will be made in our ever-expanding research fields, from genetics and developmental biology to cancer biology, metabolic biology and neurobiology. We will continue to fulfil our mission at MARC pursuing first-class science for improvement of human health with the help of animal models.

My second term as the director of MARC starts from 2021. The privileges working with a great team of devoted professors, talented students and brilliant supporting personnel give me great confidence of a bright future for MARC. The success of MARC is also owed to great supports from friends of ours over years. I wish every MARCer and all friends a happier 2023!

Shuai Chen
Director

CONTENTS

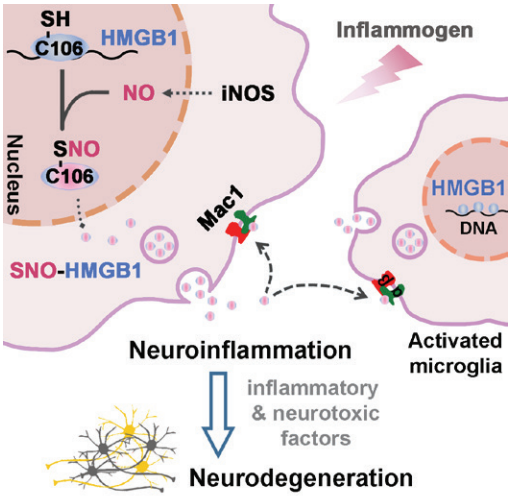
Research Highlight in 2022

Group Huiming Gao

Posttranslational S-nitrosylation modification regulates HMGB1 secretion and promotes its pro-inflammatory and neurodegenerative effects

Ru Yang, Yun Gao, Hui Li, Wei Huang, Dezhen Tu, Mengnan Yang, Xingqian Liu, Jau-Shyong Hong and Hui-Ming Gao

Nuclear protein HMGB1 (high-mobility group box 1), albeit lacking secretory signal peptide(s), can be actively secreted by inflamed immune cells and functions as a pro-inflammatory cytokine. Regulation of HMGB1 secretion is critical for treatment of HMGB1-mediated inflammation and many related diseases. HMGB1 contains three redox-sensitive cysteine residues. Here, we report S-nitrosylation (SNO; the covalent binding of nitric oxide [NO, a free radical gas and major inflammatory mediator] to cysteine thiols) by inducible nitric oxide synthase (iNOS)-derived NO at Cys106 is essential and sufficient for inflammation-elicited HMGB1 secretion. iNOS deletion/inhibition or C106S mutation, but not C23S or C45S mutation, prevents inflammation-elicited HMGB1 secretion. NO donors induce SNO of HMGB1 and reproduce inflammogen-triggered Cys106-SNO-dependent HMGB1 secretion. Determination of pathological significance of SNO-linked HMGB1 secretion shows SNO-HMGB1 induces more profound microglial activation and neurodegeneration than unmodified HMGB1 through increased binding to microglial Mac1. Intranigral HMGB1 injection replicates key features of Parkinson's disease (PD), in wildtype, but not Mac1-deficient, mice. Our findings uncover pivotal roles of SNO modification by iNOS-derived NO for HMGB1 secretion and HMGB1-Mac1 interaction for inflammatory neurodegeneration, identifying a mechanistic basis for PD development.

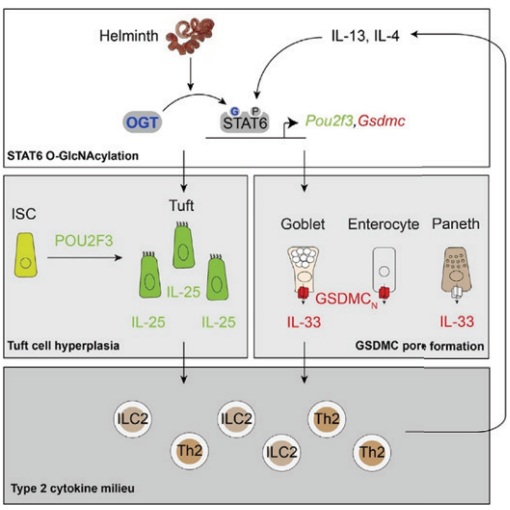


Group Zhaoyu Lin

Epithelial STAT6 O-GlcNAcylation drives a concerted anti-helminth alarmin response dependent on tuft cell hyperplasia and Gasdermin C

Ming Zhao, Kaiqun Ren, Xiwen Xiong, Yue Xin, Yujie Zou, Jason C. Maynard, Angela Kim, Alexander P. Battist, Navya Koneripalli, Yusu Wang, Qianyue Chen, Ruyue Xin, Chenyan Yang, Rong Huang, Jiahui Yu, Zan Huang, Zengdi Zhang, Haiguang Wang, Daoyuan Wang, Yihui Xiao, Oscar C. Salgado, Nicholas N. Jarjour, Kristin A. Hogquist, Xavier S. Revelo, Alma L. Burlingame, Xiang Gao, Jakob von Moltke, Zhaoyu Lin,* and Hai-Bin Ruan,*

The epithelium is an integral component of mucosal barrier and host immunity. Following helminth infection, the intestinal epithelial cells secrete "alarmin" cytokines, such as interleukin-25 (IL-25) and IL-33, to initiate the type 2 immune responses for helminth expulsion and tolerance. However, it is unknown how helminth infection and the resulting cytokine milieu drive epithelial remodeling and orchestrate alarmin secretion. Here, we report that epithelial O-linked N-Acetylglucosamine (O-GlcNAc) protein modification was induced upon helminth infections. By modifying and activating the transcription factor STAT6, O-GlcNAc transferase promoted the transcription of lineage-defining Pou2f3 in tuft cell differentiation and IL-25 production. Meanwhile, STAT6 O-GlcNAcylation activated the expression of Gsdmc family genes. The membrane pore formed by GSDMC facilitated the unconventional secretion of IL-33. GSDMC-mediated IL-33 secretion was indispensable for effective anti-helminth immunity and contributed to induced intestinal inflammation. Protein O-GlcNAcylation can be harnessed for future treatment of type 2 inflammation-associated human diseases.

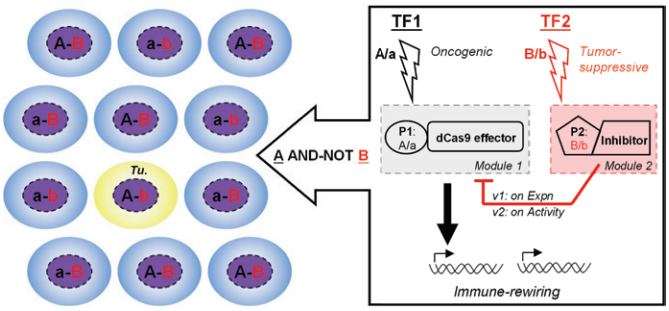


Group Jianghuai Liu

Precise tumor immune rewiring via synthetic CRISPRa gene circuits:

Yafeng Wang, Guiquan Zhang, Qingzhou Meng, Lingyun Sun, Geng Liu, Xingxu Huang and Jianghuai Liu

Reinvigoration of antitumor immunity has recently become the central theme for the development of cancer therapies. Nevertheless, the precise delivery of immunotherapeutic activities to the tumors remains challenging. Here, we explore a synthetic gene circuit-based strategy for specific tumor identification, and for subsequently engaging immune activation. By design, these circuits are assembled from two interactive modules, i.e., an oncogenic TF-driven CRISPRa effector, and a corresponding p53-inducible off-switch (NOT gate), which jointly execute an AND-NOT logic for accurate tumor targeting. In particular, two forms of the NOT gate are developed, via the use of an inhibitory sgRNA or an anti-CRISPR protein, with the second form showing a superior performance in gating CRISPRa by p53 loss. Functionally, the optimized AND-NOT logic circuit can empower a highly specific and effective tumor recognition/immune rewiring axis, leading to therapeutic effects in vivo. Taken together, our work presents an adaptable strategy for the development of precisely delivered immunotherapy.

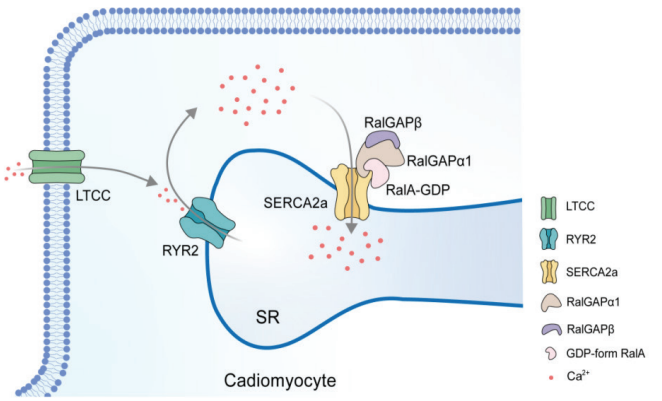


Group Shuai Chen

The RalGAPα1–RalA signal module protects cardiac function through regulating calcium homeostasis

Sangsang Zhu, Chao Quan, Ruizhen Wang, Derong Liang, Shu Su, Ping Rong, Kun Zhou, Xinyu Yang, Qiaoli Chen, Min Li, Qian Du, Jingzi Zhang, Lei Fang, Hong-Yu Wang* and Shuai Chen*

Hypertension and its associated cardiovascular and cardiac diseases are world-wide leading causes of death. Pressure overload due to hypertension causes cardiac dysfunction and leads to the development of cardiomyopathy and heart failure. However, the underlying molecular mechanisms are still incompletely understood. Sarcoplasmic/endoplasmic reticulum calcium ATPase SERCA2 mediates calcium re-uptake from the cytosol into sarcoplasmic reticulum, and its dysfunction is a hallmark of heart failure. Multiple factors have been identified to modulate SERCA2 activity, however, its regulation is still not fully understood. Here we identify a Ral-GTPase activating protein RalGAPα1 as a critical regulator of SERCA2 in cardiomyocytes through its downstream target RalA. RalGAPα1 is induced by pressure overload, and its deficiency causes cardiac dysfunction and exacerbates pressure overload-induced heart failure. Mechanistically, RalGAPα1 regulates SERCA2 through direct interaction and its target RalA. Deletion of RalGAPα1 decreases SERCA2 activity and prolongs calcium re-uptake into sarcoplasmic reticulum. GDP-bound RalA, but not GTP-bound RalA, binds to SERCA2 and activates the pump for sarcoplasmic reticulum calcium re-uptake. Overexpression of a GDP-bound RalAS28N mutant in the heart preserves cardiac function in a mouse model of heart failure. Our findings have therapeutic implications for treatment of heart failure.



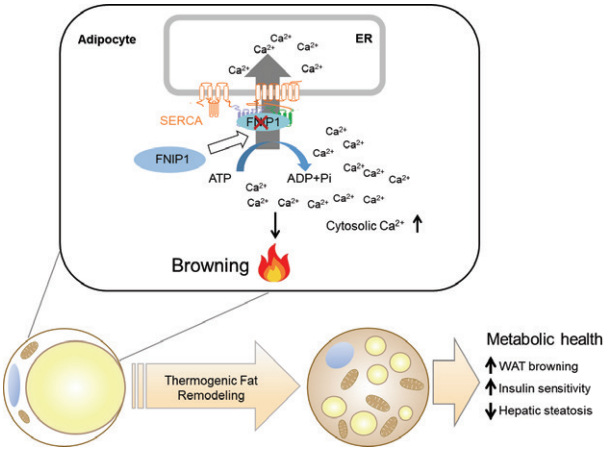
A model for the RalGAPα1–RalA signal module as a critical regulator of SERCA2a

Group Zhenji Gan

FNIP1 regulates white adipose tissue browning and systemic glucose homeostasis by shaping intracellular calcium dynamics

Yin Y#, Xu D#, Mao Y#, Xiao L, Sun Z, Liu J, Zhou D, Xu Z, Liu L, Fu T, Ding C, Guo Q, Sun W, Zhou Z, Yang L, Jia Y, Chen X, Gan Z*

Metabolically beneficial beige adipocytes offer tremendous potential to combat obesity and metabolic diseases. The folliculin interacting protein 1 (FNIP1) is implicated in the control of cellular metabolism via metabolic master regulators AMPK and mTORC1 in mice and humans. However, whether and how FNIP1 regulates the browning of white adipose tissue (WAT) is unclear. Gan Lab demonstrate that FNIP1 plays a critical role in controlling WAT browning as well as systemic glucose homeostasis. Adipocyte-specific ablation of FNIP1 promotes a broad thermogenic remodeling of WAT. FNIP1 deficient WAT was shown to have increased levels of UCP1, high mitochondrial content, and augmented capacity for mitochondrial respiration. Mechanistically, FNIP1 binds to and promotes the activity of SERCA, a main Ca^{2+} pump responsible for cytosolic Ca^{2+} removal. Loss of FNIP1 resulted in reduced SERCA Ca^{2+} pump activity, leading to enhanced intracellular Ca^{2+} signals and consequential activation of Ca^{2+}-dependent thermogenic program in white adipocytes. Furthermore, mice lacking adipocyte FNIP1 were protected against high-fat diet-induced glucose intolerance, insulin resistance, and liver steatosis. Thus, these findings reveal a pivotal role of FNIP1 as a negative regulator of beige adipocyte thermogenesis, and unravel an intriguing functional link between intracellular Ca^{2+} dynamics and WAT browning. This work was highlighted by Prof. Lawrence Kazak in JEM.



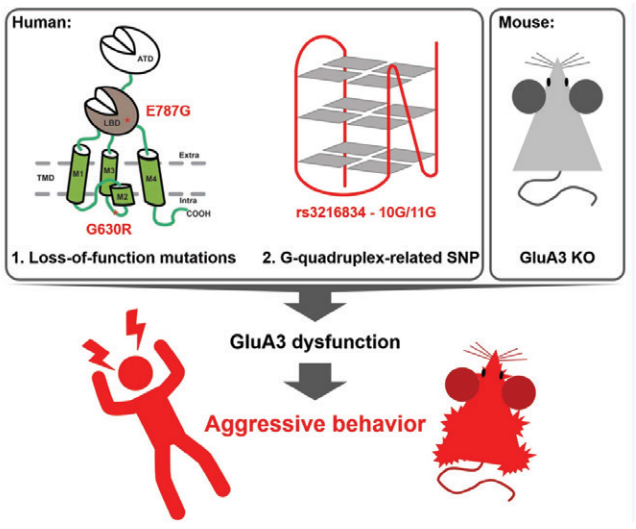
YinY, et al., …GanZJ*. J Exp Med. 2022

Group Yun Shi

Dysfunction of AMPA receptor GluA3 is associated with aggressive behavior in human

Peng SX, Pei J, Rinaldi B, Chen Jiang, Ge YH, Jia M, Wang J, Delahaye-Duriez A, Sun J, Zang YY, Shi YY, Zhang N, Gao X, Milani D, Xu X, Sheng N, Gerard B, Chen Zhang C, Bayat A, Liu N*, Yang JJ*, Shi YS* (2022).

Inappropriate aggression in humans hurts the society, families and individuals. The genetic basis for aggressive behavior, however, remains largely elusive. Here we identified two rare missense variants in X-linked GRIA3 from male patients who showed syndromes featuring aggressive outbursts. Both G630R and E787G mutations in AMPA receptor GluA3 completely lost their ion channel functions. Furthermore, a guanine-repeat single nucleotide polymorphism (SNP, rs3216834) located in the first intron of human GRIA3 gene was found to regulate GluA3 expression with longer guanine repeats (rs3216834-10G/-11G) suppressing transcription compared to the shorter ones (-7G/-8G/-9G). Importantly, the distribution of rs3216834-10G/-11G was elevated in a male violent criminal sample from Chinese Han population. Using GluA3 knockout mice, we showed that the excitatory neurotransmission and neuronal activity in the medial prefrontal cortex (mPFC) was impaired. Expressing GluA3 back into the mPFC alleviated the aggressive behavior of GluA3 knockout mice, suggesting that the defects in mPFC explained, at least partially, the neural mechanisms underlying the aggressive behavior. Therefore, our study provides compelling evidence that dysfunction of AMPA receptor GluA3 promotes aggressive behavior.



Student of the Year

Kun Zhou

Kun Zhou received his Bachelor's degree of Biological Technology in 2018 from College of Innovation and Experiment, Northwest A&F University. He joined Dr. Shuai Chen's lab at the year of 2018 to study protein phase separation in insulin signaling pathway.

For the past four years, his work focused on the exploration of protein liquid-liquid phase separation (LLPS) in insulin signaling pathway. This year, he and his colleagues reported a new LLPS-controlled insulin signal transduction model. IRS1 condensates form intracellular distributed insulin-AKT signaling hubs through recruiting critical molecules. This new model explains how does insulin signal, mainly activated AKT, deep-going into intracellular space. These findings may have important implications for the treatment of insulin resistance and AKT over-activated tumors.

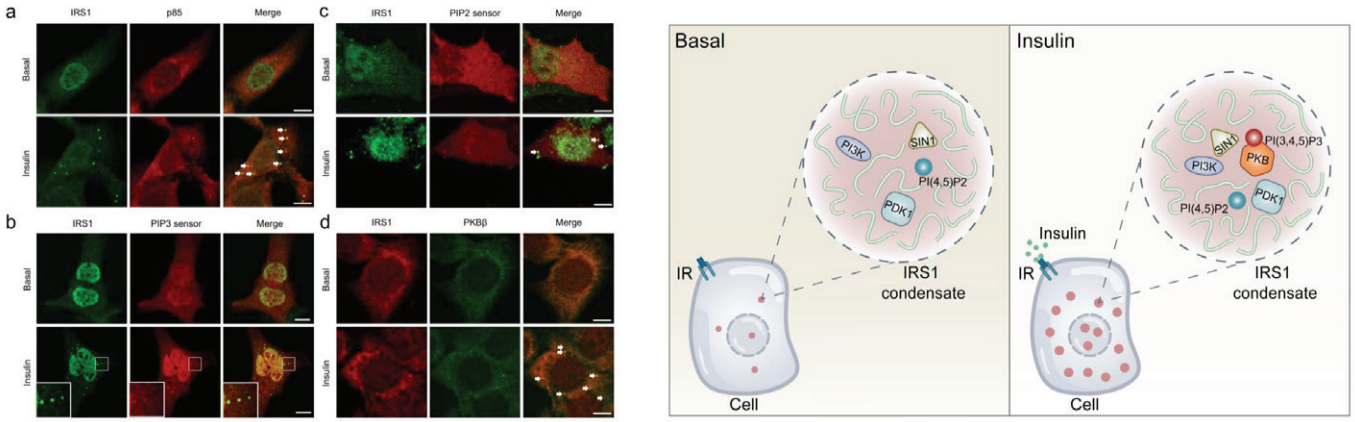


Figure 1. IRS1 responds to insulin stimulation to form intracellular signal transduction hubs by LLPS.

Selected publications

1. Zhou K, Chen QL, Chen JM, Liang DR, Feng WK, Liu MJ, Wang Q, Wang RZ, OuYang Q, Quan C and Chen S (2022) Spatiotemporal regulation of insulin signaling by liquid-liquid phase separation. Cell Discov 8(1): 64 DOI: 10.1038/s41421-022-00430-1
2. Zhu SS, Quan C, Wang RZ, Liang DR, Su S, Rong P, Zhou K, Yang XY, Chen QL, Li M, Du Q, Zhang JZ, Fang L, Wang HY and Chen S (2022) The RalGAPα1–RalA signal module protects cardiac function through regulating calcium homeostasis. Nat Commun 13: 4278 DOI: 10.1038/s41467-022-31992-z

Neurobiology

Yun Shi , Ph.D

Yun Shi received Ph.D degree in physiology at Georgia State University under the mentoring of Dr. Chun Jiang at Atlanta, USA in 2007. His Ph.D work focus on the function and regulation of vascular KATP channels. He then had postdoctoral training with Dr. Roger Nicoll in UCSF where he worked on synaptic plasticity. In 2013, he joined the Model Animal Research Center, Nanjing University as a professor and principal investigator.

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The Mechanisms of Neural Plasticity

The central neural system (CNS) is a complex network, in which the appropriate functions depend on the information exchange among neurons through a specified structure named synapse. The neurotransmitters released from presynaptic neurons bind to postsynaptic receptors, enhancing or inhibiting postsynaptic activity. Meanwhile, postsynaptic neurons also release certain regulatory information and modulate presynaptic activity, allowing the feedback to occur. Many of high neuronal functions result from the changes of neurotransmission, or so called plasticity. Dysfunction of synaptic plasticity is one of the major causes of neurodegeneration diseases. Therefore, studying the synaptic transmission not only help unreal human high neural functions but also improve our understanding on the mechanisms of neural diseases and provide cues for cure.

Glutamate is the major excitatory neurotransmitter in CNS. Two groups of glutamate receptors are located on the post-synaptic membrane, i.e., ionotropic and metabotropic glutamate receptors. Ionotropic receptors include AMPA, NMDA and Kainate receptors; each are composed of different subunits. The normal function of excitatory synapses and the generation of neural plasticity are determined by the composition and expression level of postsynaptic glutamate receptors. However, how the receptors correctly traffic to post-synaptic membrane and how the expression level is appropriately regulated remain unclear.

Hippocampus is a relative simple structure in brain, which is believed to play an essential role in learning and memory. The CA3-CA1 synapses are arguably the best studied synapses in brain. The plasticity of those synapses, including long-term potentiation (LTP) and long-term depression (LTD), are believed to be the fundament of learning and memory mechanisms.

Current research interests in our lab include: 1. The fundament of synaptic plasticity such as LTP and LTD. 2. Diseases associated with glutamate signal pathway. 3. Physiological functions of the mechanosensitive cation channel Tmem63 family.

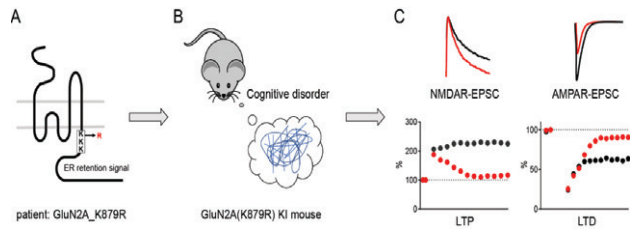

Figure 1. A GluN2A mutation impairs long-term synaptic plasticity and learning and memory (Mol Psychiatry 2022).

A. a mutation in NMDA receptor GluN2A(K879R) identified in an patient with intellectual disability.

B. Mice carrying GluN2A_K879R mutation showed impaired learning and memory.

C. Excitatory synaptic transmission and plasticity were impaired in the KI mice.

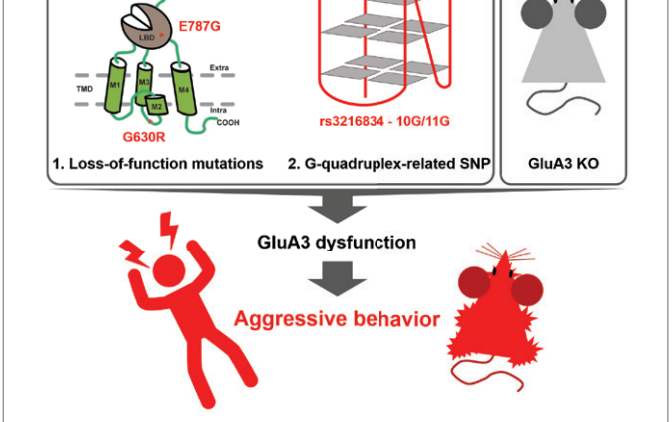

Figure 2. Loss of GluA3 function causes aggressive behavior in human. (Mol Psychiatry 2022).

Human patients with GluA3 loss-of-function mutations (E787G or G630R) showed aggressive behavior. Violent crime people had more SNP rs3216834-10G/11G compared to community control, which caused decreased GluA3 expression. GluA3 KO mice had aggressive behavior. All these observation suggest that GluA3 dysfunction leads to aggressive behavior in human and animals.

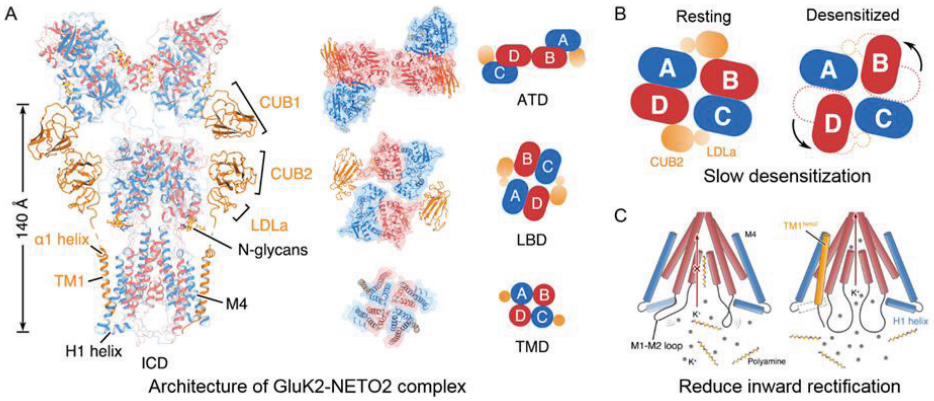

Figure 3. GluK2-NETO2 CryoEM Structure (Nature 2021).

A. The architecture of GluK2-NETO2 complex.

B. The structural mechanism for NETO2 regulation of GluK2 desensitization.

C. The mechanism for NETO2 regulation of GluK2 inward rectification.

Selected publications

1. Peng SX, Pei J, Rinaldi B, Chen Jiang, Ge YH, Jia M, Wang J, Delahaye-Duriez A, Sun J, Zang YY, Shi YY, Zhang N, Gao X, Milani D, Xu X, Sheng N, Gerard B, Chen Zhang C, Bayat A, Liu N*, Yang JJ*, Shi YS* (2022). Dysfunction of AMPA receptor GluA3 is associated with aggressive behavior in human. Mol Psychiatry. Online ahead of print.
2. Li QQ, Chen J, Hu P, Jia M, Sun JH, Feng HY, Qiao FC, Zang YY, Shi YY, Chen G, Sheng N, Xu Y, Yang JJ*, Xu Z*, Shi YS*. (2022) Enhancing GluN2A-type NMDA receptors impairs long-term synaptic plasticity and learning and memory. Mol Psychiatry. Online ahead of print.
3. He L, Sun J, Gao Y, Li B, Wang Y, Dong Y, An W, Li H, Yang B, Ge Y, Zhang XC*, Shi YS*, Zhao Y*. (2021) Kainate receptor modulation by Neto2. Nature. 599(7884):325-329.
4. Sun JH, Chen J, Ayala Valenzuela FE, Brown C, Masser-Frye D, Jones M, Romero LP, Rinaldi B, Li WL, Li QQ, Wu D, Gerard B, Thorpe E*, Bayat A*, Shi YS*. (2021) X-linked neonatal-onset epileptic encephalopathy associated with a gain-of-function variant p.R660T in GRIA3. PLoS Genet. 17(6):e1009608.
5. Jiang CH, Wei M, Zhang C*, Shi YS*. (2021) The amino-terminal domain of GluA1 mediates LTP maintenance via interaction with neuroplastin-65. PNAS. 118(9): e2019194118.
6. Du H, Ye C, Wu D, Zang YY, Zhang L, Chen C, He XY, Yang JJ, Hu P, Xu Z, Wan G*, Shi SY*. (2020) The cation channel TMEM63B is an osmosensor required for hearing. Cell Reports. 31(5):107596.
7. Duan GF, Xu S, Ye Y, Tao W, Nicoll RA, Shi YS*, and Sheng N*.(2018) Signal peptide represses GluK1 surface and synaptic trafficking through binding to amino-terminal domain. Nature Communications. 9(1):4879.
8. Niu Y, Dai Z, Liu W, Zhang C, Yang Y, Guo Z, Li X, Xu C, Huang X, Wang Y, Shi YS*, Liu JJ*. (2017) Ablation of SNX6 leads to defects in synaptic function of CA1 pyramidal neurons and spatial memory. eLife. 6. pii: e20991.
9. He XY, Li YJ, Kalyanaraman C, Qiu LL, Chen C, Xiao Q, Liu WX, Zhang W, Yang JJ, Chen G, Jacobson MP, Shi YS*. (2016) GluA1 signal peptide determines the spatial assembly of heteromeric AMPA receptors. PNAS.113(38):E5645-54.

Group members

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Jingjing Tu
Guizhou Li
Yanyu Zang

Guiquan Chen, Ph.D.

Guiquan obtained his PhD in Neuroscience at the University of Edinburgh in Scotland in 2005 and then conducted his postdoctoral research at Harvard Medical School in Boston. He joined the MARC of Nanjing University as a Principle Investigator in December of 2011. His long-term research goal is to understand molecular mechanisms by which the γ–secretase complex regulates neuronal survival and/or death. His lab uses a combination of mouse genetics, molecular biology, cellular and behavioral neuroscience techniques to address this question. Elucidation of molecular mechanisms for age-related neurodegeneration may help identify novel therapeutic targets for the prevention and the treatment of neurodegenerative diseases.

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Molecular and cellular mechanisms for neurodevelopmental and neurodegenerative diseases

1. Molecular mechanisms by which Akt regulates oligodendrocyte differentiation.

As an important protein kinase, Akt has been implicated in diseases with white matter (WM) abnormalities. To study whether and how Akt may regulate OL development, we generated oligodendrocyte (OL) lineage cells-specific Akt1/Akt2/Akt3 triple conditional knockout (Akt cTKO) mice (Fig.1). We show that deletion of Akt three isoforms causes down-regulation of Sox10 and decreased levels of phosphorylated FoxO1 (pFoxO1) in the brain (Fig.2). In vitro analysis reveals that expression of FoxO1 with mutations on phosphorylation sites for Akt significantly represses the Sox10 promoter activity (Fig.2). Together, we have identified a novel phosphorylation-dependent mechanism for Sox10 expression and OL differentiation.

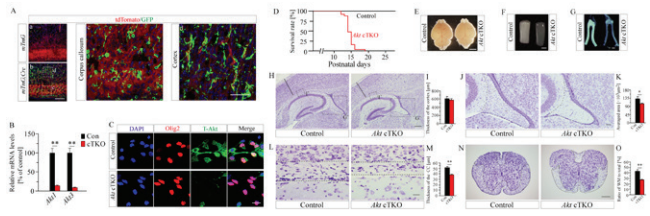

Figure 1. Deficient white matter development in Akt cTKO mice. (A-C) Characterization of Akt cTKO mice. (D) Survival rate. (E-G) Morphology of the brain, the spinal cord (SC) and the optic nerve (ON) in Akt cTKO mice. (H-O) Nissl analyses for the cortex (H), the fimbria (J), the corpus callosum (CC) (L) and the spinal cord (SC) (N).

2. Essential role of Pen-2 in governing the differentiation of oligodendrocyte precursor cells to astrocytes.

Whereas the role of γ-secretase in neurogenesis has been intensively studied, little is known about its role in astrogliogenesis. Recent evidence has demonstrated that astrocytes can be generated from OL precursor cells (OPCs). We generated OL lineage cells specific presenilin enhancer 2 (Pen-2) cKO mice (Fig.3). We show that conditional inactivation of Pen-2 in OL lineage causes enhanced generation of GFAP-expressing astrocytes (Fig.3). Mechanistic analysis reveals that deletion of Pen-2 inhibits the Notch signaling to up-regulate signal transducer and activator of transcription 3 (Stat3) (Fig.4). These findings suggest that Pen-2 may control the differentiation of OPCs to astrocytes through the Stat3 signaling.

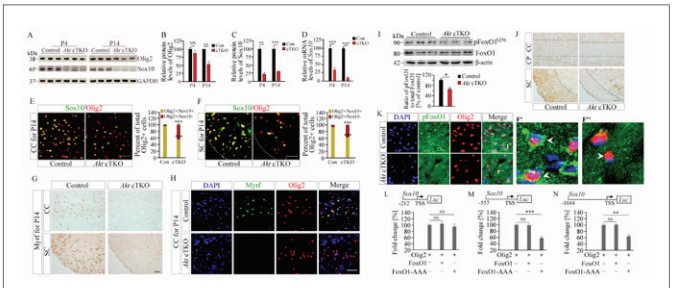

Figure 2. Down-regulation of Sox10 in Akt cTKO mice. (A-D) Decreased expression of Sox10 in Akt cTKO mice at P4 and P14. (E-F) Number of Sox10+/Olig2+ cells in the CC and the SC in Akt cTKO mice. (G,H) IHC analysis of Myrf+ cells in the CC and the SC in Akt cTKO mice at P14. (I-K) Western and IHC analyses on pFoxO1 in Akt cTKO mice. (L-M) Analysis of the promoter activity of Sox10 using cultured N2a cells.

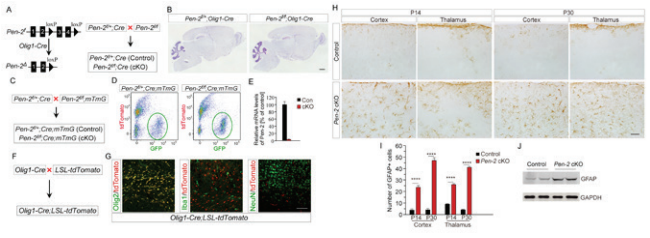

Figure 3. Enhanced generation of astrocytes in OL lineage cellS specific Pen-2 cKO mice. (A-D) Characterization of OL lineage cells specific Pen-2 cKO mice. (F,G) Co-staining of Olig2/tdTomato in Olig1-Cre;LSL-tdTomato mice. (H) IHC on GFAP in Pen-2 cKO mice. (I) Number of GFAP+ cells in Pen-2 cKO mice at P14 and P30. (J) Western analysis on GFAP in Pen-2 cKO mice.

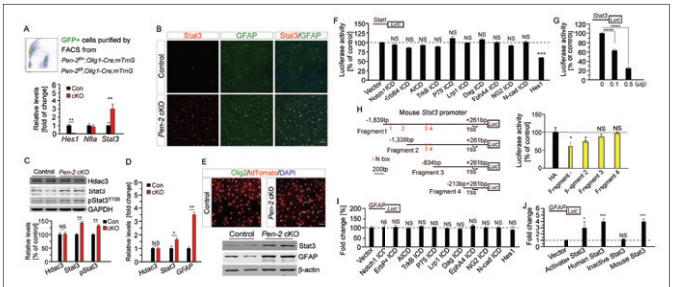

Figure 4. Increased expression of Stat3 in Pen-2 cKO mice. (A) Q-PCR analyses on Hes1, Nfia and Stat3. (B) IHC of Stat3/GFAP in Pen-2 cKO mice. (C) Western analysis on Hdac3, Stat3 and pStat3. (D) Q-PCR analyses on Hdac3, Stat3 and GFAP. (E) Western analyses on Stat3 and GFAP using cultured Pen-2 cKO OPCs. (F-H) Analysis of the promoter activity of Stat3 using cultured N2a cells. (I,J) Analysis of the promoter activity of GFAP.

Recent publications (*, Corresponding author)

1. Xia Y, Zhang Y, Xu M, Zou X, Gao J*, Ji M*, Chen G*. Presenilin enhancer2 is crucial for the transition of apical progenitors into neurons but into not basal progenitors in the developing hippocampus. Development 2022 (149):dev.200272.
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3. Teng X, Hu P, Chen Y, Zang Y, Ye X, Ou J, Chen G*, Shi YS*. A novel Lgi1 mutation causes white matter abnormalities and impairs motor coordination in mice. FASEB Journal 2022 (36):e22212.
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Neuroinflammation, neurodevelopment, and neurodegeneration

Nuclear protein HMGB1 (high-mobility group box 1) can be actively secreted from inflamed immune cells (e.g., macrophages and microglia) and functions as a pro-inflammatory cytokine. Regulation of HMGB1 secretion is critical for treatment of HMGB1-mediated inflammation and related diseases. Lacking a signal peptide, HMGB1 is secreted through unconventional secretory pathways. HMGB1 contains three redox-sensitive cysteine residues. During inflammation, nitric oxide (NO, a free radical gas), derived from inducible nitric oxide synthase (iNOS; NOS2), reaches a high level and serves as a major inflammatory mediator. We found S-nitrosylation (SNO; the covalent binding of NO to cysteine thiols) by iNOS-derived NO at Cys106 was essential and sufficient for inflammation-elicited HMGB1 secretion. iNOS deletion/inhibition (Fig. 1) or C106S and C23SC45SC106S mutations but not C23S or C45S mutation prevented LPS- and/or poly (I:C)-elicited HMGB1 secretion (Fig. 2). Secreted HMGB1 in media and cytosolic HMGB1 redirected from nuclei of LPS-/poly (I:C)-/SNP-treated microglia/macrophages, but not nuclear HMGB1 (n-HMGB1), exhibited SNO modification. NO donor SNP induced SNO of HMGB1 and reproduced inflammogen-triggered Cys106-SNO-dependent HMGB1 secretion (Fig. 1, 2).

Determination of pathological significance of SNO-linked HMGB1 secretion showed that S-nitrosylated HMGB1 (SNO-HMGB1) induced more profound microglial activation and neurodegeneration than unmodified HMGB1 through increased binding to microglial Mac1 (Fig. 3A). Intranigral HMGB1 injection induced chronic microglial activation, dopaminergic neurodegeneration and locomotor deficit, the key features of Parkinson's disease (PD), in wildtype, but not Mac1-deficient, mice (Fig. 3B-F). Collectively, this study uncovered pivotal roles of SNO modification by iNOS-derived NO for HMGB1 secretion and HMGB1-Mac1 interaction for inflammatory neurodegeneration, identifying a mechanistic basis for PD development.

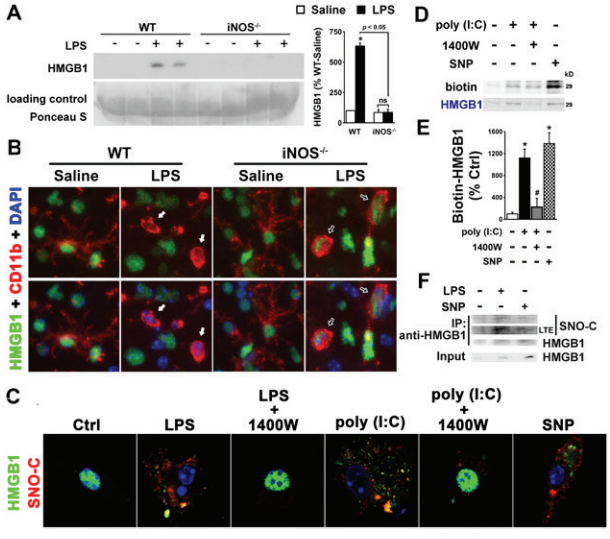

Figure 1. SNO by iNOS-derived NO regulated HMGB1 secretion.

A & B, Genetic deletion of iNOS blocked LPS-induced HMGB1 secretion. A, LPS (3 × 10^6 EU/kg; i. p.) increased serum HMGB1 in WT but not iNOS$^{-/-}$ mice. *p < 0.05 vs saline control. B, Intranigral LPS injection triggered HMGB1 secretion from activated WT microglia, as shown by faint n-HMGB1 staining (white arrows), but not from activated iNOS$^{-/-}$ microglia (with strong n-HMGB1; unfilled arrows). C, LPS-, poly (I:C)-, or SNP-treated microglia showed cytosolic S-nitroso-cysteine (SNO-C) and SNO-C^{+}HMGB1^{+} staining as well as faint SNO-C-negative n-HMGB1 staining. iNOS inhibitor 1400W prevented LPS- and poly (I:C)-induced occurrence of cytosolic SNO-C+HMGB1+ and reduction in n-HMGB1. D & E, Biotin-switch assay showed that secreted HMGB1 from poly (I:C)- or SNP-treated macrophages was biotinylated (i.e., originally S-nitrosylated). 1400W blocked poly (I:C)-elicited SNO and secretion of HMGB1. *p < 0.05 and #p < 0.05 vs vehicle- and poly (I:C)-treated cultures, respectively. F, Immunoprecipitated (IP) HMGB1 from the medium of LPS- or SNP-treated BV2 microglial cells was positive for SNO-C. LET: long-exposure time.

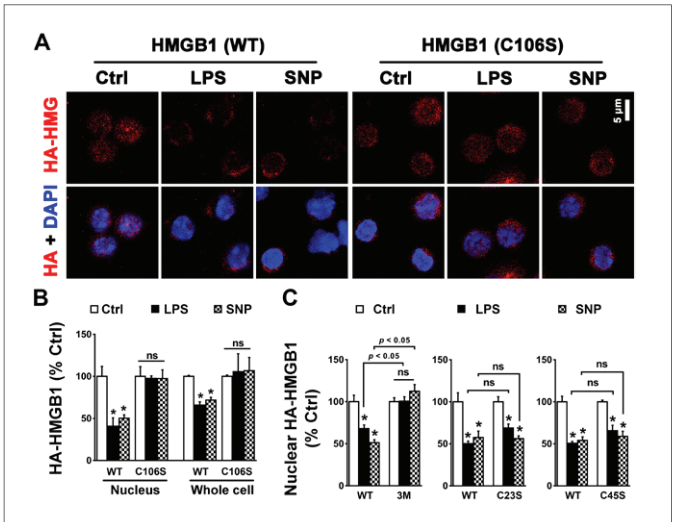

Figure 2. SNO at Cys106 was required for HMGB1 secretion.

A, LPS/SNP elicited nucleus-to-cytoplasm shuttling of WT HA-HMGB1 but not C106S mutant HA-HMGB1. B & C, C106S and C23SC45SC106S (3M) mutations, but not C23S or C45S mutation, blocked LPS-/SNP-elicited HA-HMGB1 secretion. *p < 0.05 vs the corresponding vehicle control.

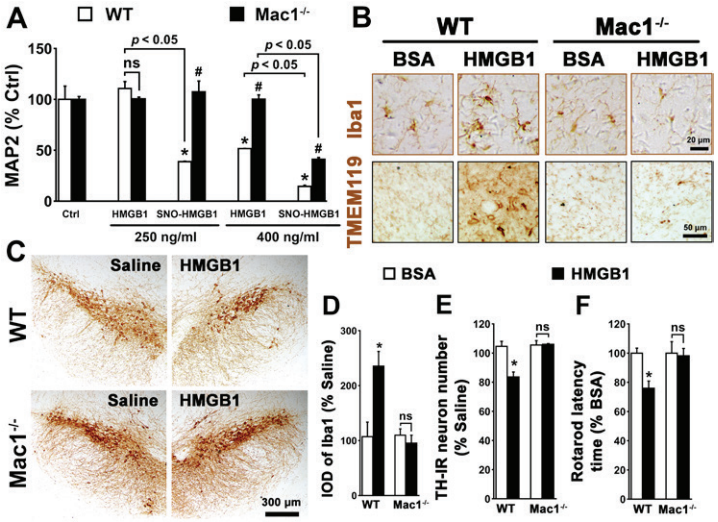

Figure 3. HMGB1 induced chronic microglial activation, dopaminergic neurodegeneration and locomotor deficit in WT but not Mac1$^{-/-}$ mice.

A, SNO-HMGB1 induced more profound neurodegeneration than unmodified HMGB1 in WT neuron-glia cultures, and the Mac1$^{-/-}$ cultures were more resistant to unmodified HMGB1 and SNO-HMGB1 than WT cultures, as shown by densitometry analysis of MAP-2 staining. *p < 0.05 vs the corresponding saline-treated control. #p < 0.05 vs the corresponding WT cultures. B-F, Intranigral HMGB1 injection induced chronic microglial activation (B, D), dopaminergic neurodegeneration (C, E) and locomotor deficit (F) in WT but not Mac1$^{-/-}$ mice.

Selected publications(* Corresponding author)

1. R. Yang, Y. Gao, Hui Li, W. Huang, D. Tu, M. Yang, X. Liu, J.-S. Hong and H.-M. Gao* (2022) Posttranslational S-nitrosylation modification regulates HMGB1 secretion and promotes its pro-inflammatory and neurodegenerative effects. Cell Reports 40, 111330
2. Liu D, Zhao Y, Qi Y, Gao Y, Tu DZ, Wang Y, Gao H-M*, Zhou H* (2020) Benzo(a)pyrene exposure induced neuronal loss, plaque deposition, and cognitive decline in APP/PS1 mice. Journal of Neuroinflammation 17(1):258
3. Gao Y, Tu DZ, Yang R, Chu CH, Hong JS and Gao H-M* (2020) Through Reducing ROS Production, IL-10 Suppresses Caspase-1-Dependent IL-1 Maturation, thereby Preventing Chronic Neuroinflammation and Neurodegeneration. Int. J. Mol. Sci., 21, 465
4. Tu DZ, Gao Y, Yang R, Guan T, Hong JS and Gao H-M* (2019) The pentose phosphate pathway regulates chronic neuroinflammation and dopaminergic neurodegeneration. Journal of Neuroinflammation 16:255
5. Chu CH, Wang S, Chen SH, Wang Q, Lu RB, Gao H-M*, and Hong JS (2016) Neurons and astroglia govern microglial endotoxin tolerance through macrophage colony-stimulating factor receptor-mediated ERK1/2 signals. Brain Behavior and Immunity 55: 260–272
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8. Gao H-M*, Zhou H, Hong J-S (2012) NADPH oxidases: novel therapeutic targets for neurodegenerative diseases. Trends in Pharmacological Sciences 33(6): 295-303

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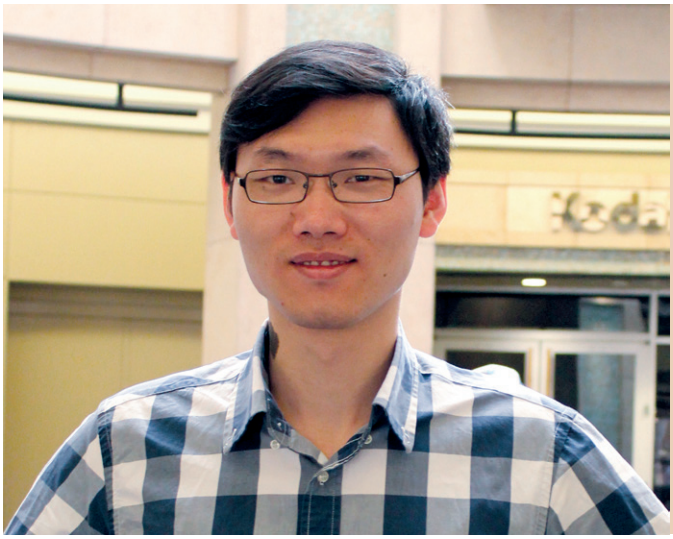

Guoqiang Wan, Ph.D.

Guoqiang Wan received both of his BSc in 2004 and PhD in 2011 from the National University of Singapore. He then had postdoctoral training with Dr Gabriel Corfas first at the Harvard Medical School/Boston Children's Hospital from 2011-2014 and then at the University of Michigan from 2014-2016. He joined MARC of Nanjing University as Principal Investigator in July 2016. Wan lab works on the genetics of hearing and deafness, development and regeneration of cochlear sensory cells and synapses, as well as applications of cochlear organoid models for hearing research.

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Development and Regeneration of Auditory Sensory Cells and Synapses

In China, 27.8 million people suffer from disabling hearing loss and this number increases by 300,000 every year. Sensorineural hearing loss (SNHL) accounts for 90% of all hearing loss and in most cases it cannot be medically or surgically treated. Mechanistically, SNHL results from damages to the sensory hair cells that are essential for sound detection and/or the spiral ganglion neurons (SGNs) that are required for transmitting the acoustic signals to the brain. In addition, even with the presence of intact sensory epithelia, hearing problems can also arise from irreversible loss of the synaptic connections between hair cells and SGNs, an auditory pathology termed as cochlear synaptopathy. Therefore, restoration of auditory functions requires not only preservation or regeneration of the sensory hair cells, neurons and non-sensory supporting cells, but also re-establishment of the cochlear synaptic connections (Figure 1). Our lab aims to identify novel molecular targets and pathways for the development and regeneration of cochlear sensory cells and synapses and to explore therapeutic potentials of these targets for treatment of sensorineural hearing loss.

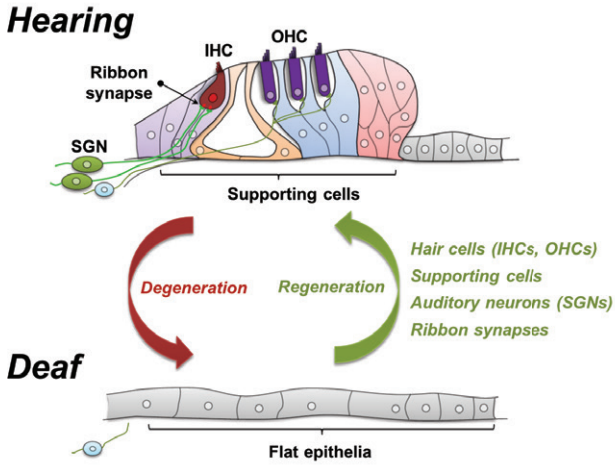

Figure 1. Research overview. In mammalian cochlea, sensory hair cells (IHC and OHC), neurons (SGN), supporting cells and the auditory ribbon synapses are required for normal hearing, damage of these cells and synapses results in deafness.

(1) Novel regulators of hair cell development and reprogramming using cochlear organoids

Loss of hair cells is the primary cause of sensorineural hearing loss. Unlike fish, birds and amphibians, mammalian hair cells do not regenerate, posing great challenge in restoration of auditory function in deaf humans. Due to the scarcity of cochlear sensory cells and lack of appropriate cell culture models, high throughput screening (HTS) for regulators of hair cells is severely limited. To circumvent this problem, we established a robust, high throughput cochlear organoid platform that facilitates 3D expansion of cochlear progenitor cells and differentiation of hair cells in a temporary-regulated manner. High throughput screening of the FDA-approved drug library identified Regorafenib, a VEGFR inhibitor, as a potent small molecule for hair cell differentiation. Regorafenib also promotes reprogramming and maturation of hair cells in both normal and neomycin-damaged cochlear explants. Mechanistically, inhibition of VEGFR suppresses TGFβ1 expression via MEK pathway and TGFβ1 downregulation directly mediates the effect of Regorafenib on hair cell reprogramming. Our study not only demonstrates the power of cochlear organoid platform in high throughput analyses of hair cell physiology, but also highlights VEGFR-MEK-TGFβ1 signaling crosstalk as a potential target for hair cell regeneration and hearing restoration (Figure 2).

(2) Progenitors and small molecule cocktails for cochlear sensory neuron reprogramming

In the mammalian cochlea, spiral ganglion neurons (SGNs) relay the acoustic information to the central auditory circuits. Degeneration of SGNs is a major cause of sensorineural hearing loss and severely affects the effectiveness of cochlear implant therapy. Cochlear glial cells are able to form spheres and differentiate into neurons in vitro. However, the identity of these progenitor cells is elusive, and it is unclear how to differentiate these cells towards functional SGNs. In this study, we found that Sox2+ subpopulation of cochlear glial cells preserves high potency of neuronal differentiation. Interestingly, Sox2 expression was downregulated during neuronal differentiation and Sox2 overexpression paradoxically inhibited neuronal differentiation. Our data suggest that Sox2+ glial cells are potent SGN progenitor cells, a phenotype independent of Sox2 expression. Furthermore, we identified a combination of small molecules that not only promoted neuronal differentiation of Sox2- glial cells, but also removed glial cell identity and promoted the maturation of the induced neurons (iNs) towards SGN fate. In summary, we identified Sox2+ glial subpopulation with high neuronal potency and small molecules inducing neuronal differentiation towards SGNs (Figure 3).

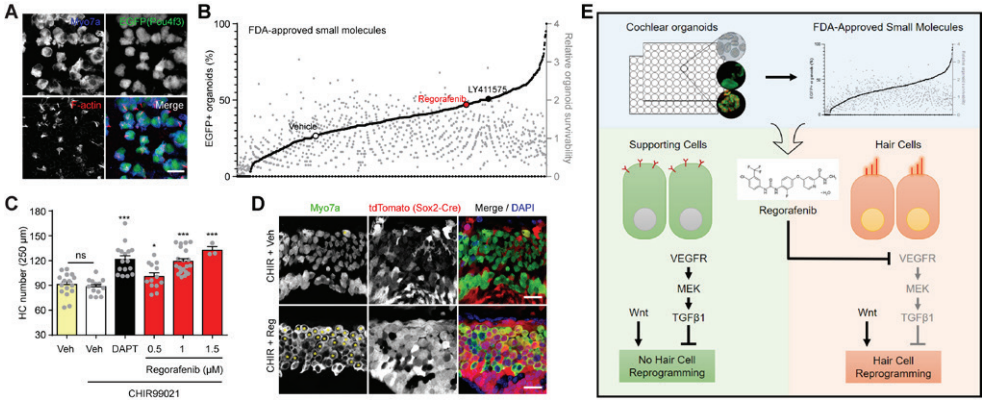

Figure 2. High throughput screening on cochlear organoids identifies VEGFR-MEK-TGFβ1 signaling promoting hair cell reprogramming. (A) Confocal images of EGFP+ hair cells in differentiated DIV26 Pou4f3(EGFP/+) reporter organoids (CHIR99021 + LY411575). Hair cells were co-labelled with Myo7a and stereocilia labelled with F-actin. Scale bar, 20 μm. (B) Screening of the FDA-approved small molecules identifies Regorafenib promoting hair cell differentiation in Pou4f3(EGFP/+) cochlear organoids. (C) Hair cell counts of cochlear explants treated with DAPT or Regorafenib at middle turn. (D) Regorafenib promoted hair cell reprogramming from Sox2+ supporting cells in cochlear explants lineage-traced by Sox2-CreER. Yellow asterisks indicate co-labelled cells. Scale bars, 20 μm. (E) A working model of VEGFR-MEK-TGFβ1 signaling axis in promoting hair cell reprogramming. (Published in Stem Cell Rep. 2021).

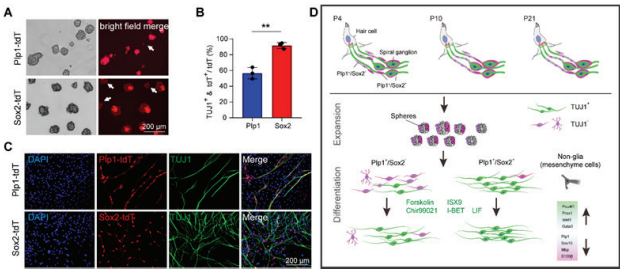

Figure 3. Cochlear Sox2+ glial cells as potent progenitors for spiral ganglion neuron reprogramming induced by small molecules. (A) Representative images of the Sox2-tdT and Plp1-tdT positive spheres culture 5 div. The white arrow represents the Plp1-tdT or Sox2-tdT negative spheres. Representative images (C) and ratios (B) of TUJ1+ neurons differentiated from Sox2-tdT or Plp1-tdT positive cells. (D) A working model of glial progenitors and small molecules for SGN reprogramming. (Published in Front Cell Dev Biol. 2021)

Selected Publications(*co-first authors, #co-senior authors)

1. Liu, Q., Zhang, L., Zhu, M.S., and Wan, G. (2021). High-throughput screening on cochlear organoids identifies VEGFR-MEK-TGFB1 signaling promoting hair cell reprogramming. Stem Cell Reports, 16:2257-2273.
2. Chen, Z., Huang, Y., Yu, C., Liu, Q., Qiu, C., and Wan, G. (2021). Cochlear Sox2+ glial cells are potent progenitors for spiral ganglion neuron reprogramming induced by small molecules. Frontiers in Cell and Developmental Biology, 9:728352.
3. Yu, C., Gao, H.M., and Wan, G. (2021). Macrophages Are Dispensable for Postnatal Pruning of the Cochlear Ribbon Synapses. Frontiers in Cellular Neuroscience, doi: 10.3389/fncel.2021.736120.
4. Zhu, G.J.*, Gong, S.*, Ma, D.B.*, Tao, T.*, He, W.Q.*, Zhang, L., Wang, F., Qian, X.Y., Zhou, H., Fan, C., Wang, P., Chen, X., Zhao, W., Sun, J., Chen, H., Wang, Y., Gao, X., Zuo, J., Zhu, M.S.#, Gao, X.#, Wan, G.# (2020). Aldh inhibitor restores auditory function in a mouse model of human deafness. PLOS Genetics, 16(9):e1009040.
5. Du, H.*, Ye, C.*, Wu, D.*, Zang, Y.Y., Zhang, L., Chen, C., He, X.Y., Yang, J.J., Hu, P., Xu, Z., Wan, G.# and Shi, Y.S.# (2020). The cation channel TMEM63B is an osmosensor required for hearing. Cell Reports, 31(5):107596.

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Jiong Chen Ph.D.

Jiong Chen received his Bachelor in Biochemistry (1995) and his Ph.D. in Molecular, Cell and Developmental Biology (2002), both from University of California, Los Angeles (UCLA). His Ph.D. thesis was carried out in Frank Laski's lab and it was focused on the genetics and developmental studies of cell movement processes in the Drosophila ovary. From 2002 to 2004, Jiong did his postdoctoral research in Drosophila eye development under the guidance of Utpal Banerjee at UCLA, and it was combined with an undergraduate teaching experience that was funded by a HHMI teaching/research grant. He joined the Faculty of Model Animal Research Center (MARC), Nanjing University full time early 2005. He is now a professor of genetics and developmental biology and a principal investigator in MARC.

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Understanding the driving forces underlying collective cell Migration

Cells do not always migrate individually; they often migrate collectively as a cluster, a sheet, or a strand under physiological, developmental and cancer metastatic conditions. Collective cell migration has recently received much attention from cell and developmental biologists, and it has emerged as an important field of study with many characteristics distinct from those of single cell migration. As a new field, collective migration still has many fundamental questions unresolved. For example, what intrinsic factors or signals pre-determine the migratory fate of a group of cells that will later collectively detach and migrate away from the host tissue (likened to a group of runners pre-selected from a larger group of candidate runners)(Figure 1)? How can the group of cells communicate with each other and collectively know the front vs. back, top vs. bottom and inside vs. outside during migration (Figures 2 and 3)? Finally, what powers the group to migrate collectively (Figure 3)?

A recent and primary focus of my lab has been to address these key questions. We utilize the border cells in Drosophila ovary to study collective migration during development, and they are genetically tractable and amenable to live imaging and optogenetic manipulation.

Cell growth regulates fate determination of border cells. Recently, my lab found that the fate determination of border cells was negatively regulated by the growth-promoting InR/Akt/TORC1 signaling pathway (Fig 1; Kang et al., Dev Cell, 2018). During development, cell growth and cell differentiation are two distinct yet coupled fundamental processes to give rise to tissues or organs. However, the mechanisms underlying the coordination or coupling between cell growth and cell differentiation are largely unknown. Our novel finding suggests that specification and differentiation of migratory cells is negatively coupled to cell growth during development.

Control of front-back polarity. It is known that the chemotactic migration of border cells is guided by the guidance receptor PVR, in response to extracellular signals secreted from oocyte. But, how guidance signaling sets up the front-back polarity of the entire border cell cluster is not well understood. We've made an interesting discovery that the guidance receptor PVR mediates the asymmetric distribution of exocyst and recycling endosome to set up the front-back polarity. (Wan et al., Development, 2013). Furthermore, we find that molecules crucial in apical-basal polarity, including aPKC and Crumbs complex, are required for the establishment of front-back polarity (Fig 3; Wang et al., Development, 2018). In addition, we find interesting coordination among the front-back, apical-basal and inside-outside polarities within the border cell cluster.

Power control of collective migration. We found that the actin depolymerizing factor Cofilin is required for the formation of actin-based lamellipodia, whose protrusion and adhesion provide force for migration of border cells (Zhang et al., Development, 2011). Moreover, Cofilin localization and phosphorylation are regulated by guidance receptor (PVR) signaling in such a way that active and unphosphorylated Cofilin are enriched in the leading border cell, resulting in the predominant protrusion forming only at the front of border cell cluster.

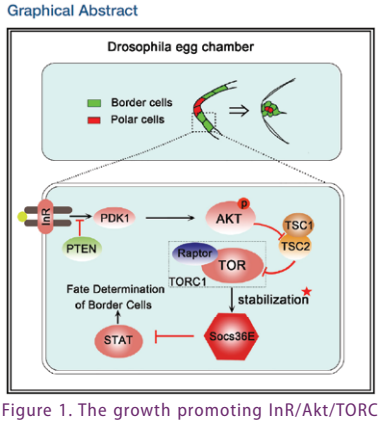


Figure 1. The growth promoting InR/Akt/TORC1 pathway attenuates migratory cell fate of border cells by down-regulation of JAK/STAT and destabilization of Socs36E. (from Kang et al., 2018)

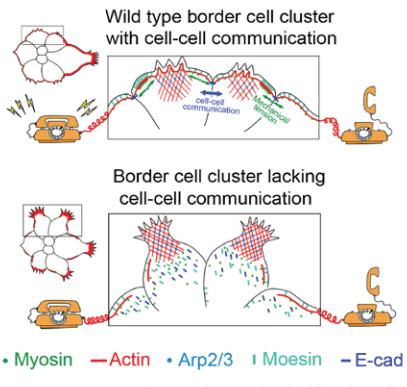


Figure 2. Diagram showing how individual border cells communicate with each other via supracellular actin-myosin network. Disruption of the supracellular network results in lack of cell-cell communication and defects in collective cell migration (from Wang H. et al., 2020)

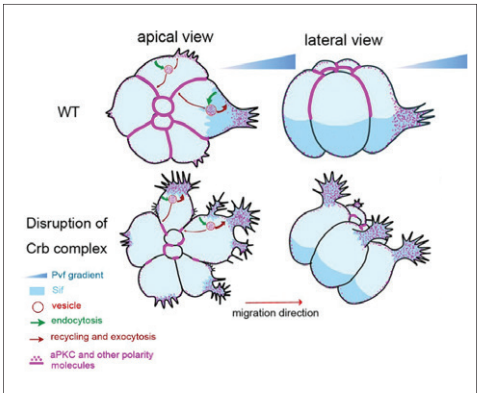
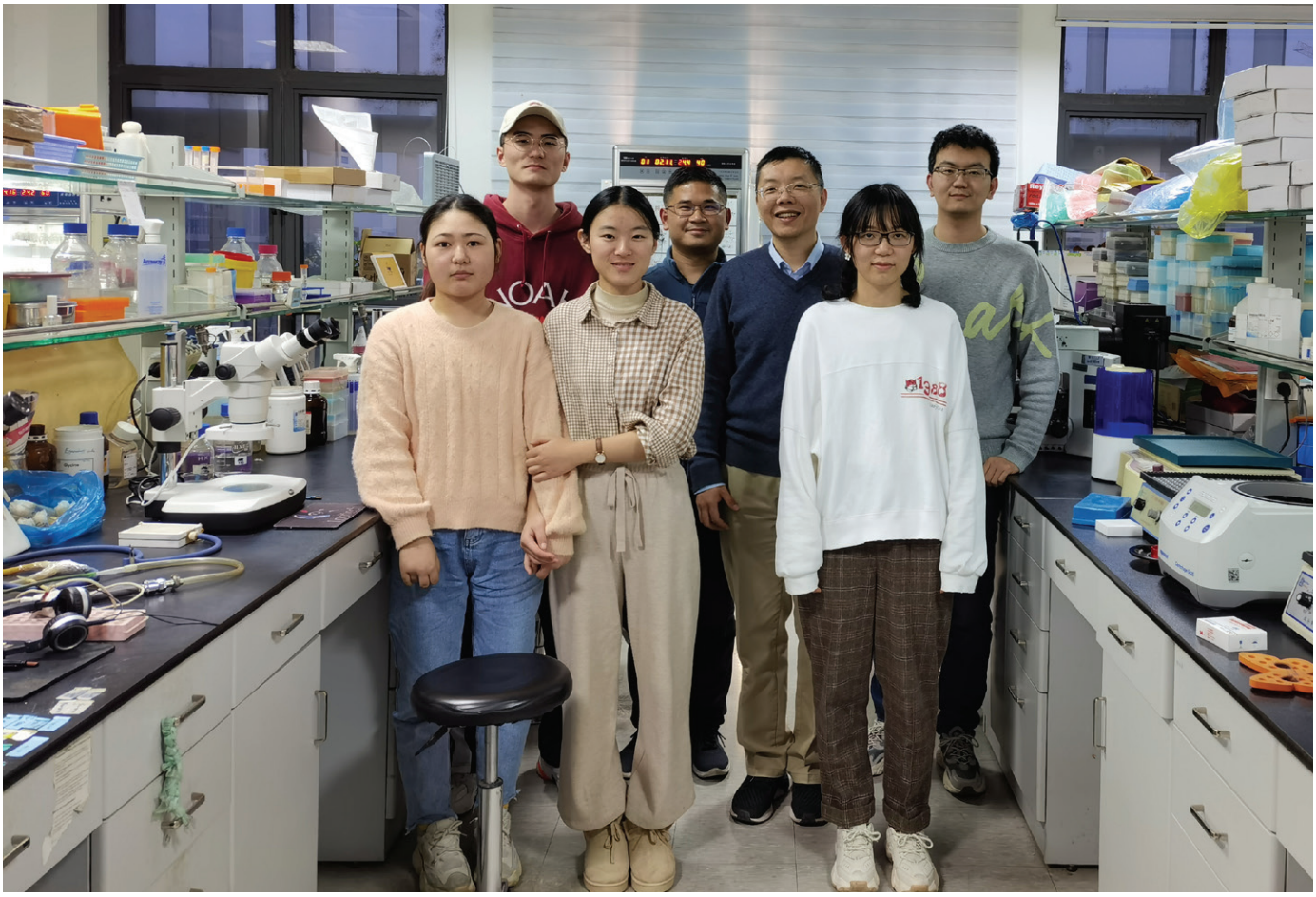


Figure 3. Diagram showing WT and Crb complex-deficient border cells in apical and lateral views. The model proposes how the two pools of aPKC and three distinct cell polarities are established by the Crb and Par complexes and endocytic recycling machinery. (from Wang et al., 2018)

Selected Publications

1. Qu, C., Yang, W., Kan, Y., Zuo, H., Wu, M., Zhang, Q., Wang, H., Wang, D. and Chen, J. * RhoA/ROCK Signaling Regulates Drp1-Mediated Mitochondrial Fission During Collective Cell Migration. Frontiers in Cell and Developmental Biology (2022)
2. Qu, C., Kan, Y., Wu, M., Dong, Z., Wang, X., Zhang, Q., Wang, H., Wang, D. and Chen, J. * Actin polymerization induces mitochondrial distribution during collective cell migration. Journal of Genetics and Genomics (2022)
3. Wang, X., Wang, H. *, Liu, L., Li, S., Emery, G. and Chen, J. * Temporal coordination of collective migration and lumen formation by antagonism between two nuclear receptors. Iscience (2020)
4. Wang, H. *, Guo, X., Wang, X., Wang, X. and Chen, J. * Supracellular actomyosin mediates cell-cell communication and shapes collective migratory morphology. Iscience (2020)
5. Guo, X., Luo, J., Wang, H.*, and Chen, J*. SERCA regulates collective cell migration by maintaining cytoplasmic Ca2+ homeostasis, Journal of Genetics and Genomics (2019)
6. Wang, H., Qiu, Z., Xu, Z., Chen, S., Luo, J., Wang, X.*and Jiong Chen*. aPKC is a key polarity molecule coordinating the function of three distinct cell polarities during collective migration, Development (2018)
7. Kang, D., Wang, D., Xu, J., Quan, C., Guo1, X., Wang, H., Luo, J., Yang, Z., Chen, S.*, Chen, J.*. The InR/Akt/TORC1 growth-promoting signaling negatively regulates JAK/STAT activity and migratory cell fate during morphogenesis, Developmental Cell (2018)
8. Wan, P., Wang, D., Luo, J., Chu, D., Wang, H., Zhang, L., and Chen, J.* Guidance receptor promotes asymmetric distribution of Exocyst and recycling endosome during collective cell migration Development (2013).
9. Zhang, L., Luo, J., Wan, P., Wu, J., Laski, F. and Chen, J.* Regulation of cofilin phosphorylation and asymmetry in collective cell migration during morphogenesis. Development 138, 455-64. (2011)



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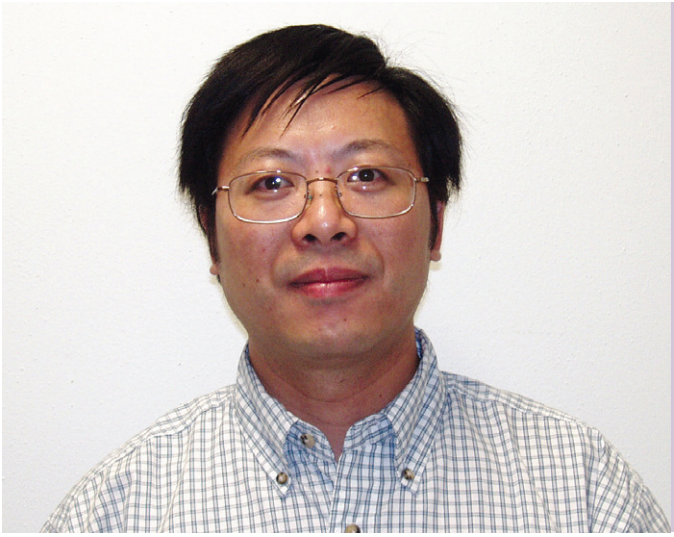

Qing Zhang, Ph.D

Qing Zhang received his Ph.D in Microbiology from Fudan University in 2002. Afterwards, he had had his postdoctoral training in Department of Developmental Biology of UT Southwestern Medical Center at Dallas for six years. In 2009, he joined the Model Animal Research Center of Nanjing University as a professor and principle investigator.

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Regulation of hedgehog signaling

Hedgehog signaling plays critical role in embryonic development and adult tissue homeostasis in species ranging from insects to human. Aberrant Hh signaling activity is associated with many human disorders including birth defects and cancers.

In Drosophila, Hh tansduces signal through binding its receptor, a 12-transmembrane protein Patched (Ptc), that alleviates suppression of ptc on Smoothened (Smo), a GPCR-like seven-transmembrane protein. Active Smo as the Hh signal transducer triggers a largely conserved signaling cascade that culminates at the activation of latent transcription factors Cubitus interruptus (Ci) which controls Hh targets decapentaplegic (dpp), ptc and engrailed (en) expression .

Based on Hh pathway is conserved among species, we take advantage of Drosophila as a model to investigate the mechanism of Hh signaling. Our work mainly focuses on getting the whole picture of around 7000 conserved genes on Hh signaling regulation and trying to answer the long-standing questions, for example, how does Ptc, the membrane receptor of Hh, inhibit Smo activity? how is hyperactive Ci degraded? and so on.

E3 ligase Herc4 regulates Hedgehog signaling through promoting Smoothened degradation

Hedgehog (Hh) signaling plays conserved roles in controlling embryonic development, its dysregulation causes many diseases including cancers. The G protein-coupled receptor Smoothened (Smo) is the key signal transducer of the Hh pathway, whose posttranslational regulation has been shown to be critical for its accumulation and activation. Ubiquitination has been reported to be an essential posttranslational regulation of Smo. Here, we identify a novel E3 ligase of Smo, Herc4, which binds to Smo and regulates Hh signaling by controlling Smo ubiquitination and degradation. Interestingly, our data suggest that Herc4-mediated Smo degradation is regulated by Hh in PKA-primed phosphorylation dependent and independent manners.

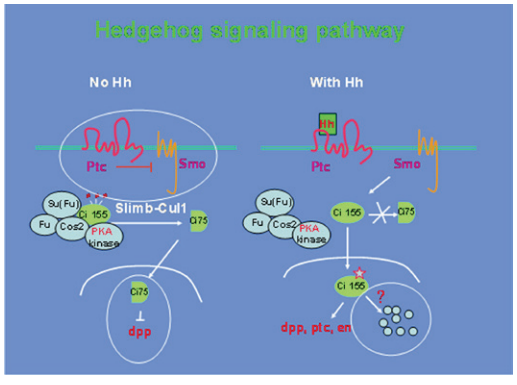

Fig1. Hedgehog signaling pathway in Drosophila.

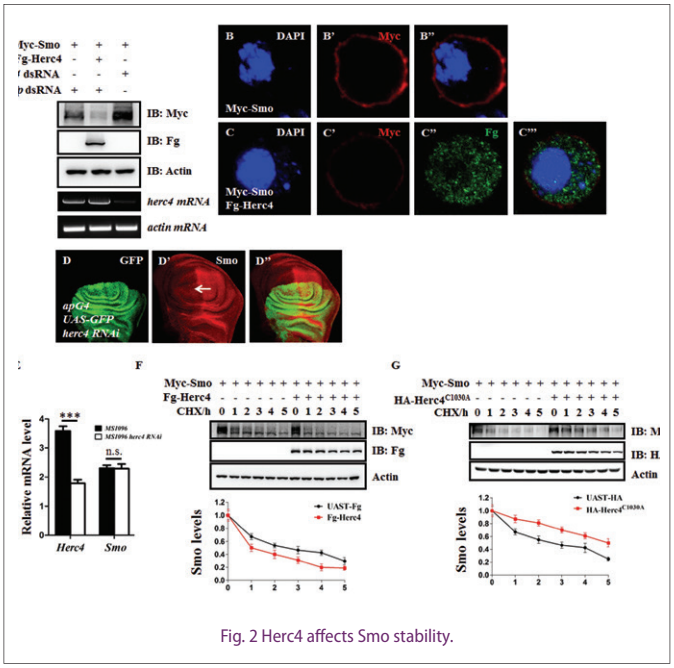

Fig. 2 Herc4 affects Smo stability.

(A) Overexpression of Fg-Herc4 downregulated Myc-Smo protein level. Knockdown of herc4 upregulated Myc-Smo protein. herc4-dsRNA could effectively knock down herc4 mRNA level in S2 cells (bottom two panels). (B-C‴) S2 cells transfected with indicated constructs were stained by Myc, Flag antibody and DAPI. Of note, Herc4 inhibited Smo cell membrane accumulation (compare Figures 2C-C‴ with Figures 2B-B″). The nuclei were showed by DAPI staining. (D-D″) Knockdown of herc4 with apG4 increased the anterior compartment Smo protein level of the wing disc. Arrows indicate the increase of Smo. (E) The relative mRNA level of smo in wing discs. (F-G) Western blots of lysates from S2 cells expressing indicated proteins and treated with CHX for the indicated time intervals. Quantification analyses were shown below. The results were presented as means±s.d. of values from three independent experiments. Of note, Herc4 could promote Smo degradation (F). Herc4^{C1030A} could hamper Smo degradation (G).

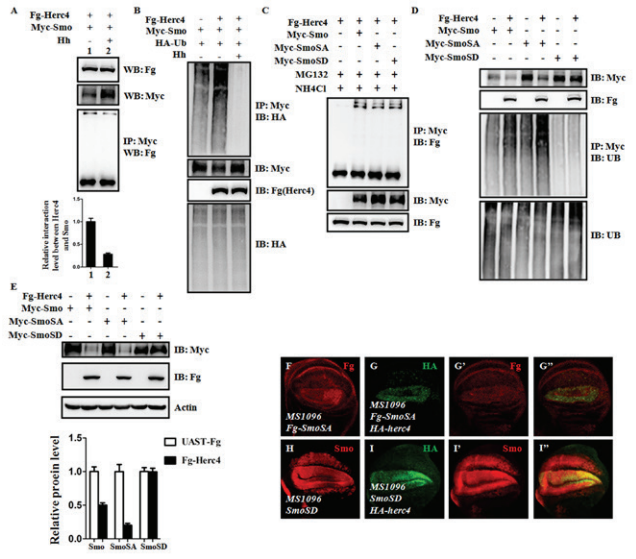

Fig. 3 Hh inhibits Herc4-mediated Smo ubiquitination.

(A) Hh treatment inhibited the interaction of Herc4 and Smo. (B) Hh decreased Smo ubiquitination mediated by Herc4. (C) S2 cells were transfected with indicated plasmids and treated with the proteasome/lysosome inhibitors MG132 and NH4Cl. Fg-Herc4 interacts equally with Myc-Smo, Myc-SmoSA and Myc-SmoSD. (D) From cell based ubiquitin assay, Herc4 upregulated the ubiquitination level of Smo and SmoSA, but did not affect the ubiquitination level of SmoSD. (E) Overexpression of Herc4 in S2 cells apparently decreased the protein level of Smo and SmoSA, but did not affect SmoSD protein level. (F-G'') Overexpression of Herc4 in wing discs with MS1096-gal4 decrease the expression level of Fg-SmoSA. (compare Figure 3G' with 3F). (H-I''') Overexpression of Herc4 in wing discs with MS1096-gal4 has no effect on the protein level of SmoSD. (compare Figure 3I' with 3H).

Selected Publications

1. Jiang W#, Yao X#, Shan Z, Li W, Gao Y, Zhang Q*. (2019) E3 ligase Herc4 regulates Hedgehog signalling through promoting Smoothened degradation. J Mol Cell Biol. 11(9):791-803.
2. Zhou Z#, Yao X#, Pang S, Chen P, Jiang W, Shan Z, Zhang Q*. (2018) The deubiquitinase UCHL5/UCH37 positively regulates Hedgehog signaling by deubiquitinating Smoothened. J Mol Cell Biol. 10(3):243-257.
3. Chen P#, Zhou Z#, Yao X, Pang S, Liu M, Jiang W, Jiang J*, Zhang Q*. (2017) Capping Enzyme mRNA-cap/RNGTT Regulates Hedgehog Pathway Activity by Antagonizing Protein Kinase A. Sci Rep. 7(1): 2891.
4. Zhou Z#, Yao X#, Li S#, Xiong Y, Dong X, Zhao Y, Jiang J*, Zhang Q*. (2015) Deubiquitination of Ci/Gli by Usp7/HAUSP Regulates Hedgehog Signaling. Dev Cell. 34(1):58-72.
5. An J, Ren S, Murphy S, Dalangood S, Chang C, Pang X, Cui Y, Wang L, Pan Y, Zhang X, Zhu Y, Wang C, Halling G, Cheng L, Sukov W, Karnes R, Vasmatzis G, Zhang Q, Zhang J, Cheville J, Yan J, Sun Y, Huang H. (2015) Truncated ERG Oncoproteins from TMPRSS2-ERG Fusions Are Resistant to SPOP-Mediated Proteasome Degradation. Mol Cell. 59:1-13.
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7. Zhang Q, Shi Q, Chen Y, Yue T, Li S, Wang B, Jiang J. (2009) Multiple Ser/Thr-rich degrons mediate the degradation of Ci/Gli by the Cul3-HIB/SPOP E3 ubiquitin ligase. PNAS . 106(50):21191-6.
8. Zhang L, Ren F, Zhang Q, Chen Y, Wang B, Jiang J. (2008) The TEAD/TEF Family of Transcription Factor Scalloped Mediates Hippo Signaling in Organ Size Control. Dev Cell. 14, 377-387.
9. Zhang Q#, Zhang L#, Wang B, Ou CY, Chien CT, Jiang J. (2006) A Hedgehog-induced BTB protein modulates Hedgehog signaling responses by degrading Ci/Gli transcription factor. Dev Cell. 10, 719-729.
10. Jia J#, Zhang L#, Zhang Q#, Tong C, Wang B, Hou F, Amanai K, Jiang J. (2005) Phosphorylation of Cubitus interruptus by Double-time/CKIe and CKIa targets it for Slimb/b-TRCP mediated proteolytic processing. Dev Cell. 9, 819-830.

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Ying Cao, Ph.D.

Cao Ying made the PhD study at the University of Essen (now University of Duisburg-Essen), Germany, from 1998 to 2002. During the period he studied developmental biology and made a research on embryonic development using an amphibian species (Xenopus leavis) as a model organism. He received the degree Dr. rer. nat. and graduated summa cum laude in 2002. Afterwards during the years from 2002 to 2008 he joined the Institute of Biochemistry, Ulm University, Germany, and continued the study on developmental biology of Xenopus. In October 2008, he set up the laboratory in MARC for developmental biology and cancer biology. The results in his group suggest that the property of neural stemness is the key to understand tumorigenicity and pluripotent differentiation potential. He proposes novel conceptual paradigms that "Tumorigenesis represents a process of loss of original cell identity and gain of properties of neural stemness" and "Neural stemness represents the ground or basal state of cell tumorigenicity and differentiation potential."

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Neural stemness unifies tumorigenicity and pluripotency.

My lab has aimed to figure out general principles governing tumorigenesis. Our previous work demonstrated that neural stemness contributes to tumorigenicity, and proposed that neural stemness represents the ground state of cell tumorigenicity and pluripotent differentiation potential. This suggested a prime importance of neural stemness for tumorigenicity and pluripotency, two fundamental cell properties in cancer biology and developmental biology. Our recent work elucidated that neural stemness unifies cell tumorigenicity and pluripotency. Loss of neural stemness in cancer cells via neuronal differentiation causes the loss of both tumorigenicity and pluripotency. Vice versa, gain of neural stemness in cancer cells leads to the enhancement of tumorigenicity and pluripotency. We found that the oncoprotein SETDB1 plays essential roles in the regulation of neural stemness, tumorigenicity and pluripotency. It maintains a regulatory network comprising of proteins involved in developmental programs and basic cellular functional machineries, including epigenetic modifications, ribosome biogenesis, translation initiation, and spliceosome assembly, thereby maintaining neural stemness in both neural stem cells and cancer cells. Interestingly, serial xenotransplantation of cancer cells showed that neural stemness, tumorigenicity, and pluripotency were simultaneously enhanced (Figure 1); these effects were accompanied by increased expression of proteins involved in developmental programs and basic machineries, including SETDB1, as well as by increased alternative splicing events. These results indicate that basic machineries work together to define a highly proliferative state with pluripotent differentiation potential and also suggest that neural stemness unifies tumorigenicity and pluripotency. Tumorigenesis representing the loss of original cell identity and gain neural stemness reminds us of a most fundamental process during embryogenesis, i.e., neural induction. Integration of evidence from developmental and cancer biology indicates that neural induction drives embryogenesis in gastrulating embryos but a similar process drives tumorigenesis in a postnatal animal (Figure 2). For detailed information, see our papers listed below.

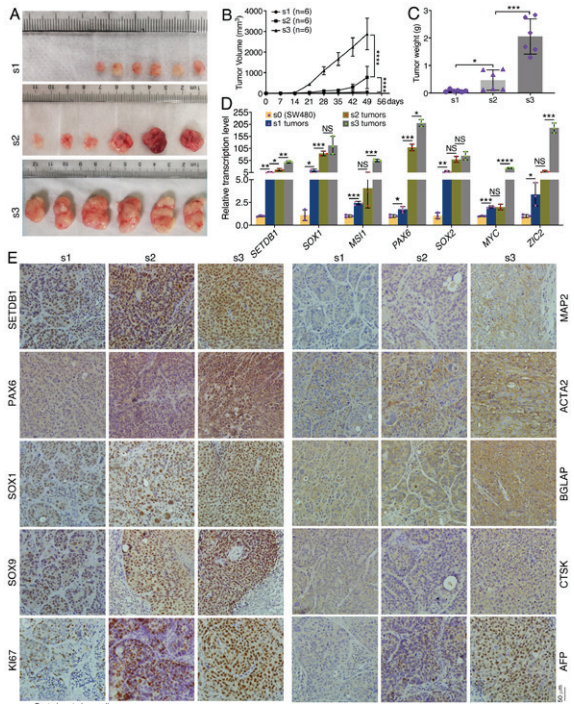

Figure 1. Serial xenotransplantation of cancer cells revealed that neural stemness, tumorigenicity and pluripotent differentiation potential enhanced simultaneously.

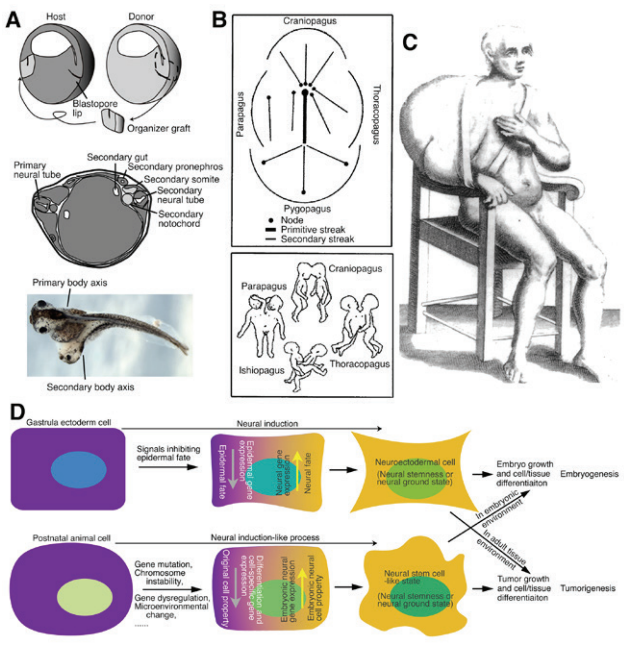

Figure 2. An ectopic neural induction event in a gastrula embryo induces secondary axis formation (A), which explains conjoined twin formation in human (B). A similar process may occur in a postnatal animal, which leads to tumor formation (C, D).

Selected publications (*Correspondence author)

1. Zhang M, Liu Y, Shi L, Fang L, Xu L, Cao Y*. Neural stemness unifies cell tumorigenicity and pluripotent differentiation potential. J Biol Chem. 2022 Jul;298(7):102106.
2. Cao Y*. Neural is Fundamental: Neural Stemness as the Ground State of Cell Tumorigenicity and Differentiation Potential. Stem Cell Rev Rep. 2022 Jan;18(1):37-55.
3. Chen L, Zhang M, Fang L, Yang X, Cao N, Xu L, Shi L, Cao Y*. Coordinated regulation of the ribosome and proteasome by PRMT1 in the maintenance of neural stemness in cancer cells and neural stem cells. J Biol Chem. 2021 Oct 4;297(5):101275.
4. Yang X, Cao N, Chen L, Liu L, Zhang M, Cao Y*. Suppression of Cell Tumorigenicity by Non-neural Pro-differentiation Factors via Inhibition of Neural Property in Tumorigenic Cells. Front Cell Dev Biol. 2021 Sep 14;9:714383.
5. Xu L, Zhang M, Shi L, Yang X, Chen L, Cao N, Lei A, Cao Y*. Neural stemness contributes to cell tumorigenicity. Cell Biosci. 2021 Jan 19;11(1):21.
6. Cao Y*. Tumorigenesis as a process of gradual loss of original cell identity and gain of properties of neural precursor/progenitor cells. Cell Biosci. 2017 Nov 7;7:61. (Review)
7. Zhang Z, Lei A, Xu L, Chen L, Chen Y, Zhang X, Gao Y, Yang X, Zhang M, Cao Y*. 2017. Similarity in gene-regulatory networks suggests that cancer cells share characteristics of embryonic neural cells. J Biol Chem. 292(31):12842-12859.
8. Lu L, Gao Y, Zhang Z, Cao Q, Zhang X, Zou J, Cao Y*. 2015. Kdm2a/b Lysine Demethylases Regulate Canonical Wnt Signaling by Modulating the Stability of Nuclear β -Catenin. Dev Cell. 33(6):660-74.

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Qingshun Zhao, Ph.D

Qingshun obtained his B.S. degree in 1987 and M.S. degree in 1990 from Nanjing University (Nanjing, Jiangsu, China), and received his Ph.D. degree in 2001 from Purdue University (West Lafayette, Indiana, USA). His Ph.D. dissertation was carried out in Dr. Paul Collodi's Lab and it was focused on identification and characterization of fibronectin isoforms during zebrafish development. From 2001 to 2003, Qingshun did his postdoctoral research on roles of retinoid signaling in zebrafish hindbrain development under the guidance of Dr. Elwood Linney in Duke University Medical Center (Durham, North Carolina, USA). In 2003, he became an associate professor and a principal investigator in Model Animal Research Center, Nanjing University. In 2006, Qingshun was promoted full professor of Nanjing University.

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Zebrafish development

The research interests of the lab focus on investigating the molecular mechanism underlying vertebrate early development using zebrafish as a model animal.

RA (retinoic acid) plays essential roles in vertebrate embryogenesis. Its homeostasis in embryos is determined by the presence of Aldh1A that produces RA and Cyp26 that metabolizes RA into bio-inactive metabolites. Unlike mammals, zebrafish only have aldh1a2, aldh1a3 and aldh8a1 but not aldh1a1. Because both aldh1a3 and aldh8a1 are expressed in late organogenesis, aldh1a2 is the gene that is responsible for RA synthesis in zebrafish early development (Liang et al, 2008). Like mammals, zebrafish possesses a third cyp26 gene (cyp26c1) (Gu et al., 2005) in addition to cyp26a1 and cyp26b1. The Cyp26c1 metabolizes RA but not retinol or retinal in a similar way to Cyp26a1, the major gene that is responsible for RA metabolism in zebrafish early development (Gu et al., 2006). Like cyp26a1, proper expression of cyp26c1 at early developmental stage is essential for the development of anterior–posterior axis and left–right symmetry in zebrafish embryos (Gu et al., 2005; Gu et al., 2006). Analyzing the promoter of cyp26a1, we reveal that zebrafish cyp26a1 possesses three conserved RAREs in response to RA signal and therefore maintains RA homeostasis in a feedback way (Hu et al., 2008; Li et al., 2012). Other than Cyp26s that can limit RA signaling, Ncor1 (nuclear receptor co-repressor) is essential for patterning the anterior–posterior axis of zebrafish hindbrain by actively repressing RA signaling (Xu et al., 2009). Consistent with these results, znfl1 whose expressions are in response to RA signaling, mediate the roles of RA in patterning zebrafish posterior neuroectoderm by acting upstream of pou5f3 and sall4 (Dong et al., 2017). Additionally, Znfl1s regulate left-right asymmetry patterning through controlling the expression of fgfr1a (Li et al., 2019).

RA signaling is also essential to vertebrate mesoderm differentiation. It plays a restrictive role in primitive myelopoiesis by inhibiting the specification of ventral mesoderm cells into anterior hemangioblasts through acting downstream of gata4/5/6 and upstream to scl in a dose dependent manner (Liang et al., 2012). Furthermore, zebrafish microRNA miR-210-5p inhibits primitive myelopoiesis by silencing foxj1b and slc3a2a mRNAs downstream of gata4/5/6 transcription factor genes (Figure 1; Jia et al., 2019). Moreover, RA is also essential for valvulogenesis by affecting endocardial cushions formation in zebrafish embryos (Li et al., 2016). Additionally, Ncor1 and Ncor2 play essential but distinct roles in zebrafish primitive myelopoiesis (Li et al., 2014). On the other hand, the differentiation of ventral mesoderm is affected by environmental factors, excessive sodium nitrite affects zebrafish valve leaflet formation by producing too much NO signaling (Li et al., 2014).

RA signaling is genetically controlled by upstream genes. Foxc1a is a member of the forkhead transcription factors. By generating foxc1a knockout zebrafish using TALEN (transcription activator-like effector nuclease) technology, we demonstrate that foxc1a is essential for somitogenesis by controlling Fgf and Notch signaling through restricting the expression of aldh1a2 in zebrafish paraxial mesoderm directly (Li et al., 2015) and plays essential roles in zebrafish cardiogenesis by directly activating the expression of nkx2.5, encoding a transcriptional regulator of cardiac progenitor cells (Yue et al., 2018), and directly inhibiting the expression of aldh1a2 in foxc1a-expressing cells (Gu et al., Unpublished data). In human cells, we demonstrate that FOXC1 does regulate human NKX2-5 expression in a dose-dependent manner via direct binding to its proximal promoter. A comparison of FOXC1 mutant function in the rat cardiac cell line H9c2 and zebrafish embryos suggested that the zebrafish embryos might serve as a more representative model system than the H9c2 cells. Three of the Axenfeld-Rieger syndrome FOXC1 mutations tested increased whereas a fourth repressed the expression of NKX2-5 implying that mutant FOXC1s might play etiological roles in CHD by abnormally regulating NKX2-5 in the patients. To sum up, zebrafish embryos can serve as a useful in vivo platform for rapidly evaluating disease-causing roles of mutated genes (Zhang et al., 2020).

Engineered endonuclease including ZFN, TALEN and CRISPR/Cas9 are powerful tools to create genome edited animals without species limitation. Employing ZFN and TALEN, we produced heritable targeted inactivation of myostatin genes in yellow catfish, the first endogenous gene knockout in aquaculture fish (Dong et al., 2011, Dong et al., 2014), and the mstna null yellow catfish exhibit double muscle phenotype with muscle hyperplasia (Zhang et al., 2019). By co-microinjecting yfp-nanos3 mRNA with genome editing tools to make founders and then screen them with the help of tentatively fluorescent-labeled PGCs, we invent a new method that significantly increases the ease and speed of generating heritable knockin animals with CRISPR/Cas9 (Dong et al., 2014). Using this method, we develop "two-step strategy" to generate an aldh1a2 floxed zebrafish line (aldh1a2flox/flox) by first inserting mloxP sites into its 3rd intron and then into its 4th intron. With the systemic expression of Cre in the eggs of aldh1a2flox/flox zebrafish, we obtained an aldh1a2 conventional knockout zebrafish line ($aldh1a2^{+/-}$) (Gu et al., Unpublished data). Interestingly, the embryos whose primordial germ cells are eliminated at early development grow up as all-male-like sterile zebrafish (Zhou et al., 2018). Collaborating with the groups of Professors Zhou and Zhu, we developed an alternative novel tool for DNA editing (SGN: structure-guided nuclease) without target sequence limitation (Xu et al., 2016). Unfortunately, our further efforts do not support that the system works in human colorectal carcinoma cell line (HCT116), nor in producing any germline transmission zebrafish mutants (Zhang et al., Unpublished data)

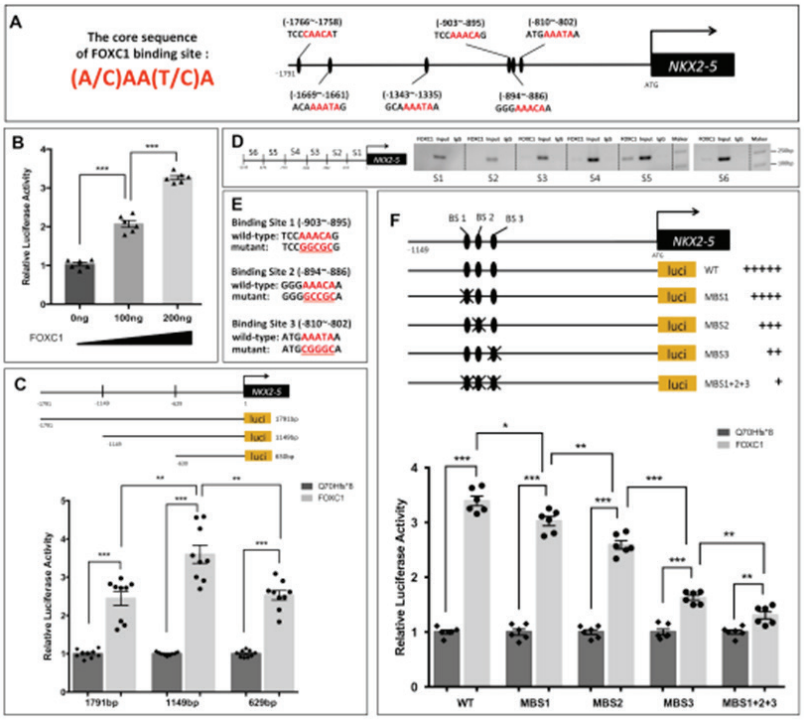

Figure 1. FOXC1 directly regulates the expression of NKX2-5 by binding to its proximal promoter in H9c2 cells.

A, Schematic showing putative FOXC1 transcription factor binding sites in 1791 bp 5′-flanking sequence upstream of NKX2-5 translation start site (ATG). B, Results of Dual-Luciferase Reporter Assay showing the responses of NKX2-5 promoter to different doses of FOXC1. C, Schematic (top) showing the firefly luciferase reporter expression constructs comprising the different lengths of upstream regulatory sequence ofNKX2-5, namely 1791 bp, 1149 bp or 630 bp, and the coding sequences of NKX2-5 or firefly luciferase, and the results (below) of Dual-Luciferase Reporter Assay on the three expression constructs. D, Schematic (left) showing the dissection of the 1149 bp regulatory sequences ofNKX2-5 into S1-S6 regions and the results of ChIP-PCR assay (right) indicating that S5contains FOXC1-binding sites. E, The wild-type sequences and location of FOXC1-binding sites (BS) in S5 of NKX2-5 regulatory sequence (top), and the mutant FOXC1 binding sites (MBS) with changed core sequence. F, Schematic (top) showing the reporter expression constructs carrying wild-type BS or MBS of FOXC1, and the results (below) of Dual-Luciferase Reporter Assay on the five expression constructs. X-axis (B, C, F): The amount of overexpressed FOXC1 (B), the reporter expression constructs with different lengths of regulatory sequences (C), or the reporter expression constructs carrying wild-type BS or MBS of FOXC1 (F). Light grey columns (C, F): transfected with wild-type FOXC1;Dark grey columns (C, F): transfected with the same amount of functional null mutated FOXC1(p.Q70Hfs*8) as control. Y-axis (B, C, F): Relative activity of firefly luciferase reporter.

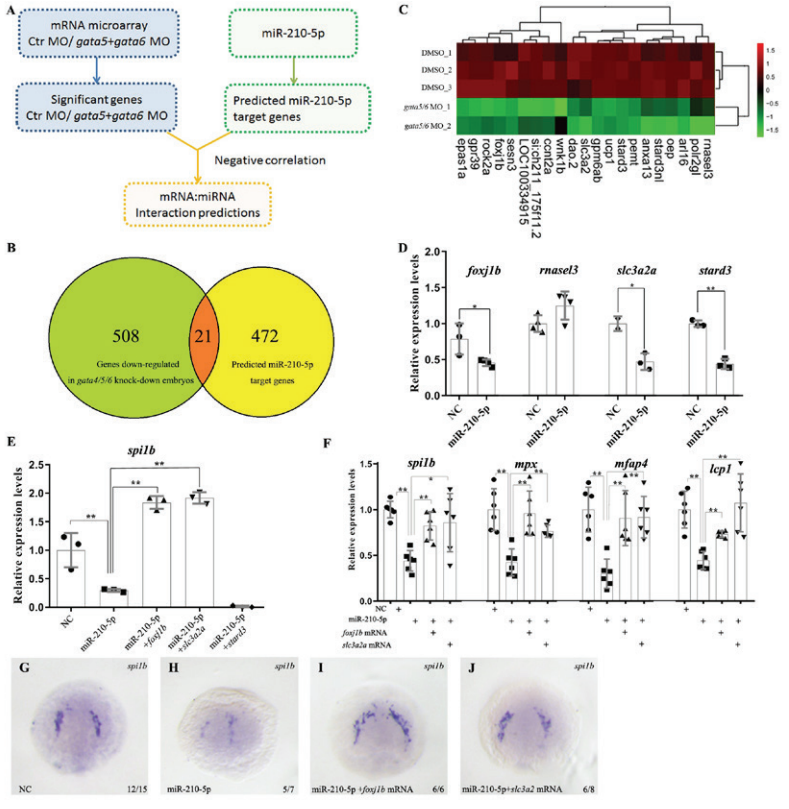

Figure 2. Both foxj1b and slc3a2a work downstream of miR-210-5p to mediate its role in inhibiting zebrafish primitive myelopoiesis.

(A) Schematic showing the workflow to screen the candidate downstream genes of miR-210-5p to mediate its role in inhibiting zebrafish primitive myelopoiesis. (B) Venn map showing the candidate target genes of miR-210-5p to mediate its roles in inhibiting zebrafish primitive myelopoiesis. (C) Heat map showing the clustering analysis of 21 candidate genes working downstream of miR-210-5p to inhibit the primitive myelopoiesis. (D) qRT-PCR results showing the expression changes of candidate target genes in the miR-210-5p overexpressed embryos at 14 hpf. (E) Overexpressions of foxj1b and slc3a2a but not stard3 effectively rescued the expression of spi1b that was inhibited in the 14 hpf embryos overexpressed with miR-210-5p. (F) Overexpressions of foxj1b and slc3a2a effectively rescued the expressions of spi1b, mpx, mfap4 and lcp1 in the zebrafish embryos at 14 hpf and 26 hpf, respectively. (G-J) Whole mount in situ hybridization results showing the expressions of spi1b in the 14 hpf embryos microinjected with NC, miR-210-5p mimic alone or miR-210-5p mimic together with foxj1b or slc3a2a mRNA, respectively.

Selected Publications (*corresponding author;**co-corresponding author)

1. Li N, Jiang D, He L, Yue Y, Zhang Q, Wang S, Zhang Y, Wei Y, Zhao Q. Both prokaryotes and eukaryotes produce an immune response against plasmids with 5'-GTTTGTT-3'. Cell Biosci. 2022 Jun 11;12(1):87. doi: 10.1186/s13578-022-00825-3. PMID: 35690839; PMCID: PMC9188160.
2. Dong X, Jiang D, Wang L, Zhao J, Yu L, Huang Y, Wu X, Zhu Y, Zhao Y, Zhao Q, Zhang G, Li X. VPS28 regulates brain vasculature by controlling neuronal VEGF trafficking through extracellular vesicle secretion. iScience. 2022 Mar 9;25(4):104042. doi: 10.1016/j.isci.2022.104042. PMID: 35330682; PMCID: PMC8938284.
3. Qinxin Zhang, Dong Liang, Yunyun Yue, Luqingqing He, Nan Li, Dongya Jiang, Ping Hu, Qingshun Zhao*. 2020. Axenfeld-Rieger syndrome-associated mutants of the transcription factor FOXC1 abnormally regulate NKX2-5 in model zebrafish embryos. The Journal of Biological Chemistry, 295(33):11902-11913.
4. Wenshuang Jia, Dong Liang; Nan Li, Meijing Liu, Zhangji Dong, Jingyun Li, Xiaohua Dong, Yunyun Yue, Ping Hu, Jihua Yao, Qingshun Zhao*. 2019. Zebrafish microRNA miR-210-5p inhibits primitive myelopoiesis by silencing foxj1b and slc3a2a mRNAs downstream of gata4/5/6 transcription factor genes. The Journal of Biological Chemistry, 294(8):2732-2743.
5. Yunyun Yue, Mingyang Jiang, Luqingqing He, Zhaojunjie Zhang, Qinxin Zhang, Chun Gu, Meijing Liu, Nan Li, Qingshun Zhao*. 2018. The transcription factor Foxc1a in zebrafish directly regulates expression of nkx2.5, encoding a transcriptional regulator of cardiac progenitor cells. The Journal of Biological Chemistry, 293(2):638-650.
6. Xiaohua Dong, Jingyun Li, Luqingqing He, Chun Gu, Wenshuang Jia, Yunyun Yue, Jun Li, Qinxin Zhang, Lele Chu, Qingshun Zhao*. 2017. Zebrafish Znfl1s control the expression of hoxb1b in the posterior neuroectoderm by acting upstream of pou5f3 and sall4. The Journal of Biological Chemistry, 292(31):13045-13055.
7. Shu Xu, Shasha Cao, Bingjie Zou, Yunyun Yue, Chun Gu, Xin Chen, Pei Wang, Xiaohua Dong, Zheng Xiang, Kai Li, Minsheng Zhu**, Qingshun Zhao**, Guohua Zhou*. 2016. An alternative novel tool for DNA editing without target sequence limitation: the structure-guided nuclease. Genome Biology. 17(1):186.
8. Junbo Li, Yunyun Yue, Qingshun Zhao*. 2016. Retinoic acid signaling is essential for valvulogenesis by affecting endocardial cushions formation in zebrafish embryos. Zebrafish, 13(1):9-18. (Cover)
9. Jingyun Li, Yunyun Yue, Xiaohua Dong, Wenshuang Jia, Kui Li, Dong Liang, Zhangji Dong, Xiaoxiao Wang, Xiaoxi Nan, Qinxin Zhang, Qingshun Zhao*. 2015. Zebrafish foxc1a plays a crucial role in early somitogenesis by restricting the expression of aldh1a2 directly. The Journal of Biological Chemistry, 290(16):10216-28.
10. Zhangji Dong, Xiaohua Dong, Wenshang Jia, Shasha Cao, Qingshun Zhao*. 2014. Improving the efficiency for generation of genome-edited zebrafish by labelling primordial germ cells. The International Journal of Biochemistry & Cell Biology, 55:329-34.

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Metabolism and Immunity

Xiang Gao, Ph.D.

Xiang was an alumina of Nanjing University. He received his Ph.D. degree from Thomas Jefferson University in 1994, then did his postdoctoral training at the Jackson Laboratory and University of North Carolina at Chapel Hill. In 2000, Xiang was recruited back to Nanjing University. He later founded both MARC and National Resource Center of Mutant Mice of China. He is also the current director for the State Key Laboratory of Pharmaceutical Biotechnology. Xiang is the recipient for Cheung Kong Scholar from Ministry of Education and Distinguished Young Scholar from National Science Foundation.

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Physiological regulation and metabolic homeostasis

The advance of modern technologies, especially the NGS and gene editing, transform the biomedical fields. The complicated metabolic regulatory networks crossing the variety of tissues and organs are becoming tangible with these new tools. We are excited to embrace these promising progresses for identifying the previous unsolvable biological questions. In my laboratory, we are more interested in defining the global regulators for crucial physiological processes. Following are some of our publications:

1. Uncovering that magnesium enhances survival of sepsis by blocking pyroptosis (Figure 1)

Hypomagnesemia is a significant risk factor for critically ill patients to develop sepsis, a life-threatening disease with a mortality rate over 25%. Our clinic data analysis showed that hypomagnesemia is associated with a decreased monocyte count in septic patients. At the cellular level, we found that Mg2+ inhibits pyroptosis. Specifically, Mg2+ limits the oligomerization and membrane localization of gasdermin D N-terminal (GSDMD-NT) upon the activation of either the canonical or non-canonical pyroptotic pathway. Mechanistically, we demonstrated that Ca2+ influx is a prerequisite for the function of GSDMD-NT. Mg2+ blocks Ca2+ influx by inhibiting the ATP-gated Ca2+ channel P2X7, thereby impeding the function of GSDMD-NT and inhibiting lipopolysaccharide (LPS)-induced non-canonical pyroptosis. Furthermore, Mg2+ administration protects mice from LPS-induced lethal septic shock. Together, our data reveal the underlying mechanism of how Mg2+ inhibits pyroptosis and suggest potential clinic applications of magnesium supplementation for sepsis prevention and treatment. (Wang et al, Cell Death Differ)

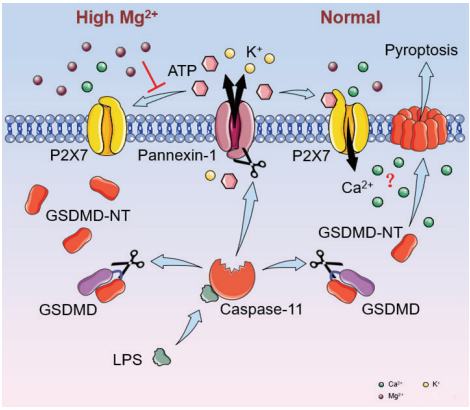

Figure 1. Magnesium enhances survival of sepsis by blocking pyroptosis

2. A SNP of bacterial blc disturbs gut lysophospholipid homeostasis and induces inflammation through epithelial barrier disruption (Figure 2)

Alteration of commensal bacterial composition is associated with many inflammatory diseases. However, few studies pinpointed the specific bacterial genes that may suppress host immune responses against microbes. By screening 3,983 E. coli mutants, we discovered that 9 bacterial genes, when deleted, activate innate immunity in the host Caenorhabditis elegans. The gene encoding bacterial lipocalin (blc), among these 9 genes, shown a distinctive SNP in many clinic pathogenic bacteria. We found bacteria with this SNP, which converts the Blc G84 to Blc E84, are highly enriched in the fecal of inflammatory bowel disease (IBD) patients. Exposure to the BlcE84-encoding bacteria resulted in epithelial barrier disruption and immune activation both in worm and mouse. Detailed analysis indicated the infection of the BlcE84-encoding bacteria causes a significant decrease in lysophosphatidylethanolamine (LPE) levels in the intestine, and subsequently the disruption of gut epithelial integrity in mice. Consistently, the levels of LPE in IBD patients are significantly lower compared to that of health people. Finally, supplement of LPE, which activating the LPA1/PLCβ/PKC signaling, can reverse all the defects induced by the BlcE84-encoding bacteria. Our results identified a novel bacterial gene in E. coli that regulates gut integrity and immunity. (Zou et al, Ebiomedicine).

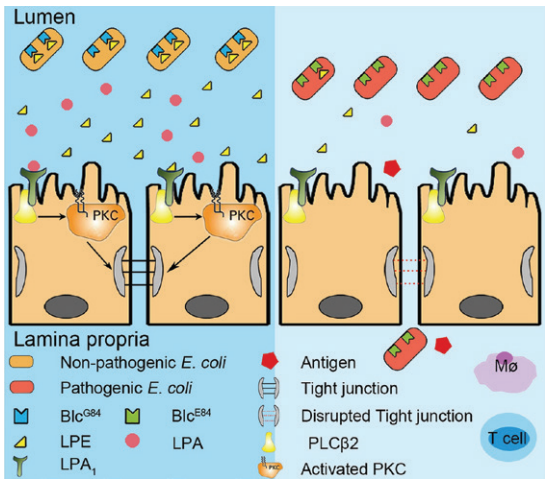

Figure 2. A SNP of bacterial blc disturbs gut lysophospholipid homeostasis and induces inflammation through epithelial barrier disruption

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1. Zou, Y., J. Pei, Y. Wang, Q. Chen, M. Sun, L. Kang, X. Zhang, L. Zhang, X. Gao*, and Z. Lin*, The Deficiency of SCARB2/LIMP-2 Impairs Metabolism via Disrupted mTORC1-Dependent Mitochondrial OXPHOS. Int J Mol Sci, 2022. 23(15).
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3. Chen, Q., J. Zheng, D. Wang, Q. Liu, L. Kang, X. Gao*, and Z. Lin*, Nitrosonisoldipine is a selective inhibitor of inflammatory caspases and protects against pyroptosis and related septic shock. Eur J Immunol, 2021. 51(5): p. 1234-1245.
4. Zou, D., J. Pei, J. Lan, H. Sang, H. Chen, H. Yuan, D. Wu, Y. Zhang, Y. Wang, D. Wang, Y. Zou, D. Chen, J. Ren*, X. Gao*, and Z. Lin*, A SNP of bacterial blc disturbs gut lysophospholipid homeostasis and induces inflammation through epithelial barrier disruption. EBioMedicine, 2020. 52: p. 102652.
5. Wang, D., J. Zheng, Q. Hu, C. Zhao, Q. Chen, P. Shi, Q. Chen, Y. Zou, D. Zou, Q. Liu, J. Pei, X. Wu, X. Gao*, J. Ren*, and Z. Lin*, Magnesium protects against sepsis by blocking gasdermin D N-terminal-induced pyroptosis. Cell Death Differ, 2020. 27(2): p. 466-481.
6. Zheng, M., D. Li, Z. Zhao, D. Shytikov, Q. Xu, X. Jin, J. Liang, J. Lou, S. Wu, L. Wang, H. Hu, Y. Zhou, X. Gao*, and L. Lu*, Protein phosphatase 2A has an essential role in promoting thymocyte survival during selection. Proc Natl Acad Sci U S A, 2019. 116(25): p. 12422-12427.
7. Xu, Q., X. Jin, M. Zheng, D. Rohila, G. Fu, Z. Wen, J. Lou, S. Wu, R. Sloan, L. Wang, H. Hu, X. Gao*, and L. Lu*, Phosphatase PP2A is essential for TH17 differentiation. Proc Natl Acad Sci U S A, 2019. 116(3): p. 982-987.
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Dr. Shuai Chen received his Ph.D. degree from University of Halle-Wittenberg (Germany) in 2005. After his postdoctoral training in the field of cell signaling and molecular physiology at the MRC Protein Phosphorylation Unit, University of Dundee (UK) from 2006 to 2011, Dr. Chen joined MARC as a principle investigator and a professor in Metabolic Biology in 2012. He is the recipient for Distinguished Young Scholars from the National Natural Science Foundation (2020) and New Century Excellent Talents from the Ministry of Education (2013). He currently serves as Director of MARC and Director of Nanjing Biomedical Research Institute of Nanjing University (NBRI).

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Metabolic Signaling, Physiology and Diseases

Metabolic diseases including type 2 diabetes mellitus (T2DM), obesity and non-alcoholic fatty liver disease (NAFLD) have become prevalent world-wide in the last few decades, which urges a better understanding of their pathogenesis as well as new therapeutic strategies to combat these diseases. Insulin resistance is a common cause for the pathogenesis of these metabolic diseases, whose underlying mechanism is still not clear. Insulin actions exhibit a tissue/pathway-dependent manner. Therefore, the goal of my laboratory is to understand the molecular basis of tissue/pathway-specific insulin actions, the pathogenic mechanisms of metabolic diseases, and discoveries of leading compounds to combat these diseases. We are currently running three research programmes in the laboratory: (1) protein modifications in mediating insulin actions, (2) tissue/pathway-specific insulin actions and diabetic complications, (3) discoveries of therapeutic targets and agents for metabolic diseases.

The recent progresses of my lab is as follows:

1. Tissue- and pathway-specific insulin actions and diabetic complications

We took a proteomic approach to identify novel proteins that are regulated by insulin in various organs including heart and liver. In the liver, we identified an E3 ligase TRIM24 as a critical distal component of insulin signaling, which translocates from the nucleus into the cytosol to regulate P-body function in response to insulin (Wei W., Chen Q.L., Liu M.J., Sheng Y., …, Wang H.Y.*, Chen S.* 2022 Nat Commun). In the heart, we have shown that the protein kinase SPEG is a key component in insulin signaling, which is activated by PKB/Akt and in turn phosphorylates Ca2+ pump SERCA2a. To delineate the in vivo role of SERCA2a phosphorylation, we generated a knockin mouse model in which the insulin responsive phosphorylation site Thr484 was mutated to a non-phosphorylatable alanine. Using this model, we show that SERCA2a is both a target and a regulator of the insulin pathway, linking insulin signaling with Ca2+ homeostasis. Impaired phosphorylation of SERCA2a-Thr484 due to insulin resistance underlies the early pathogenesis of diabetic cardiomyopathy. These findings have therapeutic implications for treatment of diabetic cardiomyopathy. (Quan C., …, Wang H.Y.*, Chen S.* 2022 Life Metab).

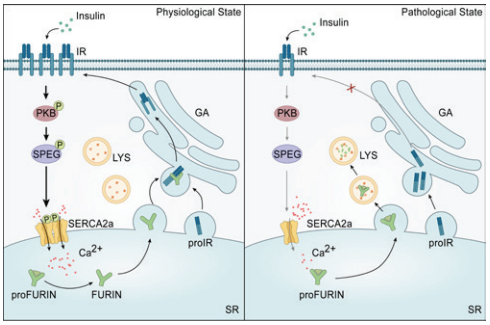

Figure 1 SERCA2a as both a target and a regulator of insulin signaling in the pathogenesis of diabetic cardiomyopathy.

2. Spatiotemporal regulation of insulin signaling by liquid-liquid phase separation

Insulin signals through its receptor to recruit insulin receptor substrates (IRS) and phosphatidylinositol 3-kinase (PI3K) to the plasma membrane for production of phosphatidylinositol-3,4,5-trisphosphate (PIP3) from phosphatidylinositol-4,5-bisphosphate [PI(4,5)P2]. PIP3 consequently recruits protein kinase B (PKB, also known as Akt) to the plasma membrane where PKB is activated by PDK1 and mTORC2. How insulin signals transduce from the plasma membrane into the cytosol is not clearly understood. Our recent study shows that liquid-liquid phase separation (LLPS) plays a critical role in spatiotemporal control of insulin signaling through regulating multiple components including IRS1. Both protein concentration and insulin stimulation can drive the formation of intracellular IRS1 condensates through LLPS. Components including PI(4,5)P2, p85-PI3K and PDK1 are constitutively present in IRS1 condensates whereas production of PIP3 and recruitment of PKB in them are induced by insulin. Thus, IRS1 condensates function as intracellular signal hubs to transduce insulin signals deep into the cells, whose formation is impaired in insulin resistant cells. Collectively, these data reveal an important function of LLPS in spatiotemporal control of insulin signaling. (Zhou K., Chen Q.L.., …, Quan C.*, Chen S.* 2022 Cell Discov).

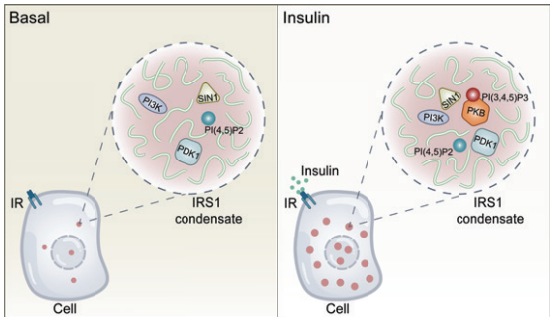

Figure 2 A model of IRS1 condensates as intracellular insulin signal hubs.

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1. Quan C, Zhu SS, Wang RZ, Chen JM, Chen QL, Li M, Su S, Du Q, Liu MJ, Wang HY* and Chen S* (2022) Impaired SERCA2a phosphorylation causes diabetic cardiomyopathy through impinging on cardiac contractility and precursor protein processing. Life Metabolism DOI: 10.1093/lifemeta/loac013 (* corresponding authors)
2. Zhu SS, Quan C, Wang RZ, Liang DR, Su S, Rong P, Zhou K, Yang XY, Chen QL, Li M, Du Q, Zhang JZ, Fang L, Wang HY* and Chen S* (2022) The RalGAPα1–RalA signal module protects cardiac function through regulating calcium homeostasis. Nat Commun 13: 4278 DOI: 10.1038/s41467-022-31992-z (* corresponding authors)
3. Wei W, Chen QL, Liu MJ, Sheng Y, OuYang Q, Feng WK, Yang XY, Ding LF, Su S, Zhang JZ, Fang L, Vidal-Puig A, Wang HY* and Chen S* (2022) TRIM24 is an insulin-responsive regulator of P-bodies. Nat Commun 13: 3972 DOI: 10.1038/s41467-022-31735-0 (* corresponding authors)
4. Zhou K, Chen QL, Chen JM, Liang DR, Feng WK, Liu MJ, Wang Q, Wang RZ, OuYang Q, Quan C* and Chen S* (2022) Spatiotemporal regulation of insulin signaling by liquid-liquid phase separation. Cell Discov 8(1): 64 DOI: 10.1038/s41421-022-00430-1 (* corresponding authors)
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6. Yang XY, Chen QL, OuYang Q, Rong P, Feng WK, Quan C, Li M, Jiang Q, Liang H, Zhao TJ, Wang HY* and Chen S*. (2021) Tissue-specific splicing and dietary interaction of a mutant As160 allele determine muscle metabolic fitness in rodents. Diabetes 70(8): 1826-1842(* corresponding author)
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8. Quan C, Li M, Du Q, Chen QL, Wang H, Campbell D, Fang L, Xue B, MacKintosh C, Gao X, Ouyang KF, Wang HY and Chen S* (2019) SPEG controls calcium re-uptake into the sarcoplasmic reticulum through regulating SERCA2a by its second kinase-domain. Circ Res 124(5): 712-726 (* corresponding author)

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Di Chen, Ph.D.

Di Chen got his Ph.D. in Genetics from the University of Missouri-Columbia, USA in 2004. He was supervised by Dr. Donald L. Riddle to study how the nematode C. elegans respond to genetic and environmental cues to enter and exit developmental diapause. He did post-doctoral training in Dr. Pankaj Kapahi's lab at the Buck Institute for Research on Aging, USA, where he studied the molecular mechanisms of aging in C. elegans. He joined the Model Animal Research Center, Nanjing University as a Principal Investigator in 2013.

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Aging and Metabolism Using C. elegans as a Model

Aging is a process of function decline accompanied with increased mortality rate over time. The evolutionary theory of aging proposed that aging takes place because the natural selection favors genes that confer benefit early on life at the cost of deterioration later in life (antagonistic pleiotropy). Using model organisms, researchers have demonstrated that aging can be modulated by highly conserved signaling pathways. Appropriate genetic or environmental modulations not only extend lifespan but also delay age-related pathologies. Many exciting discoveries on the molecular basis of aging were initially made in C. elegans, which is a great system for biology research because of the ease of genetics analysis and the conservation with higher species.

The highly conserved Insulin/IGF-1 signaling (IIS) and Target of Rapamycin (TOR) pathway play an important role in aging in many species. To characterize how IIS and TOR pathway interact with each other to modulate aging, we constructed a double mutant in DAF-2 (IGF-1 receptor) and the TOR target RSKS-1 (ribosomal S6 kinase). Surprisingly, this daf-2 rsks-1 mutant shows a nearly 5-fold, synergistic lifespan extension (Figure 1A). Using transcriptome profiling, we demonstrated that the underlying mechanisms involve positive feedback regulation of the DAF-16/FOXO transcription factor via the key energy homeostasis regulator AMPK (Figure 1B, C). We then performed polysomal profiling coupled with mRNA-Seq to identify genes that are translationally regulated in the daf-2 rsks-1 mutant and characterize their roles in aging (Figure 1D). Eventually, we identified a translationally regulated non-autonomous mitochondrial stress response mechanism in the modulation of lifespan by insulin-like signaling and S6K (Figure 1E).

Dietary restriction (DR) is one of most robust environmental manipulations that slow down aging in various species. However, the molecular mechanisms of DR remain largely unknown. Previously, we demonstrated that the hypoxia inducible factor-1 (HIF-1) plays an important role in DR-induced lifespan extension by regulating the IRE-1 ER stress pathway. To gain better insights on the relationship between nutrients and aging, we performed an RNAi-based genetic screen and identified a key mediator of DR. Mutations in this gene block DR-induced lifespan and healthspan extension in a tissue-specific manner (Figure 2).

Currently, our research focuses on the following aspects:

1) Cell non-autonomous regulation of mitochondrial stress response.

2) Lipid metabolism in dietary restriction-induced lifespan extension.

3) RNA metabolism in aging and age-related diseases.

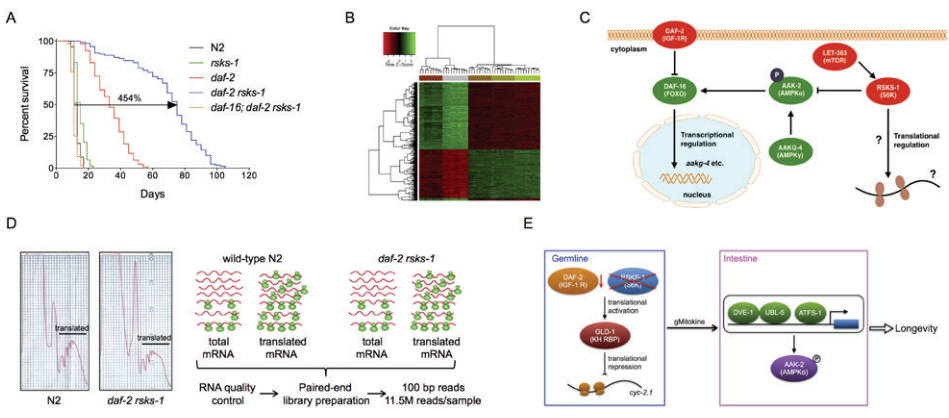

Figure 1. Functional genomics analysis of the super long-lived daf-2 rsks-1 mutant.
(A) Double mutations in DAF-2 (IGF-1 receptor) and RSKS-1 (ribosomal S6 kinase) leads to nearly 5-fold synergistic lifespan extension, which requires the DAF-16 (FOXO) transcription factor. (B) Transcriptome profiling via microarrays helped to identify genes that are differentially expressed in the daf-2 rsks-1 double mutant. (C) A model depicting the positive feedback regulation of DAF-16 via AMPK in the super long-lived daf-2 rsks-1 double mutant. (D) Polysomal profiling and mRNA-Seq were performed to identify genes that are regulated at the translational level in the daf-2 rsks-1 mutant. (E) A model depicting the translational repression of CYC-2.1 (cytochrome c) by the RNA-binding protein GLD-1 in the germline non-autonomously activates mitochondrial unfolded protein response (UPRmt) and AMPK in the intestine via germline-produced mitokine (gMitokine) signaling, which leads to significant lifespan extension in the daf-2 rsks-1 mutant.

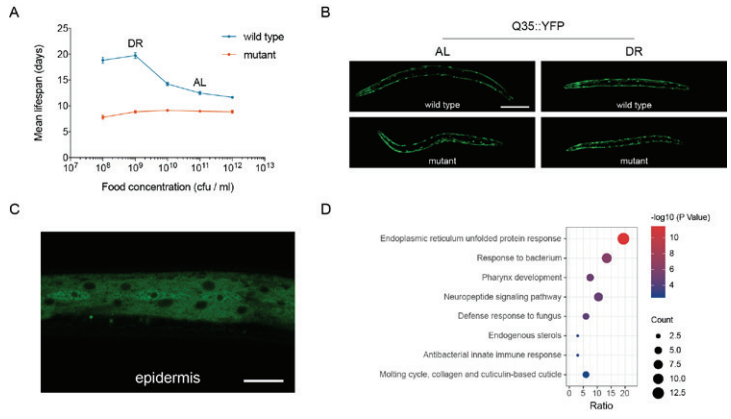

Figure 2. Characterization of a key mediator of dietary restriction-induced delay of aging.

(A) Inhibition of a lipid metabolism gene completely blocks the lifespan extension induced by DR. (B) Inhibition of the key DR mediator gene abolishes the DR-induced protection on proteostasis in a polyQ model. (C) The key DR mediator gene is expressed in the epidermis. (D) Mutation in the key DR mediator gene causes ER stress.

Recent publications (*, corresponding authors):

1. Meng Huang, Minjie Hong, Xinhao Hou, Chengming Zhu, Di Chen*, Xiangyang Chen*, Shouhong Guang*, Xuezhu Feng*, H3K9me1/2 Methylation Limits the Lifespan of daf-2 Mutants in C. elegans. eLife, 2022, 11: e74812.
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4. Jianfeng Lan#, Jarod A. Rollins#, Xiao Zang#, Di Wu#, Lina Zou, Zi Wang, Chang Ye, Zixing Wu, Pankaj Kapahi*, Aric N. Rogers*, Di Chen*, Translational Regulation of Non-autonomous Mitochondrial Stress Response Promotes Longevity. Cell Reports, 2019, 28(4): 1050-1062.e6. (#, co-first authors)
5. Lina Zou, Di Wu, Xiao Zang, Zi Wang, Zixing Wu, Di Chen*, Construction of a Germline-specific RNAi Tool in C. elegans. Scientific Reports, 2019, 9(1): 2354
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7. Lei Hou#, Dan Wang#, Di Chen#, Yi Liu, Yue Zhang, Hao Cheng, Chi Xu, Na Sun, Joseph McDermott, William B. Mair, Jing-Dong J. Han, A Systems Approach to Reverse Engineer Lifespan Extension by Dietary Restriction. Cell Metabolism, 2016, 23(3): 529-540. (#, co-first authors)
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10. Di Chen*, Patrick Wai-Lun Li, Benjamin A. Goldstein, Waijiao Cai, Emma Lynn Thomas, Fen Chen, Alan E. Hubbard, Simon Melov, Pankaj Kapahi*, Germline Signaling Mediates the Synergistically Prolonged Longevity Produced by Double Mutations in daf-2 and rsks-1 in C. elegans, Cell Reports, 2013, 5(6): 1600-1610.

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Chao-Jun Li received his Ph. D in Physiology from Nanjing University in 1994. He did his postdoctoral training at the Hong Kong University of Science and Technology from 1996-1998 and the Medical School of Yale University from 1999-2000. 1994-2008, he was a professor at Nanjing Normal University. 2008-2019, he was a professor of Cell Biology and a principal investigator in MARC and the Medical School of Nanjing University, He is now a professor of State Key Laboratory of Reproductive Medicine of Nanjing Medical University. He was elected as the vice-president of Chinese Society for Cell Biology since 2014.

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Ketone bodies are a vital alternative metabolic fuel source for all the domains of life, eukarya, bacteria, and archaea. In mammals, ketone bodies are produced predominantly in the liver from FAO-derived acetyl-CoA, and they are transported to extrahepatic tissues for terminal oxidation. Although they are energetically rich, ketone bodies exert provocative 'non-canonical' signaling roles in cellular homeostasis. For example, β-hydroxybutyrate (β-HB) inhibits Class I HDACs, which increases histone acetylation and thereby induces the expression of genes that curtail oxidative stress. β-HB is also an effector via G-protein coupled receptors and then exerts multi-roles such as suppressing sympathetic nervous system activity and reducing total energy expenditure. Our research on ketone bodies originated from the discovery that the ketogenic rate-limiting enzyme HMGCS2 was transiently highly expressed in multiple neonatal organs, for example heart, and ketone body β-HB was also transiently accumulated in the neonatal blood. Further research found that ketone body β-HB maintained β-hydroxybutyrylation but inhibited acetylation of mitochondrial proteins, which was responsible for the enzyme activity in mitochondria, thereby regulating mitochondrial functional maturation and heart development after birth.What is more, our research showed that ketone body displayed marked potential in the organ-to-organ communication. We found that liver responded to lipid overload first and sent ketone body β-HB targeting adipocytes to regulate adipose expansion to maintain lipid homeostasis. In addition, ketone bodies also play an important role in early reproductive development. Neonatal serum ketone body production could determine the quantity and quality of the primordial follicle pool by reducing ROS-induced primordial follicle apoptosis during follicular reserve formation in the early life.

1. The neonatal ketone body is important for primordial follicle pool formation and regulates ovarian ageing in mice

Adverse nutritional conditions during the perinatal stage are related to early menopause in adulthood; however, the underlying mechanism is still unclear. Herein, we revealed that colostrum-activated ketone body elevation during the postnatal stage regulated primordial follicle reservoir size and then affected ovarian ageing. We found that the expression of the ketogenesis rate-limiting enzyme 3-hydroxy-3-methylglutaryl-CoA synthase 2 (Hmgcs2) was largely enhanced during primordial follicle pool formation after birth and might be activated in the ovaries by colostrum. Reactive oxygen species (ROS) elevation in the ovaries leads to follicle apoptosis to deplete damaged follicles, while Hmgcs2 deficiency enhances follicle apoptosis and thus decreases the size of the primordial follicle pool and leads to premature ovarian ageing (POA), which might be related to the activation of cellular endogenous antioxidant system. All these defects could be rescued by ketone body administration, which suppressed ROS-activated follicle apoptosis. Our results suggest that the internal metabolic homeostasis of new-born mice is critical for the primordial reservoir and that any intrauterine and perinatal undernutrition could result in POA.

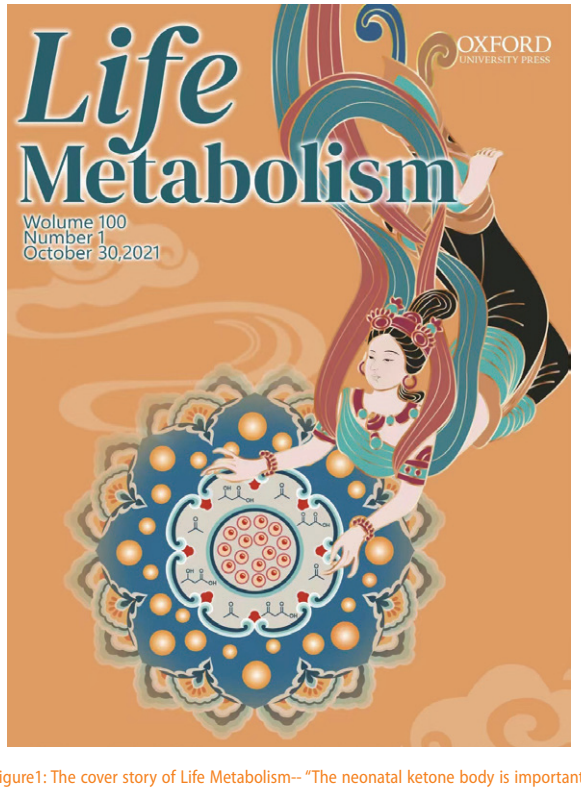

Figure1: The cover story of Life Metabolism-- "The neonatal ketone body is important for primordial follicle pool formation and regulates ovarian ageing in mice "

2. Ketone body promote adipose expansion to alleviate liver steatosis in response to ketogenic diet

Ketogenic diet is commonly utilized as the synergic intervention to decrease visceral adipose tissue and fatty infiltration of the liver, as well as modulate and improve inflammation and body composition. However, as a kind of low-carbohydrate but high-fat diet, here we show that acute ketogenic diet can cause the transient hepatic lipid accumulation within one week. Meanwhile, the phenomenon is accompanied with white adipose tissue expansion by enhanced adipogenesis and lipogenesis of adipocytes. Thus, we propose the hypothesis that liver senses metabolic stress and sends corresponding signals, such as ketone body, to promote adipocytes differentiation to adapt to metabolic challenge. To confirm the hypothesis, we eliminated ketone bodies in mice by constructing Hmgcs2 knockout mice. We found that ketogenesis deficiency whole body cannot improve hepatic lipid overload and promote adipose expansion with ketogenic diet feeding.In vitro, we also find ketone body can indeed enhance adipocytes differentiation. Thus, we highlight an inter-organ mechanism whereby liver senses metabolic stress and sends corresponding signalsto promote adipocytes differentiation to maintain lipid homeostasis.

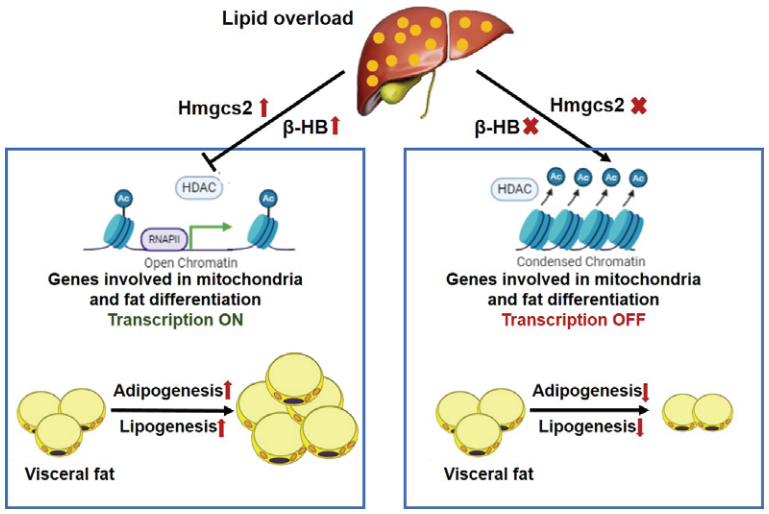

Figure2: Working model for Ketone body promote adipose expansion to alleviate liver steatosis in response to ketogenic diet

3. Ketone bodies alleviate childhood IBD by maintaining intestinal barrier and flora homeostasis

During early life, the gut (including the gut barrier and immune system) and the microbiota develop gradually, and a balanced relationship is established between them. Abnormal gut development or dysbiosis may lead to intestinal diseases such as inflammatory bowel disease. Ketone bodies are synthesized transiently during the neonatal period. Our previous studies found that the concentration of ketone bodies in the gut of newborn mice was high, but gradually decreased after about 1 week of birth, and almost no ketone bodies were detected in adulthood. To study the regulatory effect of ketone bodies on intestinal development and flora homeostasis in neonatal period, we eliminated ketone bodies in mice by constructing Hmgcs2 knockout mice. We found that Hmgcs2 knockout mice showed typical symptoms of IBD such as intestinal immune cell infiltration and shortened colon length at weaning period (3 weeks old). And the intestinal barrier of Hmgcs2 knockout mice was severely damaged. In addition, Hmgcs2-knockout mice had a decreased diversity of gut microbiota and a reduced abundance of beneficial flora such as Lactobacillus, which was similar to the gut flora characteristics of IBD patients. Furthermore, intestinal epithelial cells of Hmgcs2 knockout mice undergo significant apoptosis. Considering that ketone bodies can maintain mitochondrial function and resist oxidative stress, we speculate that ketone bodies in the neonatal period maintain the survival of intestinal epithelial cells by promoting mitochondrial function and resisting oxidative stress, thereby promoting the establishment of intestinal barrier and microbiota and ultimately resist the occurrence of IBD.

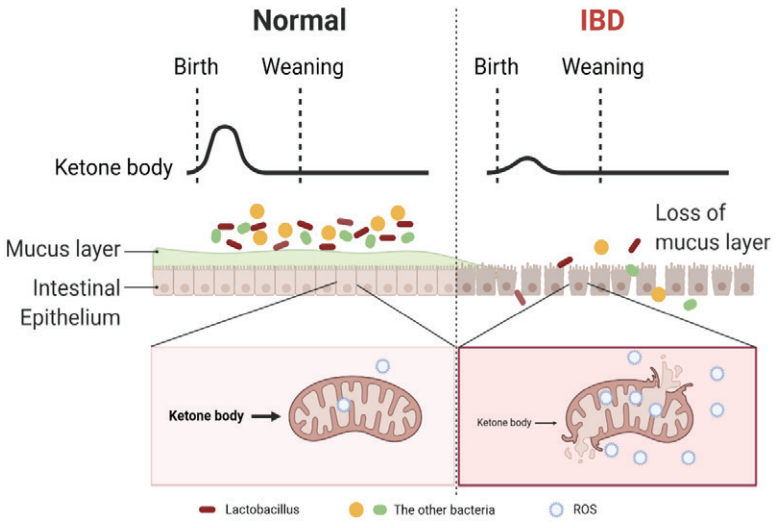

Figure 3: Working model for ketone body regulating intestinal barrier and flora homeostasis

Selected Publications (#:Co-first authors; *:Co-corresponding authors)

1. Dan-Yang Chong#, Ya-Yun Gu#, Tong-Yu Zhang#, et al., Li Xu*, Zhi-Bin Hu*, Chao-Jun Li*. Neonatal ketone body elevation regulates postnatal heart development by promoting cardiomyocyte mitochondrial maturation and metabolic reprogramming. Cell Discovery, 2022;8(1):106.doi: 10.1038/s41421-022-00447-6.
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3. Chao-Fan Yang#, Wen-Wen Liu#, et al., Gui-Jun Yan*, Chao-Jun Li*, Hai-Xiang Sun*. Gonadal white adipose tissue is important for gametogenesis through maintenance of local metabolic and immune niches in mice. J Biol Chem. 2022;298(5):101818.doi: 10.1016/j.jbc.2022.101818.
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6. Chen WW, et al., Li CJ*, and Sun LY*. Lipocalin-2 Exacerbates Lupus Nephritis by Promoting Th1 Cell Differentiation JASN 31:, 2020. doi: ASN.2019090937
7. Liu J#, Jiang S#, Zhao Y#, et al., Xue B*, Li C*. Geranylgeranyl diphosphate synthase (Ggps1) regulates non-alcoholic fatty liver disease (NAFLD)-fibrosis progression by determining hepatic glucose/fatty acid preference under high-fat diet conditions. J Pathol. 2018 Nov;246(3):277-288. (Commentary by Fullerton MD. Does prenylation predict progression in NAFLD? J Pathol. 2018; Oct 30)
8. Chen Z, et al., Li C.*. Geranylgeranyl pyrophosphate synthase facilitates the organization of cardiomyocytes during mid-gestation through modulating protein geranylgeranylation in mouse heart. Cardiovasc Res. 2018 Jun 1;114(7):965-978. (Commentary by Helen M. Phillips. Protein geranygeranylation: a possible new player in congenital heart defects. Cardiovasc Res. 2018;114:922-924)

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Zhenji Gan, Ph.D.

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Energy metabolism and muscle fitness

Muscle fitness is an important determinant of health and disease. Exercise training enhances muscle endurance and performance by augmenting the capacity of mitochondria to burn fuels and by increasing the proportion of slow oxidative fibers and blood supply. Conversely, reduced physical activity such as occurs with chronic illness, obesity, and aging results in de-trained muscle (Fig. 1). Our lab focuses on fundamental mechanisms that control muscle energy metabolism in physiological and disease states. We are particularly interested in exploring the regulatory networks involved in the coordinate control of energy metabolic and structural programs that define muscle fitness and their potential for therapeutic development.

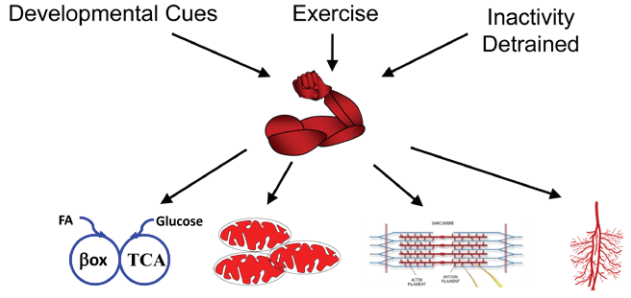

Fig 1. The muscle fitness is determined by developmental as well as physiological inputs. These inputs coordinately control the programs including fuel burning, mitochondrial ATP production, contraction, and angiogenesis.

Disuse-associated loss of muscle LONP1 that controls mitochondrial function and skeletal muscle mass.

Mitochondrial proteolysis is an evolutionarily conserved quality-control mechanism to maintain proper mitochondrial integrity and function. However, the physiological relevance of stress-induced impaired mitochondrial protein quality remains unclear. Here, we demonstrate that LONP1, a major mitochondrial protease resides in the matrix, plays a role in controlling mitochondrial function as well as skeletal muscle mass and strength in response to muscle disuse. In humans and mice, disuse-related muscle loss is associated with decreased mitochondrial LONP1 protein. Skeletal muscle-specific ablation of LONP1 in mice resulted in impaired mitochondrial protein turnover, leading to mitochondrial dysfunction. This caused reduced muscle fiber size and strength. Mechanistically, aberrant accumulation of mitochondrial-retained protein in muscle upon loss of LONP1 induces the activation of autophagy-lysosome degradation program of muscle loss. Overexpressing a mitochondrial retained mutant ornithine transcarbamylase (ΔOTC), a known protein degraded by LONP1, in skeletal muscle induces mitochondrial dysfunction, autophagy activation, and cause muscle loss and weakness. Thus, these findings reveal a role of LONP1-dependent mitochondrial protein quality-control in safeguarding mitochondrial function and preserving skeletal muscle mass and strength, and unravel a link between mitochondrial protein quality and muscle mass maintenance during muscle disuse. (Fig. 2).

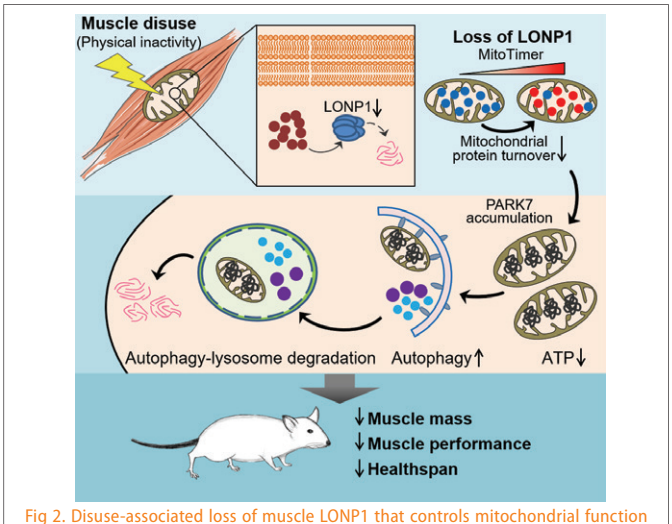

Fig 2. Disuse-associated loss of muscle LONP1 that controls mitochondrial function and skeletal muscle mass. Mitochondrial protease LONP1 protein is sensitive to muscle disuse in mice and humans.

Skeletal muscle mitochondrial unfolded protein response controls systemic metabolism.

Mitochondrial quality in skeletal muscle is crucial for maintaining energy homeostasis during metabolic stresses. However, how muscle mitochondrial quality is controlled and its physiological impacts remain unclear. Here, we demonstrate that mitoprotease LONP1 is essential for preserving muscle mitochondrial proteostasis and systemic metabolic homeostasis. Skeletal muscle–specific deletion of Lon protease homolog, mitochondrial (LONP1) impaired mitochondrial protein turnover, leading to muscle mitochondrial proteostasis stress. A benefit of this adaptive response was the complete resistance to diet-induced obesity. These favorable metabolic phenotypes were recapitulated in mice overexpressing LONP1 substrate ΔOTC in muscle mitochondria. Mechanistically, mitochondrial proteostasis imbalance elicits an unfolded protein response (UPRmt) in muscle that acts distally to modulate adipose tissue and liver metabolism. Unexpectedly, contrary to its previously proposed role, ATF4 is dispensable for the long-range protective response of skeletal muscle. Thus, these findings reveal a pivotal role of LONP1-dependent mitochondrial proteostasis in directing muscle UPRmt to regulate systemic metabolism. (Fig. 3).

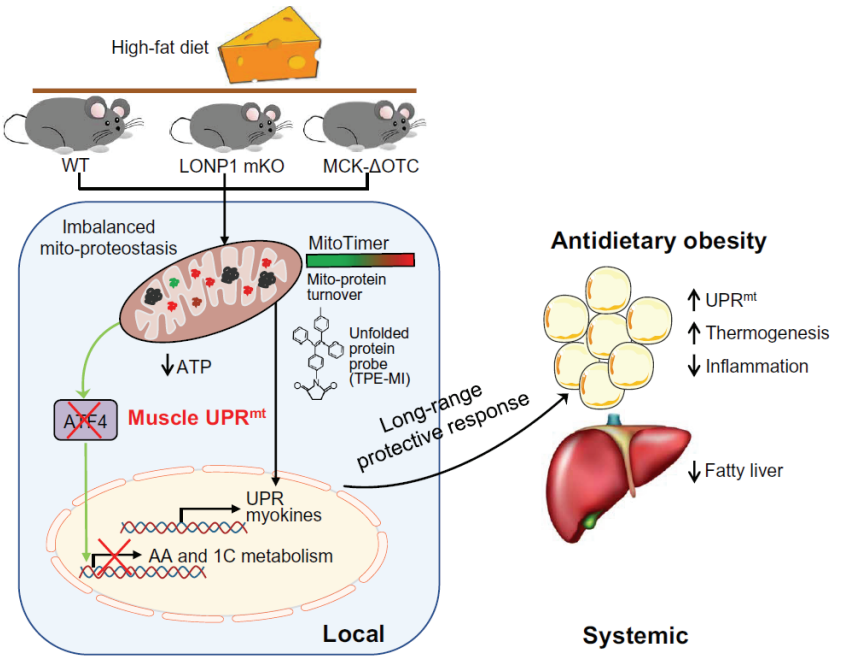

Fig 3. Skeletal muscle mitochondrial unfolded protein response controls systemic metabolism. Mitochondrial proteostasis stress in skeletal muscle directs a long-range metabolic response to alleviate dietary obesity independent of ATF4.

- LONP1-dependent mitochondrial protein quality-control is essential for safeguarding muscle mitochondrial proteostasis and preserving systemic metabolic homeostasis upon HFD metabolic stress
- Mitochondrial proteostasis imbalance elicits an UPRmt in muscle that acts distally to modulate the response of the adipose tissue and liver to HFD
- Muscle UPRmt acts independently of ATF4 to drive a long-range protective response, emphasizing an ATF4-independent UPRmt in mammals

FNIP1 controls intracellular $Ca2^+$ homeostasis and WAT browning.

Metabolically beneficial beige adipocytes offer tremendous potential to combat metabolic diseases. The folliculin interacting protein 1 (FNIP1) is implicated in controlling cellular metabolism via AMPK and mTORC1. However, whether and how FNIP1 regulates adipocyte browning is unclear. Here, we demonstrate that FNIP1 plays a critical role in controlling adipocyte browning and systemic glucose homeostasis. Adipocyte-specific ablation of FNIP1 promotes a broad thermogenic remodeling of adipocytes, including increased UCP1 levels, high mitochondrial content, and augmented capacity for mitochondrial respiration. Mechanistically, FNIP1 binds to and promotes the activity of SERCA, a main $Ca2^+$ pump responsible for cytosolic $Ca2^+$ removal. Loss of FNIP1 resulted in enhanced intracellular $Ca2^+$ signals and consequential activation of $Ca2^+$-dependent thermogenic program in adipocytes. Furthermore, mice lacking adipocyte FNIP1 were protected against high-fat diet–induced insulin resistance and liver steatosis. Thus, these findings reveal a pivotal role of FNIP1 as a negative regulator of beige adipocyte thermogenesis and unravel an intriguing functional link between intracellular $Ca2^+$ dynamics and adipocyte browning. (Fig. 4).

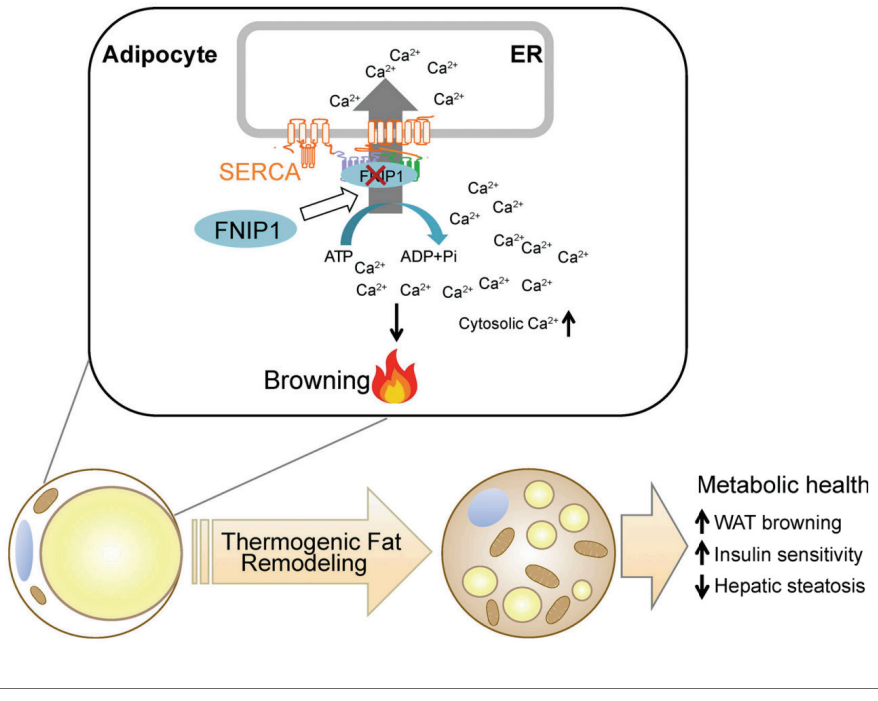

Fig 4. FNIP1 controls intracellular Ca^{2+} homeostasis and WAT browning. FNIP1 binds to the ATP hydrolysis site of SERCA, and this leads to increased SERCA Ca^{2+} pump activity, thereby dampening Ca^{2+} -dependent thermogenic program. Importantly, adipocyte-specific ablation of FNIP1 led to a broad thermogenic remodeling of WAT and protected the mice from HFD-induced glucose intolerance, insulin resistance, and liver steatosis.

- FNIP1 as a negative regulator of beige adipocyte thermogenesis
- FNIP1 directly binds to and promotes the activity of SERCA, a main Ca^{2+} pump responsible for cytosolic Ca^{2+} removal, thereby dampening Ca^{2+}-dependent thermogenic program
- The FNIP1 pathway exerts profound effects on whole-body glucose homeostasis and hepatic steatosis

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Mechanisms of metabolic disorder

The liver is a key organ in vertebrates, which has a wide range of functions, including detoxification of various metabolites, protein synthesis, and the production of chemicals for digestion. Nonalcoholic fatty liver disease (NAFLD) is a range of condition caused by the hepatic fat accumulation, which is also considered the hepatic manifestation of metabolic syndrome affecting about one-third of the population worldwide. Up to 25% of NAFLD patients develop a progressive inflammatory and damaged liver disease termed non-alcoholic steatohepatitis (NASH) that may progress towards cirrhosis, hepatic carcinoma, and the need for liver transplantation. Yet, the pathogenesis of NAFLD/NASH has not been completely elucidated. However, insulin resistance, inflammatory cytokines, and oxidative stress are thought to be important in the development and/or progression of the disease. Lifestyle modification with exercise and diet has been the first step in NAFLD/NASH treatment.

Our laboratory aims to understand the molecular mechanisms of the development and progression of NAFLD/NASH. Lipidomics, biochemistry, cell biology and transgenics approaches are applied to identify novel components for diagnosis and intervention of NAFLD/NASH progressions.

Insulin stimulates hepatic lipogenesis through mechanisms that are still incompletely understood. We took a proteomic approach to identify novel insulin-responsive proteins in the liver and identified an E3 ligase TRIM24 as a PKB substrate. Upon insulin stimulation, PKB phosphorylates TRIM24 on its Ser1043 and stimulates its shuttling from the nucleus into the cytoplasm. TRIM24 interacts with several critical components of P-bodies in the cytoplasm, promoting their polyubiquitylation, which consequently stabilises Pparγ mRNA. Inactivation of TRIM24 E3-ligase activity or prevention of its Ser1043 phosphorylation via knockin mutations in mice promotes hepatic Pparγ degradation via P-bodies. Consequently, both knockin mutations alleviate hepatosteatosis in mice fed on a high-fat diet. Our results demonstrate the critical role of TRIM24 in linking insulin signalling to P-bodies and have therapeutic implications for the treatment of hepatosteatosis. (Wei W., Chen Q.L., Liu M.J., Sheng Y., …, Wang H.Y.*, Chen S.* 2022 Nat Commun)

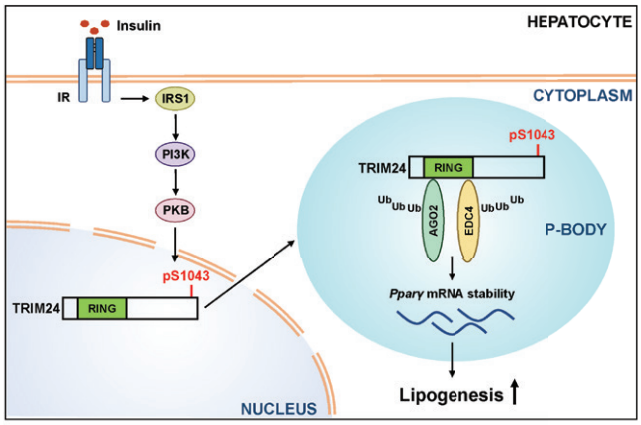

Figure 1 TRIM24 as an insulin-responsive regulator of P-bodies.

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1. Quan C, Zhu SS, Wang RZ, Chen JM, Chen QL, Li M, Su S, Du Q, Liu MJ, Wang H-Y* and Chen S* (2022) Impaired SERCA2a phosphorylation causes diabetic cardiomyopathy through impinging on cardiac contractility and precursor protein processing. Life Metabolism DOI: 10.1093/lifemeta/loac013 (* corresponding authors)
2. Zhu SS, Quan C, Wang RZ, Liang DR, Su S, Rong P, Zhou K, Yang XY, Chen QL, Li M, Du Q, Zhang JZ, Fang L, Wang H-Y* and Chen S* (2022) The RalGAPα1–RalA signal module protects cardiac function through regulating calcium homeostasis. Nat Commun 13: 4278 DOI: 10.1038/s41467-022-31992-z (* corresponding authors)
3. Wei W, Chen QL, Liu MJ, Sheng Y, OuYang Q, Feng WK, Yang XY, Ding LF, Su S, Zhang JZ, Fang L, Vidal-Puig A, Wang H-Y* and Chen S* (2022) TRIM24 is an insulin-responsive regulator of P-bodies. Nat Commun 13: 3972 DOI: 10.1038/s41467-022-31735-0 (* corresponding authors)
4. Yang XY, Chen QL, Ouyang Q, Rong P, Feng WK, Quan C, Li M, Jiang Q, Liang H, Zhao T-J, Wang H-Y* and Chen S*. Tissue-Specific Splicing and Dietary Interaction of a Mutant As160 Allele Determine Muscle Metabolic Fitness in Rodents. 2021 Diabetes Aug; 70(8): 1826-1842.
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8. Chen Q, Xie B, Zhu S, Rong P, Sheng Y, Ducommun S, Chen L, Quan C, Li M, Sakamoto K, MacKintosh C, Chen S* and Wang H-Y* (2016) A TBC1D1Ser231Ala knockin mutation partially impairs 5-aminoimidazole-4-carboxamide-1-ß-D-Ribofurano-side- but not exercise-induced muscle glucose uptake in mice. Diabetologia 60(2): 336-345 (*corresponding author)

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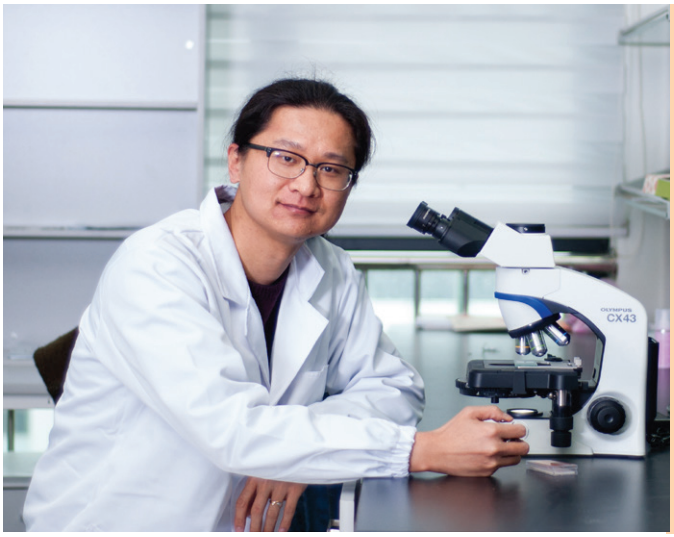

Yan LI Ph.D.

Dr. Yan Li received his Ph.D. degree from MIT in 2012 under the Singapore-MIT alliance program with supervision of Professor Jianzhu CHEN. From 2012 to 2016, he completed his post-doctoral training with Prof. James DI SANTO at Institut Pasteur, Paris. He worked as an assistant professor at Institut Pasteur from 2016 to 2018. In 2018, he became a full professor of Nanjing University and a principal investigator at the State Key Laboratory of Pharmaceutical Biotechnology. In 2019, he was qualified as a doctoral advisor, also a principal investigator of the Chemistry and Biomedicine Innovation Center. He is awarded as Jiangsu Provincial "Innovative and Entrepreneurial Talent " and "Distinguished Professor", and received the grant support from the National Key R&D Program Youth Program (formerly the Youth 973 Program). In 2020, he was awarded the leading talent of Jiangsu Innovative and Entrepreneurial Team Program. In 2021, he was supported by National Science Fund for Excellent Young Scholars.

His research has been published in prestigious journals such as Cell, Nature Methods, Nature Communications, etc. He has been invited for presentation at international conferences for 10 times, and filed one international patent. Dr. Yan Li is currently a member of the Immune Cell Branch of the Chinese Society of Cell Biology, a member of the Chinese Anti-Cancer Association, a board member of the Jiangsu Society of Cell and Developmental Biology. He is also an associate editor of the Frontiers in Immunology humanized mouse topic, and a member of the Scientific Committee of the International Workshops on Humanized Mice. In addition, he also reviews manuscripts for journals such as PNAS, European Journal of Immunology, and Frontiers in Immunology.

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Next-generation humanized mouse models for translational medicine

Human immune system (HIS) mice provide a valuable platform to investigate and modulate human hematopoiesis development and immune cell function in vivo. It bridges the gap between mouse study and translational research. During the past decade, we have been using the classical HIS mice model for human hematopoiesis and immune function study. Nevertheless, the current HIS mice model is not perfect, and we are also dedicating to develop the next generation of HIS mice with proper functional human immune system (Figure 1). With the developing of next generation HIS mice, we are expanding our research field from those common research fields (hematopoiesis, cancer research, etc.) to new areas such as full-human antibody generation and autoimmune diseases (Figure 2).

At present, the laboratory is supported by the National Natural Science Foundation of China/Ministry of Science and Technology, Jiangsu Provincial Department of Science and Technology/Education and Nanjing University. We have undertaken some projects including the National Key Research Plan, Jiangsu Innovative and Entrepreneurial Team, and the Fundamental Research Funds for the Central Universities.

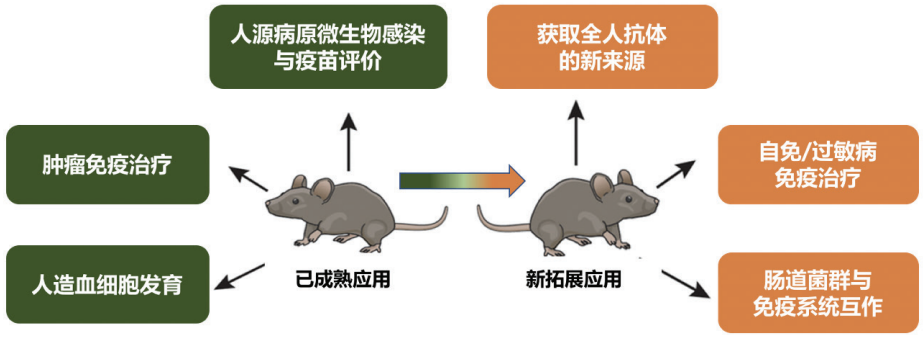

Figure 1 Current research fields in the laboratory

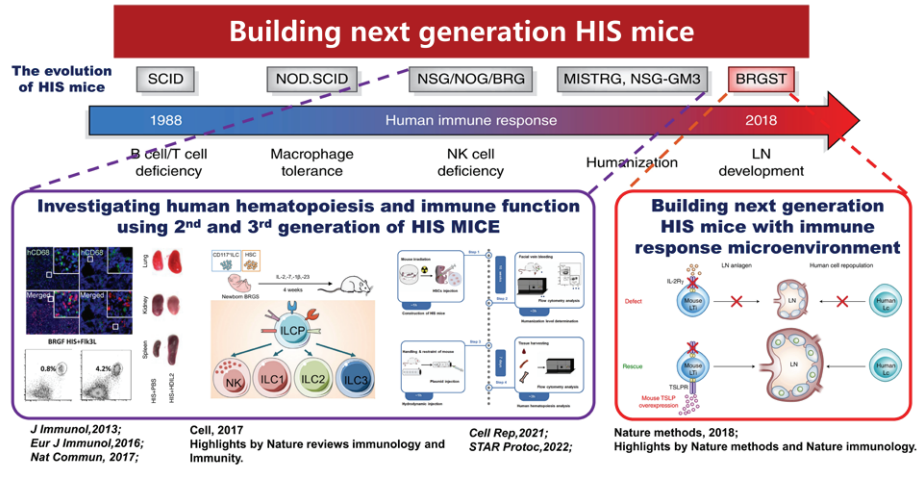

Figure 2 Current research fields in the laboratory

Selected Publications

1. Deshan REN, Wei Liu, Shuai Ding, Yan Li (2022). Protocol for generating human immune system mice and hydrodynamic injection to analyze human hematopoiesis in vivo, STAR Protoc. 3(1):101217.
2. Xiufei Chen, Wenjie Zhou, Ren-Hua Song, Shuang Liu, Shu Wang, Yujia Chen, Chao Gao, Chenxi He, Jianxiong Xiao, Lei Zhang, Tianxiang Wang, Peng Liu, Kunlong Duan, Chen Zhang, Jinye Zhang, Yiping Sun, Felix Jackson, Fei Lan, Yun Liu, Yanhui Xu, Justin Jong-Leong Wong, Pu Wang, Hui Yang, Yue Xiong, Tong Chen*, Yan Li, Dan Ye (2022). Tumor suppressor CEBPA interacts with and inhibits DNMT3A activity, Science Advances. 8(4):eabl5220.
3. Ana Cardoso, Ana Catarina Martins, Ana Raquel Maceiras, Wei Liu, Isabel Castro, Antonio G. Castro, Antonio Bandeira, James P. Di Santo, Ana Cumano, Yan Li, Paulo Vieira, and Margarida Saraiva (2021). Interleukin-10 induces interferon-g-dependent emergency myelopoiesis. Cell Reports. 37, 109887.
4. Yan Yan, Wei Zhao, Wei Liu, Yan Li, Xu Wang, Jingna Xun, Chantsalmaa Davgadorj (2021). CCL19 enhances CD8+ T-cell responses and accelerates HBV clearance. J Gastroenterol. 56:769-785.
5. Wei Liu, Hua Song, Xiaojing Li, Deshan Ren, Shuai Ding, Yan Li (2021). Lipid Metabolism in Tumor-associated Myeloid Derived Suppressor Cells. Lipid Metabolism in Tumor Immunity (Book), Springer.
6. Tao Cheng, Shuai Ding, Shanshan Liu, Yan Li, Lingyun Sun (2021). Human umbilical cord-derived mesenchymal stem cell therapy ameliorates lupus through increasing CD4+ T cell senescence via MiR-199a-5p/Sirt1/p53 axis. Theranostics. 11(2):893-905.
7. Shuai Ding, Siqi Li, Shujie Zhang and Yan Li (2020). Genetic alterations and checkpoint expression: mechanisms and models for drug discovery. Regulation of Cancer Immune Checkpoints: Molecular and Cellular Mechanisms and Therapy (Book), Springer.
8. Yan Li and James P. Di Santo (2019). Modeling infectious diseases in mice with a 'humanized' immune system. Bacteria and Intracellularity Microbiol Spectrum 7(2):BAI-0019-2019. doi:10.1128/microbiolspec.BAI-0019-2019.
9. Guillemette Masse-Ranson, Mathilde Dusséaux, Oriane Fiquet, Sylvie Darche, Maud Boussand, Yan Li, Silvia Lopez-Lastra, Nicolas Legrand, Erwan Corcuff, Antoine Toubert, Mireille Centlivre, Timothée Bruel, Hergen Spits, Olivier Schwartz, Yves Lévy, Hélène Strick-Marchand and James P. Di Santo (2019). Accelerated thymopoiesis and improved T cell responses in HLA-A2/-DR2 transgenic BRGS- based human immune system mice. European Journal of Immunology. 49(6):954-965.
10. Yan Li*, Guillemette Masse Ranson, Zacarias Garcia, Timothee Bruel, Ayrin Kok-Harunova, Helene Strick-Marchand, Gregory Jouvion, Nicolas Serafini, Ai Ing Lim, Mathilde Dusseaux, Thierry Hieu, Frank Bourgade, Antoine Toubert, Daniela Finke, Oliver Schwartz, Phillippe Bousso, Hugo Mouquet and James P. Di Santo. (2018) A human immune system mouse model with robust lymph node development. Nature Methods. DOI: 10.1038/s41592-018-0071-6.
11. Emmanuel Clave, Itauá Leston Araujo, Cécile Alanio, Etienne Patin, Jacob Bergstedt, Alejandra Urrutia, Silvia Lopez-Lastra, Yan Li, Bruno Charbit, Cameron Ross MacPherson, Milena Hasan, Breno Luiz Melo-Lima, Noemie Saut, Marine Germain, David-Alexandre Tregouet, Pierre-Emmanuel Morange, Magnus Fontes, Darragh Duffy, James P. Di Santo, Lluis Quintana-Murci, Matthew L.Albert, Antoine Toubert, for The Milieu Intérieur Consortium. (2018) Human thymopoiesis is influenced by a common genetic variant within the TCRA-TRCD locus. Science Translational Medicine. DOI: 10.1126/scitranslmed.aao2966

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Immune and metabolic regulation of physical homeostasis

Immune and metabolism is the key factors to maintain the physical homeostasis. The disruption of immune or metabolic regulation of physical homeostasis will lead to the occurrence of complex diseases, like autoimmune disease, obesity, cancer, cardiovascular disease and Alzheimer's disease. In our laboratory, we are interest in analysis of functions and the underlying molecular mechanisms of the disease related genes in immune or metabolic homeostasis.

Recently, we focus on a new discovered immunoregulatory protein family-Gasdermin. Our lab analyzed the roles of Gasdermin family in physical status and autoimmune diseases. Gsdmd and Gsdme are demonstrated to be the executors of pyroptosis, which is a type of pro-inflammatory programmed cell death. We discovered that Gasdermin directly trigger cell death and inflammation in 2015. Our recent works are mainly about the regulation of Gsdmd in pyroptosis (Figure 1). We found that inhibition of

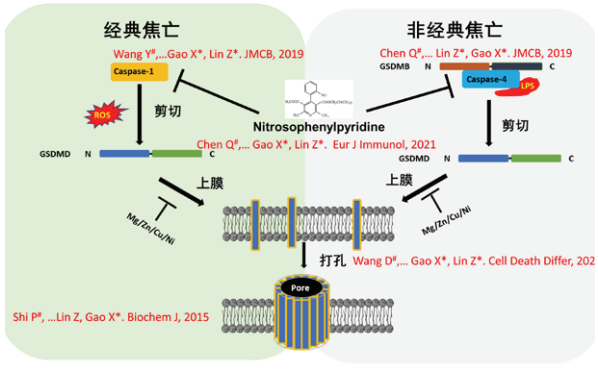

Figure 1. Gsdmd could be the target to block pyroptosis in autoimmune diseases

ROS reduces the cleavage of Gsdmd in canonical pyroptosis and inhibition of GSDMB reduces the cleavage of GSDMD in non-canonical pyroptosis. We developed several methods to block pyroptosis in autoimmune diseases. Magnesium could block the membrane translocation of Gsdmd-N-terminals and greatly enhance the survival rate of sepsis mice model. We also found that nitrosonisoldipine is a selective inhibitor of inflammatory caspases and protects against pyroptosis and related septic shock.

The physiological functions of Gasdermins is not only induce pyroptosis, but also lead to rapid cytokine secretion without cell death. Recently, we found that GSDMC activation drives anti-helminth responses and intestinal inflammation through promoting IL33 secretion. STAT6 O-GlcNAcylation promotes the expression of GSDMC. GSDMC N-terminus pores on cell membrane enable IL-33 rapid secretion. GSDMC-mediated IL-33 secretion was indispensable for effective anti-helminth immunity and contributed to induced intestinal inflammation (Figure 2).

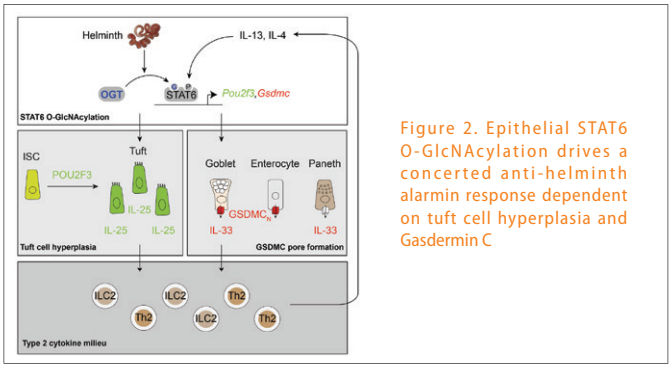

Figure 2. Epithelial STAT6 O-GlcNAcylation drives a concerted anti-helminth alarmin response dependent on tuft cell hyperplasia and Gasdermin C

We are also interesting with the relationship between obesogenic memory and immunity. Obesity, as a rapidly emerging public health problem, are associated with many severe diseases/complications, resulting in significantly compromised life quality of the patients. These patients can be greatly benefited by weight management. However, weight is very often regained during and after the treatments for obesity. This phenomenon is named obesogenic memory, leading to the failure of weight management and more importantly, of controlling the obesity-associated health problems including diabetes. Therefore, understanding the mechanisms regulating obesogenic memory, is especially beneficial for the patients with obesity. In the previous work, we has demonstrated that among immune cells, CD4+ T cells are the direct carrier, which is necessary and sufficient to induce and maintain obesogenic memory in mice. Recently, we found that obesogenic memory related CD4+ T cells are a subpopulation of central memory T cells with high expression of CD300C, which is a receptor of phosphatidylethanolamine (PE), an essential group of phospholipids in the cell membrane (Figure 3).

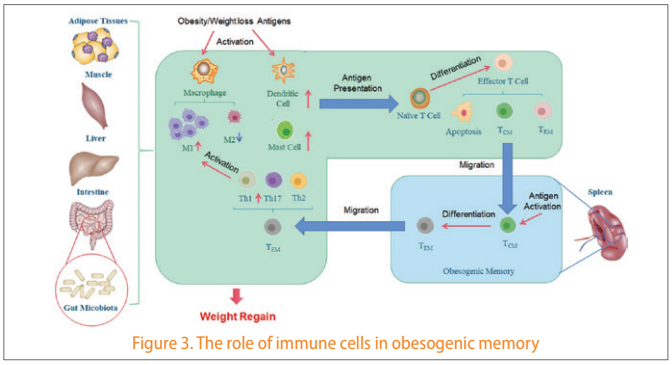

Figure 3. The role of immune cells in obesogenic memory

Selected Publications

1. Zou, Y., J. Pei, Y. Wang, Q. Chen, M. Sun, L. Kang, X. Zhang, L. Zhang, X. Gao*, and Z. Lin*, The Deficiency of SCARB2/LIMP-2 Impairs Metabolism via Disrupted mTORC1-Dependent Mitochondrial OXPHOS. Int J Mol Sci, 2022. 23(15).
2. Zhao, M., K. Ren, X. Xiong, Y. Xin, Y. Zou, J.C. Maynard, A. Kim, A.P. Battist, N. Koneripalli, Y. Wang, Q. Chen, R. Xin, C. Yang, R. Huang, J. Yu, Z. Huang, Z. Zhang, H. Wang, D. Wang, Y. Xiao, O.C. Salgado, N.N. Jarjour, K.A. Hogquist, X.S. Revelo, A.L. Burlingame, X. Gao, J. von Moltke, Z. Lin*, and H.B. Ruan*, Epithelial STAT6 O-GlcNAcylation drives a concerted anti-helminth alarmin response dependent on tuft cell hyperplasia and Gasdermin C. Immunity, 2022. 55(4): p. 623-638.e5.
3. Chen, Q., J. Zheng, D. Wang, Q. Liu, L. Kang, X. Gao*, and Z. Lin*, Nitrosonisoldipine is a selective inhibitor of inflammatory caspases and protects against pyroptosis and related septic shock. Eur J Immunol, 2021. 51(5): p. 1234-1245.
4. Zou, D., J. Pei, J. Lan, H. Sang, H. Chen, H. Yuan, D. Wu, Y. Zhang, Y. Wang, D. Wang, Y. Zou, D. Chen, J. Ren*, X. Gao*, and Z. Lin*, A SNP of bacterial blc disturbs gut lysophospholipid homeostasis and induces inflammation through epithelial barrier disruption. EBioMedicine, 2020. 52: p. 102652.
5. Wang, D., J. Zheng, Q. Hu, C. Zhao, Q. Chen, P. Shi, Q. Chen, Y. Zou, D. Zou, Q. Liu, J. Pei, X. Wu, X. Gao*, J. Ren*, and Z. Lin*, Magnesium protects against sepsis by blocking gasdermin D N-terminal-induced pyroptosis. Cell Death Differ, 2020. 27(2): p. 466-481.
6. Sun, M., S. Zheng, X. Gao*, and Z. Lin*, The role of immune cells in obesogenic memory. Cell Mol Immunol, 2020.
7. Wang, Y., P. Shi, Q. Chen, Z. Huang, D. Zou, J. Zhang, X. Gao*, and Z. Lin*, Mitochondrial ROS promote macrophage pyroptosis by inducing GSDMD oxidation. J Mol Cell Biol, 2019. 11(12): p. 1069-1082.
8. Chen, Q., P. Shi, Y. Wang, D. Zou, X. Wu, D. Wang, Q. Hu, Y. Zou, Z. Huang, J. Ren*, Z. Lin*, and X. Gao*, GSDMB promotes non-canonical pyroptosis by enhancing caspase-4 activity. J Mol Cell Biol, 2019. 11(6): p. 496-508.

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Cancer and Stem Cell Biology

Geng Liu, Ph.D.

Geng Liu received his B.S. degree in Biochemistry from Wuhan University, China and his Ph.D. degree in Gene & Development from University of Texas Graduate School of Biomedical Sciences at Houston in 1999. After his postdoctoral training at University of Texas M.D. Anderson Cancer Center, Dr. Geng Liu joined the Model Animal Research Center of Nanjing University as a principal investigator and professor of Genetics in 2006.

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Probing and Understanding Cellular Metabolism and Stress Response

Integral to their functions, various cell behaviors are dictated by extrinsic and intrinsic stimuli through a network of signaling mechanisms. Our laboratory is interested in studying the determinants of cell behaviors and their close connections with stress responses and cellular metabolism in the contexts of tissue homeostasis as well as cancer. We investigated how stress response as mediated by the p53 signaling pathway regulated cell behaviors including cell proliferation, cell competition, inflammation, and Epithelial-Mesenchymal transition. On the other hand, cellular metabolisms are not only required for the execution of proper cell functions but also serve as a signaling module in adapting the cells to certain behaviors. In addition, cell metabolisms are intrinsically connected to cellular redox state and stress response. Therefore, dissecting the intricate interplay between cell behaviors, stress responses and metabolism, facilitated with the establishment and utilization of probes and reporters may allow us to fully understand the complex cell behaviors in many fundamental processes including development, ageing and tumorigenesis.

p53 stress response pathway influences cell behaviors in distinctive manners

p53 is extremely important for stress response and tumor suppression as exemplified by its mutations found in over 50% of human cancers. p53 protein is undetectable in normal tissues. With the BAC transgenic p53 reporter mice, we revealed a regulatory mechanism controlling p53 expression and activity selectively in the proliferating cellular compartments during mouse development, postnatal growth, tissue homeostasis, regeneration and tumorigenesis (Chen, et al., 2015). The close monitoring of cellular proliferation state by p53 also serves as a base to generate the first genetic tool for proliferation tracing in studying the cardiomyocyte proliferation during heart regeneration (Xiao, et al., 2017). On the other hand, in collaboration with Dr. Jianghuai Liu's laboratory, the successful identification and marking of p53 deficient cells also offers a unique and highly specific strategy for the future development of targeted cancer prevention and therapy (Wang, et al., 2022).

In the presence of stress, p53 is activated to exert its role in influencing the cell fate. Various degree of stresses results in different level of p53 activation. Instead of directing the classic pathways of cell cycle arrest, senescence or apoptosis, we demonstrated that low dose X-ray induced mild p53 activation affected the EMT process during valvuloseptal morphogenesis of cardiac development and resulted in congenital heart defects in mice (Zhang, et al., 2012). p53 also play a crucial role in macrophage polarization in the tumor microenvironment to affect tumorigenesis in a non-cell autonomous manner (He, et al., 2015). Our recent study found that mild p53 activation in cells renders them less competitive in multi-cellular context during mouse embryogenesis, possibly contributing to the control of tissue fitness (Zhang, et al, 2017). These results indicate that p53 signaling pathway critically and delicately influence cell behaviors and functions in distinctive manners.

Probing, manipulating and understanding cellular metabolic states and their maintenance in vivo

To study the influence of cellular metabolism on cell behaviors and function in a multitude of in vivo contexts, we established mouse models in imaging and probing the metabolic heterogeneity within the tissues, in which we reveal highly stringent quality control mechanisms for an active mitochondrial state (Fig.1, Xie et al., 2022). Extending from the in vivo observations, we focused on further elucidating the regulatory network of mitochondrial oxidative metabolism and redox homeostasis using various approaches including drug screening and expression profiling (manuscript in preparation).

In addition, we have established a series of mouse models involved in promoting specific metabolic pathways in a controlled manner. Our results showed that cellular metabolisms could be manipulated in vivo and may have great impact on either cell behavior or systemic homeostasis (Xiang et al., 2021). Aiming to discover new strategies to boost cancer immune therapy, we found that specific manipulation and alteration of T cell metabolism could potently stimulate the anti-tumor immune response, revealing interesting insights for the intrinsic regulatory roles of the specific metabolic route on T cell differentiation and function (manuscript in preparation).We believe these attempts will greatly impact on our abilities in the understanding and fighting against a variety of diseases, especially those linked to cancer and ageing, in the perspectives of cellular metabolism and stress response.

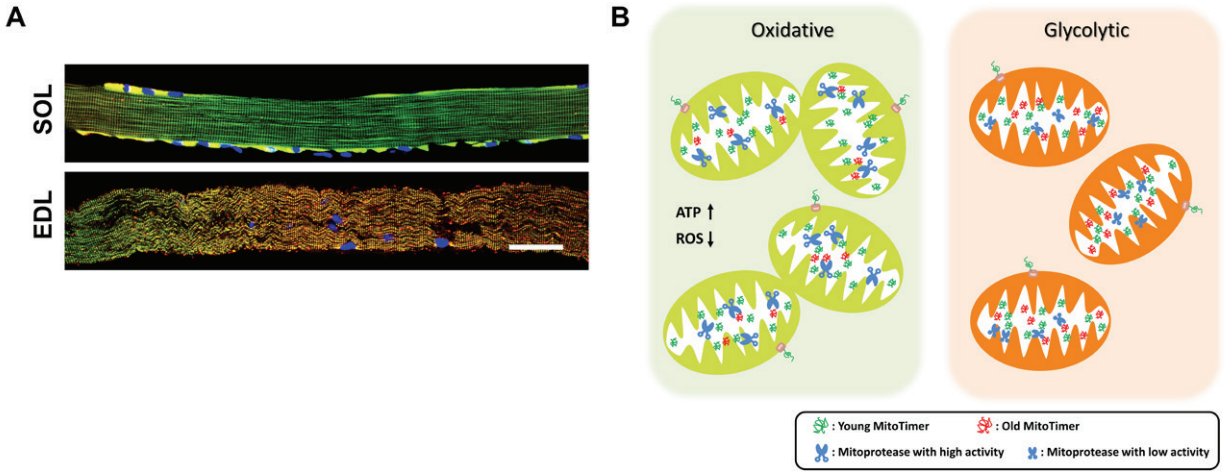

Figure 1. MitoTimer fluorescence reveals an active mitochondrial state tightly coupled with mitoproteolysis in the mouse oxidative skeletal muscle fibers.

Representative MitoTimer fluorescence images of freshly isolated fibers in mouse soleus (oxidative) and EDL (glycolytic) muscles following doxycycline induction from 1 month to 3 months of age. Scale bar, 50μm. Note the green predominant fluorescence as well as the lack of red puncta (indicating mitophagy) in the soleus fiber. (B) A schematic summarizes the results that the energy coupled mitoproteolytic activity dictates MitoTimer fluorescence spectrum and marks an active and well-maintained mitochondrial state.

Selected Publications

1. Xie YY#, Zhang YN#, Sun AN#, Peng YM, Hou WK, Xiang C, Zhang GX, Lai BB, Hou XS, Zheng FF, Wang F and Liu G*. The coupling of mitoproteolysis and oxidative phosphorylation enables tracking of an active mitochondrial state through MitoTimer fluorescence. Redox Biology 2022, 56,102447.
2. Wang Y, Zhang G, Meng Q, Huang S, Guo P, Leng Q, Sun L, Liu G*, Huang X* and Liu J*. Precise tumor immune rewiring via synthetic CRISPRa circuits gated by concurrent gain/loss of transcription factors. Nat Commun 2022, 13(1):1454.
3. Xiang C, Zhang YN, Chen QL, Sun AN, Peng YM, Zhang GX, Zhou DX, Xie YY, Hou XS, Zheng FF, Wang F, Gan Z, Chen S*, Liu G*. Increased glycolysis in skeletal muscle coordinates with adipose tissue in systemic metabolic homeostasis. J Cell Mol Med. 2021, 25:7840–7854.
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5. Xiao Q, Zhang GX, Wang HJ, Chen L, Lu SS, Pan DJ, Liu G*, Yang ZZ*. A p53 based genetic tracing system to follow postnatal cardiomyocyte expansion in heart regeneration. Development. 2017, 144(4):580-589.
6. He XY, Xiang C, Zhang CX,Xie YY, Chen L, Zhang GX, Liu G*. p53 in myeloid lineage modulates an inflammatory microenvironment limiting initiation and invasion of intestinal tumors. Cell Rep. 2015, 13(5):888-97.
7. Chen L, Zhang GX, He XY, Zhang CX, Xie YY, Liu G*. BAC transgenic mice provide evidence that p53 expression is highly regulated in vivo. Cell Death Dis. 2015, 6: e1878.
8. Zhang Q, He X, Chen L, Zhang C, Gao X, Yang Z, Liu G*. Synergistic regulation of p53 by Mdm2 and Mdm4 is critical in cardiac endocardial cushion morphogenesis during heart development. J Pathol. 2012, 228(3):416-28.

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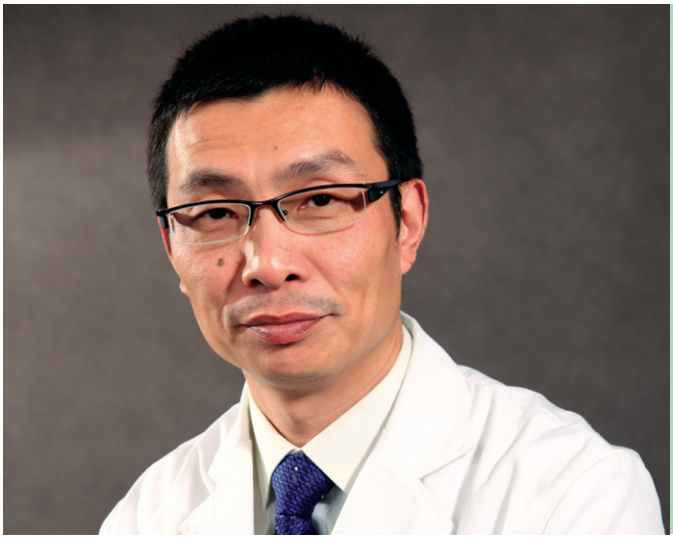

Qing Jiang Ph.D., M.D.

Chief physician / Professor / Doctoral supervisor; The head of the department of Sports Medicine and Adult Reconstructive Surgery, Nanjing Drum Tower Hospital/ The vice president of school of medicine, Nanjing University/ Director, institute of medical 3D printing, Nanjing University. Prof. Jiang, the first sports medicine clinical doctor cultivated by China, had been engaged in orthopedic and sports medicine clinical and basic research since 1989 and got the PhD degree in Beijing Medical University in 1999. In 2008, he was appointed professor in Nanjing University and Adjunct Professor in Model Animal Research Center (MARC). Prof. Jiang won the National Science Fund for Distinguished Young Scholars in 2011. The department of Sports Medicine and Adult Reconstructive Surgery is the only joint disease treatment center identified by Jiangsu Provincial Health Department, and also the training base of artificial joint and arthroscopic techniques in Jiangsu province. Jiang's team has established human gene bank of bone and joint disease including osteoarthritis (OA), developmental dysplasia of the hip (DDH), deep venous thrombosis (DVT), ankylosing spondylitis (AS) and osteoporosis (OP), and published 266 Chinese core articles, 162 SCI articles, included Nature Medicine, Nature Genetic, Science Translational Medicine, Advanced Functional Materials, Annals of the Rheumatic Diseases, Biomaterials, Small. Prof. Jiang is the first domestic scholars who hold the post of committee member of the OARSI, who also is vice chair of China branch of ICRS, vice chairman of sports medicine branch of Chinese medical association, chairman of sports medicine branch of Jiangsu medical association, vice chairman of orthopedics branch, vice chairman of trauma branch, and next chairman orthopaedic branch of Jiangsu medical association, etc.

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Skeletal System Disease

Osteoarthritis (OA) is a progressive degenerative disease of the joints and its progression is closely associated with an imbalance in M1/M2 synovial macrophages. On one hand, we pioneer the reprogramming of mitochondrial dysfunction with a camouflaged meta-Defensome, which can transform M1 synovial macrophages into the M2 phenotype with a high efficiency of 82.3%. The meta-Defensome recognizes activated macrophages via receptor-ligand interactions and accumulates in mitochondria through electrostatic attraction. In vitro results indicate that the meta-Defensomes successfully reprogram mitochondrial metabolism of RAW 246.7-differentiated M1 macrophages by restoring aerobic respiration by scavenging mtROS and inhibiting mtNOS, thereby increasing TFAM expression. The camouflaged metaDefensomes are a promising therapeutic agent for impeding OA progression in clinical practice (Zhang, 2022). On the other hand, we collected synovial tissues from normal patients and patients with OA. The role of methyltransferase-like 3 (METTL3) in autophagy regulation was explored using N6-methyladenosine (m6A)-methylated RNA and RNA immunoprecipitation assays. Senescent fibroblast-like synoviocytes (FLSs) were markedly increased with the progression of OA in patients and mouse models. We determined that impaired autophagy occurred in OA-FLS, resulting in the upregulation of senescence-associated secretory phenotype (SASP). Re-establishment of autophagy reversed the senescent phenotype by suppressing GATA4. Silencing METTL3 enhanced autophagic flux and inhibited SASP expression in OA-FLS. Intra-articular injection of synovium-targeted METTL3 siRNA suppressed cellular senescence propagation in joints and ameliorated DMM-induced cartilage destruction. Our study revealed the important role of FLS senescence in OA progression. Targeted METTL3 inhibition could alleviate the senescence of FLS and limit OA development in experimental animal models, providing a potential strategy for OA therapy (Chen, 2022).

Developmental dysplasia of the hip (DDH) is the most frequent inborn deformity of the locomotors apparatus. Genetic factors play a considerable role in pathogenesis of DDH. We have performed GWAS study of DDH, and detected several novel genes and signaling pathways previously. In a recent study, we identified likely pathogenic variants in the LRP1 (lowdensity lipoprotein receptor-related protein 1) gene in two families and seven unrelated patients. The heterozygous Lrp1 knockout (KO) mouse (Lrp1+/-) showed phenotypes recapitulating the human DDH phenotypes, indicating Lrp1 loss of function causes DDH. Lrp1 knockin mice with a missense variant corresponding to a human variant identified in DDH (Lrp1R1783W) also presented DDH phenotypes, which were milder in heterozygotes and severer in homozygotes than those of the Lrp1 KO mouse. The timing of triradiate cartilage development was brought forward 1 or 2 wk earlier in the LRP1 deficient mice, which leads to malformation of the acetabulum and femoral head (Figure 1). Furthermore, Lrp1 deficiency caused a significant decrease of chondrogenic ability in vitro and the expression of chondrocyte markers was rescued by PNU-74654 (a β-catenin antagonist) in an shRNA-Lrp1–expressed ATDC5 cell (Yan, 2022). Our study reveals a critical role of LRP1 in the etiology and pathogenesis of DDH, opening an avenue for its treatment.

Crosstalk between the liver and bone. Hepatic osteodystrophy (HOD) is a metabolic bone disease that is often associated with chronic liver disease and is marked by bone loss. We demonstrate that hepatic expression of the phosphatase PP2Aca is upregulated during HOD, leading to the downregulation of expression of the hepatokine lecithin-cholesterol acyltransferase (LCAT) (Figure 2). Loss of LCAT function markedly exacerbates the bone loss phenotype of HOD in mice. In addition, we found that alterations in cholesterol levels are involved in the regulation of osteoblast and osteoclast activities. We also found that LCAT improves liver function and relieves liver fibrosis in the mouse HOD model by promoting reversal of cholesterol transport from the bone to the liver (Lu & Shi, 2022). In summary, defects in a liver-bone axis occur during HOD that can be targeted to ameliorate disease progression.

Deep vein thrombosis (DVT). Lots of retrospective and prospective experiments had conducted to study how to prevent DVT in our group. Total joint arthroplasty (TJA) is a form of high-risk postoperative venous thromboembolism (VTE). We determined the relationship between preoperative Soleal veins (SVs) diameter and postoperative DVT after TJA. A large SV diameter was significantly associated with postoperative total DVT after TJA, and also symptomatic DVT after TJA. The bank for DVT patients is still enlarging.

Main research projects currently going on in the lab.

1. (Key projects of NSFC 8173000209) The mechanism study of the cartilage and subchondral bone defect reconstruction using a hydrogel with sustained release of small molecule kartogenin.

2. (Major Projects of NSFC) Study on the role and regulatory mechanisms of bone-derived factors in maintaining homeostasis of the body--Manipulating bone derived factors to develop therapeutic strategies for extra-bone diseases

3. (National Key Project SQ2021YFA1200083) Clinical study on electromagnetic therapy of degenerative orthopedic diseases based on magnetic micro-nano devices

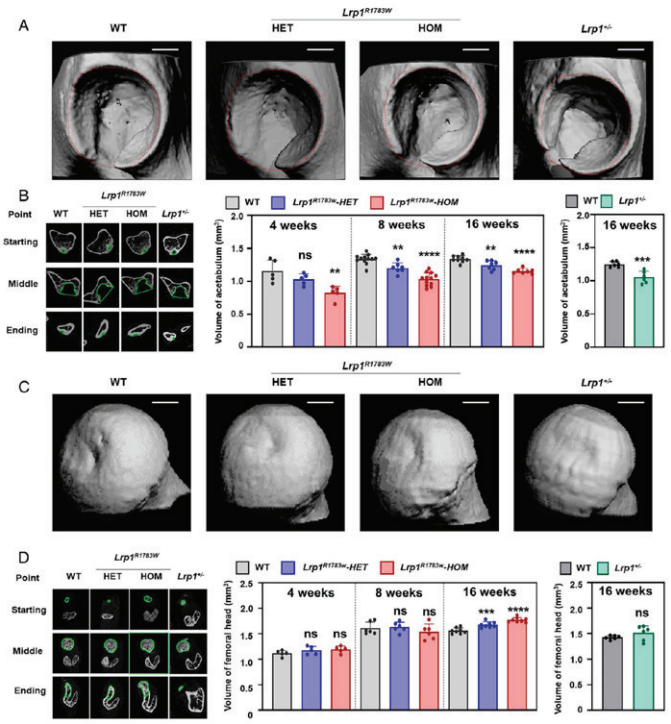

Fig. 1. 3D micro-CT images of the DDH phenotype of heterozygous and homozygous Lrp1R1783W mice, Lrp1+/ mice, and their WT littermates at 8 wk.

(A) Micro-CT images of the acetabulum.

(B) The volume of the acetabulum measured according to the ROI.

(C) Micro-CT images of the femoral head.

(D) The volume of the femoral head. Volumes were measured according to the ROI.

Scale bar: 1 mm. Values = means ± SD; ns, not significant; **P < 0.01; ***P < 0.001; ****P < 0.0001; HET, heterozygote; HOM, homozygote.

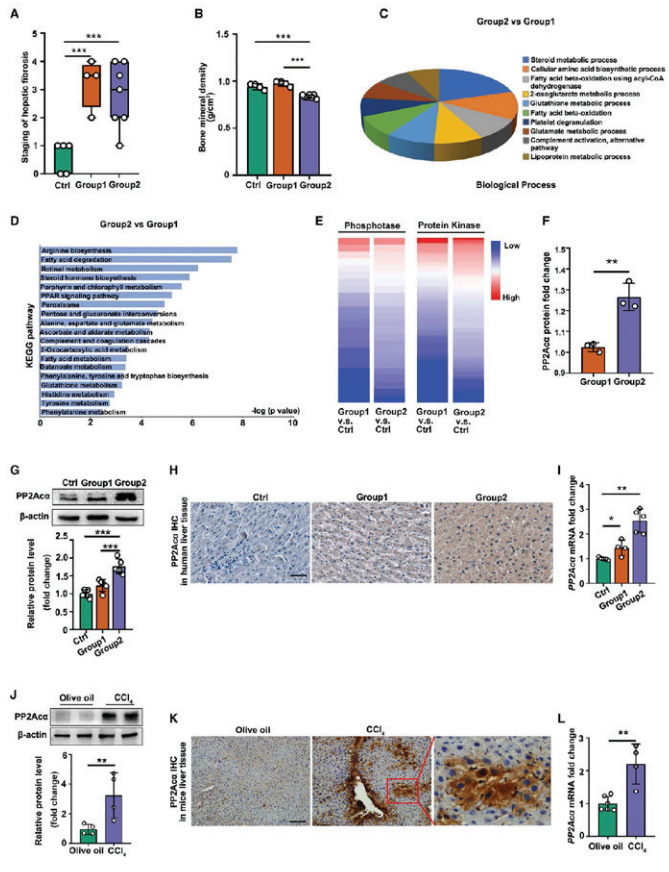

Fig. 2. The upregulation of liver PP2Aca correlates with HOD severity.

(A and B) The stages of hepatic fibrosis (A) and bone mineral density (BMD) (B) of individuals from control group (n = 5), Group1 (n = 4), and Group2 (n = 7). The liver tissues were collected from age-matched individuals with hepatic hemangioma (as control group) and liver cirrhosis with normal BMD (as Group1) and individuals with liver cirrhosis and low BMD (as Group2 or HOD group).

(C and D) Analysis of biological process (C) and KEGG pathway (D) from upregulation of liver proteins in Group2 compared to Group1 (1 technical replicate of 3 biological replicates per group).

(E) Comparative analysis of phosphatase and protein kinase between Group2 and Group1 (1 technical replicate of 3 biological replicates per group).

(F) Fold changes in PP2Aca expression in Group2 compared with Group1 (n = 3 paired biological replicates).

(G) Representative western blots (n = 5 in total) of PP2Aca expression in liver tissues of individuals (top) and its quantification (bottom).

(H) Representative IHC of PP2Aca expression in liver tissues of the groups indicated (control, n = 5; Group1, n = 4; Group2, n = 5; 20 areas per individual were analyzed).

(I) qRT-PCR of relative PP2Aca mRNA levels of the groups indicated, with the level in the control group arbitrarily set to 1 (control, n = 5; Group1, n = 4; Group2, n = 5).

(J) Representative western blots (n = 4 in total) of PP2Aca expression in a mouse model of liver injury after low doses of CCl4 injection for 6 weeks (top) and its quantification (bottom) (olive oil, 2 technical replicates of 2 biological replicates; CCl4, 2 technical replicates of 2 biological replicates).

(K) Representative IHC of PP2Aca expression in liver tissues of a mouse model of liver injury after low doses of CCl4 injection for 6 weeks (scale bar, 200 mm) (olive oil, n = 6; CCl4, n = 4; 7 areas per mouse were analyzed).

(L) qRT-PCR of relative PP2Aca mRNA levels of the groups indicated, with the level in the olive oil group arbitrarily set to 1 (olive oil, n = 6; CCl4, n = 4).

*p < 0.05, **p < 0.01, ***p < 0.001; NS, no significant difference. Rank-sum test (A), two-tailed Student's unpaired t test (F, J, and L), or one-way ANOVA followed by Tukey's multiple comparisons test (B, G, and I). Data are represented as mean ± SEM. See also Figure S1 and Table S1.

Selected publications

1. Yan W, Zheng L, Xu X, Hao Z, Zhang Y, Lu J, Sun Z, Dai J, Shi D, Guo B, Jiang Q*. (2022) Heterozygous LRP1 deficiency causes developmental dysplasia of the hip by impairing triradiate chondrocytes differentiation due to inhibition of autophagy. Proc Natl Acad Sci U S A. 119(37):e2203557119. IF = 12.779 Q1
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Immune cell plasticity and rewiring

In recent years, our laboratory has engaged in the development of new genetic tools (based on the principle of synthetic biology and genome editing) to accurately reprogram cell functions. Thanks to our past background in immunology, we started by developing new tools for rewiring immune regulation.

The complex interface of tumor and immunity presents a highly dynamic system that is key to disease progression and cancer treatment. By establishing novel genetic tools to operate in a condition- or subset-specific manner, we are seeking new generation of therapeutic agents capable of reinvigorating tumor-specific immune surveillance. We are also dedicated to developing cutting-edge genome editing tools, which shall hold potential for broad applications in medicine and agriculture.

Some ongoing projects in the lab are described in the following section:

1. Precise tumor immune rewiring via synthetic circuits gated by concurrent gain/loss of transcription factors:

Reinvigoration of antitumor immunity has recently become the central theme for the development of cancer therapies. Nevertheless, the precise delivery of immunotherapeutic activities to the tumors remains challenging. Here, we explored a synthetic gene circuit-based strategy to specifically identify tumors by their concurrent gain/loss of transcription factor (TF) activities, and to subsequently rewire them toward immune activation (Fig. 1A). We have provided evidence that this synthetic gene circuit enabled specific activation of an IFN program ONLY in p53-deficient, but not -sufficient, tumor cells (Fig. 1B). Additionally, the logic circuit empowered a highly specific and effective immune surveillance against tumors (Fig. 1C). This work has presented an adaptable strategy for the development of precisely delivered immunotherapy.

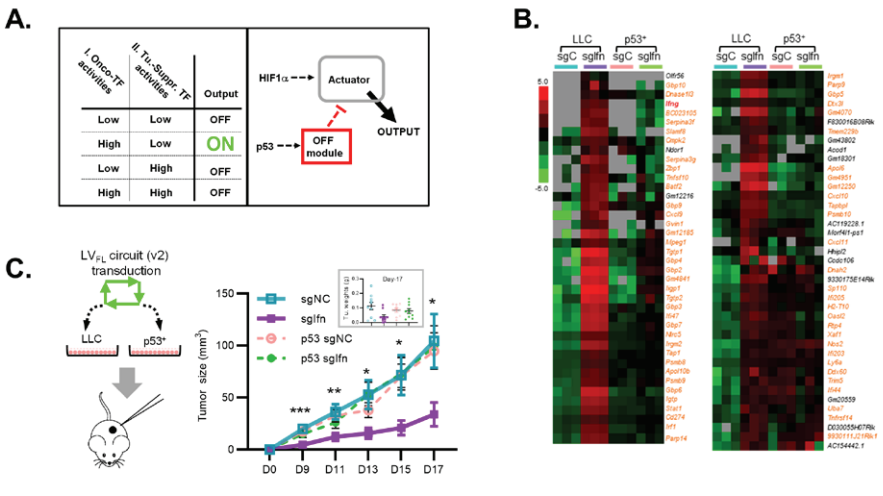

Figure 1: Synthetic gene circuit for tumor-targeted immune intervention.

(A) A scheme shows a synthetic cell classifier that can sense multi-parameter cell states and drive functional outputs accordingly. (B and C) Such a circuit empowered a p53-deficiency-dependent immune activation program in LLC tumor cells, leading to inhibition of tumor growth.

2. Enhancement of prime editing via xrRNA motif-joined pegRNA.

editors (PEs) have shown great promise for precise genome modification (Fig. 2A). However, their suboptimal efficiencies present a significant technical challenge. Here, we harnessed viral exoribonuclease-resistant RNA motifs (xrRNA) for enhancing the activities of pegRNAs (Fig. 2B, C). This upgraded PE platform (xrPE) showed substantially enhanced editing efficiencies in multiple cell lines (Fig. 2D). The appendment of the mechanically rigid xrRNA motif to the 3′-extended portion of pegRNAs led to their increased resistance against degradation (Fig. 2E, F). Of note, parallel comparison of xrPE to the most recently developed epegRNA-based PE system shows their largely equivalent editing performances. Our study establishes a highly adaptable platform of improved PE that shall have broad implications.

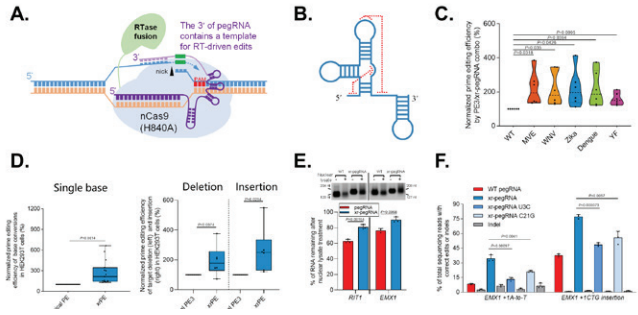

Figure 2: Enhancement of prime editing via xrRNA motif-joined pegRNA.

(A) An illustration of the basic prime editor [PE]. (B) Common structural features of an xrRNA motif. (C) Comparison of effects by xrRNAs derived from different flaviviruses for enhancement of PE. (D) xrPE enabled significantly enhanced editing at various genomic loci. (E) The xr-pegRNAs have increased stability. (F) Mutant xrRNA deficient in mechanical rigidity also showed reduced effect on improving PE.

Selected publications: (*corresponding author)

1. Zhang G†, Liu Y†, Huang S†, Qu S, Cheng D, Yao Y, Ji Q, Wang X*, Huang X* and Liu J*. Enhancement of prime editing via xrRNA motif-joined pegRNA. Nat Commun 2022, 13(1):1856.
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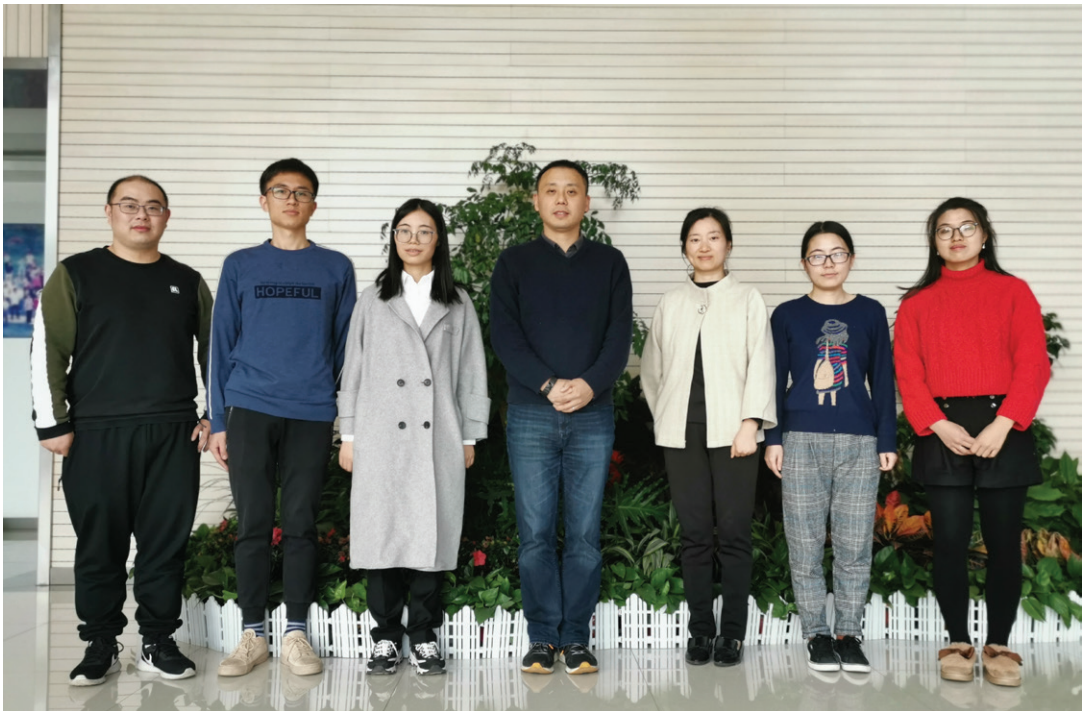

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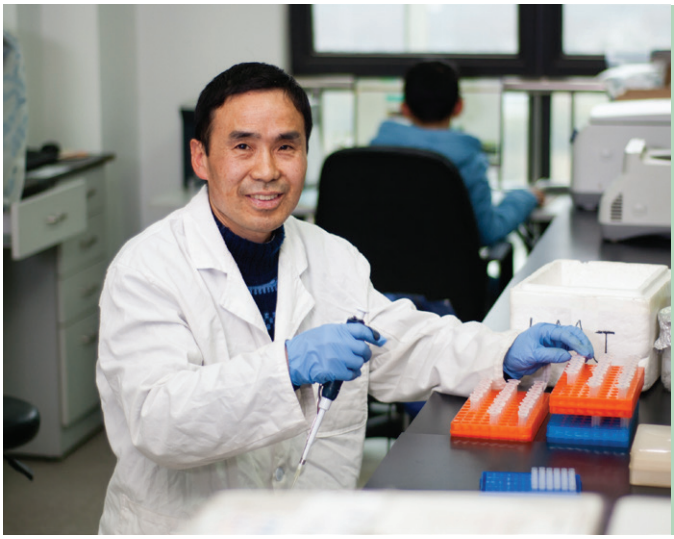

Jinzhong Qin , Ph.D.

Jinzhong Qin received his Ph.D. from Cleveland State University (Ohio, USA) in 2004 after completing a research project at Department of Immunology, Cleveland Clinic Foundation. His research at Cleveland Clinic was focused on the regulation of Innate Immune signaling pathways. From 2005 to 2008, Jinzhong did his postdoctoral fellowship at the Massachusetts General Hospital Cancer Center, Harvard Medical School in Boston, USA, and he was promoted to Assistant in Genetics within the same Institution in 2008. Using murine genetics, he described an essential role of L3mbtl2-containing atypical Polycomb Repressive Complex 1 (PRC1) in embryonic stem cells (ESCs) proliferation and early embryonic development. He joined the Faculty of Model Animal Research Center (MARC), Nanjing University in 2013. He is now a Professor of genetics and developmental biology and a Principal Investigator in MARC.

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Roles of the polycomb group proteins in stem cells & early development

1. Mga safeguards embryonic stem cells from acquiring extraembryonic endoderm fates.

Polycomb group (PcG) proteins form multiprotein complexes that affect stem cell identity and fate decisions by still largely unexplored mechanisms. Here, by performing a CRISPR-based loss-of-function screen in embryonic stem cells (ESCs), we identify PcG gene Mga involved in the repression of endodermal transcription factor Gata6. We report that deletion of Mga results in peri-implantation embryonic lethality in mice. We further demonstrate that Mga-null ESCs exhibit impaired self-renewal and spontaneous differentiation to primitive endoderm (PE). Our data support a model in which Mga might serve as a scaffold for PRC1.6 assembly and guide this multimeric complex to specific genomic targets including genes that encode endodermal factors Gata4, Gata6, and Sox17. Our findings uncover an unexpected function of Mga in ESCs, where it functions as a gatekeeper to prevent ESCs from entering into the PE lineage by directly repressing expression of a set of endoderm differentiation master genes.

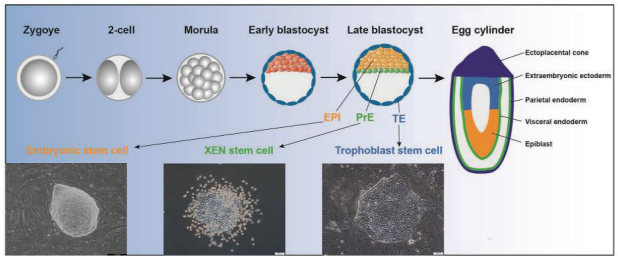

Figure 1. A process of progressive cell fate specification during mouse preimplantation development. Three distinct cell lineages are present in the implanting blastocyst: the extraembryonic trophectoderm (TE), the primitive endoderm (PE, also called the hypoblast), and the embryonic epiblast (EPI).

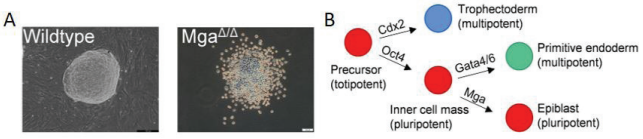

Figure 2. Mga is required for maintenance of the pluripotent state in ESCs.

(A) Mga deficiency results in dynamic changes in cell morphology and gene expression characteristic of Xen cells. (B) Mga blocks XEN lineage formation by repressing the expression of Gata4/6.

2. Rbbp4 suppresses premature differentiation of embryonic stem cells.

Polycomb group (PcG) proteins exist in distinct multi-protein complexes and play a central role in silencing developmental genes, yet the underlying mechanisms remain elusive. Here, we show that defificiency of retinoblastoma binding protein 4 (RBBP4), a component of the Polycomb repressive complex 2 (PRC2), in embryonic stem cells (ESCs) leads to spontaneous differentiation into mesendodermal lineages. We further show that Rbbp4 and core PRC2 share an important number of common genomic targets, encoding regulators involved in early germ layer specifiification. Moreover, we fifind that Rbbp4 is absolutely essential for genomic targeting of PRC2 to a subset of developmental genes. Interestingly, we demonstrate that Rbbp4 is necessary for sustaining the expression of Oct4 and Sox2 and that the forced co-expression of Oct4 and Sox2 fully rescues the pluripotency of Rbbp4-null ESCs. Therefore, our study indicates that Rbbp4 links maintenance of the pluripotency regulatory network with repression of mesendoderm lineages.

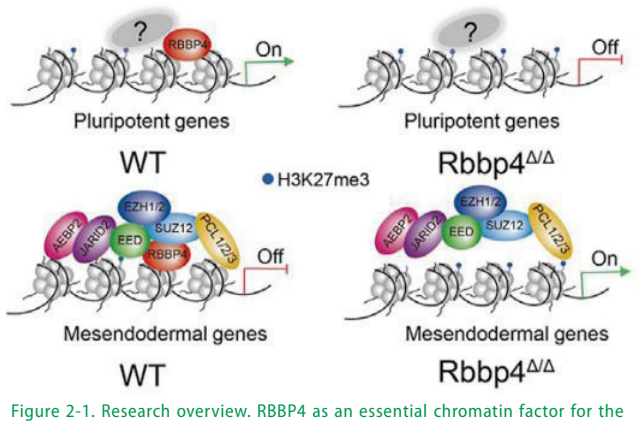

Figure 2-1. Research overview. RBBP4 as an essential chromatin factor for the maintenance of ESC pluripotency and it functions within the PRC2 complex to repress mesendoderm specifiification.

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Crosstalk between IL-15Rα^{+} TAM and breast cancer cells reduces CD8^{+} T cell recruitment

IL-15 is a promising immunotherapeutic agent owing to its powerful immune-activating effects. Here, we found that macrophages contributed to the resistance of tumor cells to IL-15. Further investigation showed that IL-15Rα^{+} TAMs reduced the protein levels of chemokine CX3C chemokine ligand 1 (CX3CL1) in tumor cells to inhibit the recruitment of CD8^{+} T cells by releasing the IL-15/IL-15Rα complex (IL-15Rc). Administration of an IL-15Rc blocking peptide markedly suppressed breast tumor growth and overcame the resistance of cancer cells to PD-1 antibody immunotherapy. Interestingly, Granulocyte-macrophage colony-stimulating factor (GMCSF) induced γ chain expression to promote tumor cell-macrophage crosstalk, which facilitated tumor resistance to IL-15. Additionally, we observed that the non-transcriptional regulatory function of HIF-1α was essential for IL-15Rc to regulate CX3CL1 expression in tumor cells. Therefore, the IL-15Rc-HIF-1α-CX3CL1 signaling pathway might serve as a crosstalk between macrophages and tumor cells in the tumor microenvironment of breast cancer. And targeting this pathway will provide a potential therapeutic strategy for enhancing the efficacy of cancer immunotherapy.

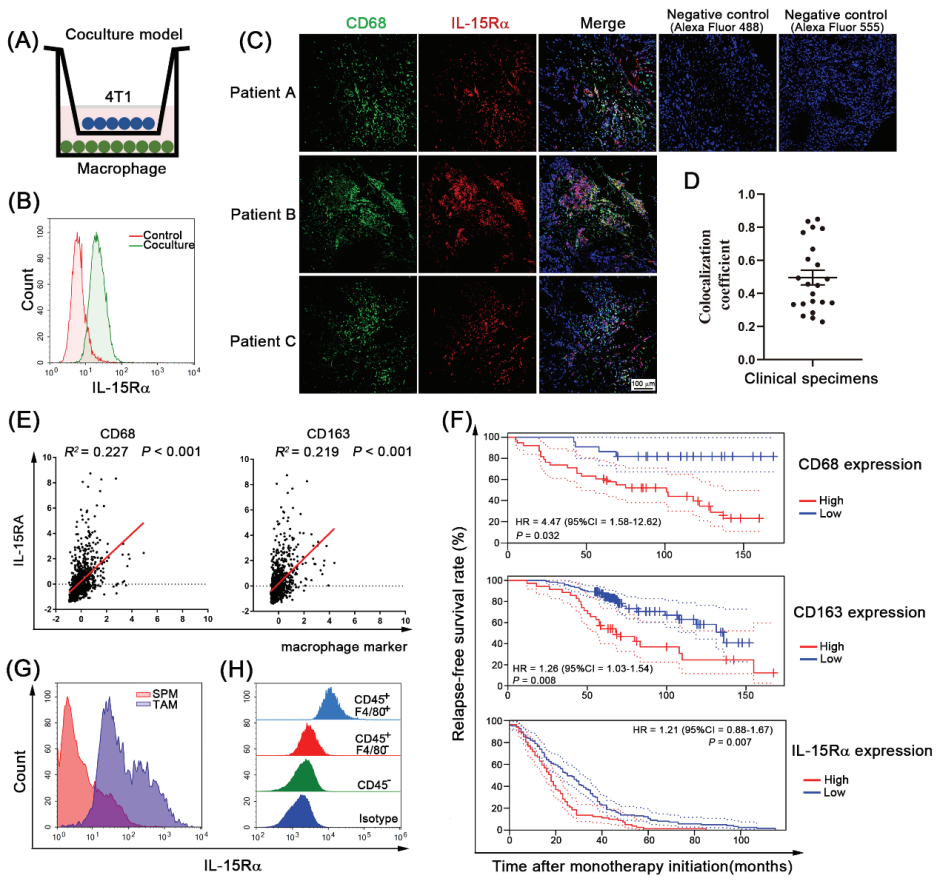
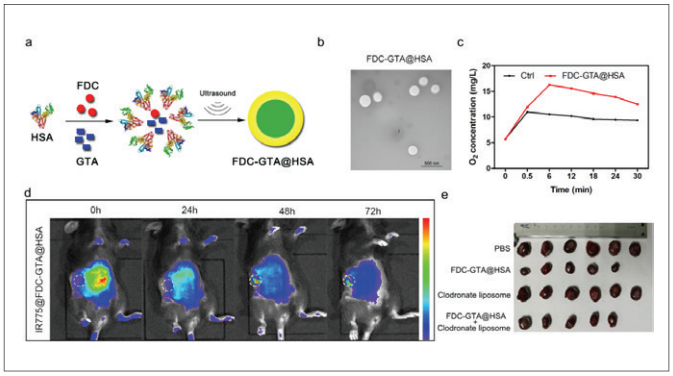


Figure 1. A TAM subset with high IL-15Rα expression is associated with poor survival in breast cancer patients.

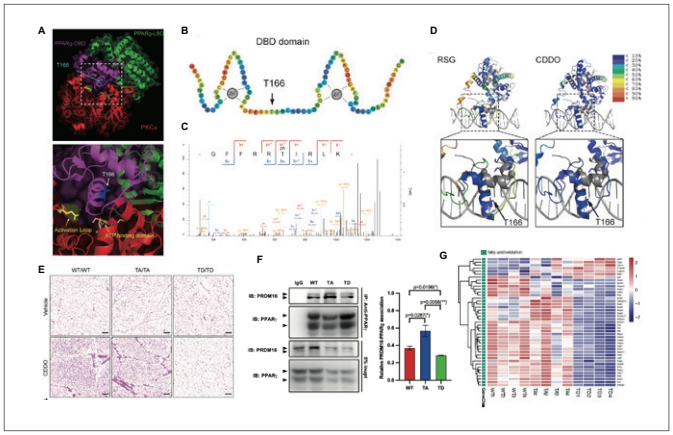
In situ nanoparticle-based editing for the reprogramming of anti-tumor actions of TAMs

Shaping the actions of tumor-associated macrophages (TAMs) is one of the current goals in cancer immunotherapy. Based on our previous findings that pyruvate carboxylase (PCB) is suppressed in TAMs by the hypoxic tumor microenvironment and that elevating PCB level significantly modifies the anti-tumor actions of TAMs, we fabricated a novel nanoparticle named FDC-GTA@HSA, which was produced by encapsulating perfluorodecalin (FDC), oxygen, glycerin triacetate (GTA) into human serum albumin (HSA). This nanoparticle can accumulate at tumor site dependent on EPR (enhanced permeability and retention) effect. By mitigating tumor hypoxia and activating TAMs-intrinsic PCB, FDC-GTA@HSA reprograms TAMs in situ, resulting in the restoration of anti-tumor immunity and the inhibition of tumor progression. Our study suggests that FDC-GTA@HSA may offer a unique approach to edit the functions of TAMs, which can be an adjunct form of immunotherapy.



Blockage of PPARγ T166 phosphorylation enhances the inducibility of beige adipocytes and improves metabolic dysfunctions

Beige adipocytes in mammalian white adipose tissue (WAT) can reinforce fat catabolism and energy expenditure. Promoting beige adipocyte biogenesis is a tantalizing tactic for combating obesity and its associated metabolic disorders. Here, we report that a previously unidentified phosphorylation pattern (Thr166) in the DNA-binding domain of PPARγ regulates the inducibility of beige adipocytes. This unique posttranslational modification (PTM) pattern influences allosteric communication between PPARγ and DNA or coactivators, which impedes the PPARγ-mediated transactivation of beige cell-related gene expression in WAT. The genetic mutation mimicking T166 phosphorylation (p-T166) hinders the inducibility of beige adipocytes. In contrast, genetic or chemical intervention in this PTM pattern favors beige cell formation. Moreover, inhibition of p-T166 attenuates metabolic dysfunction in obese mice. Our results uncover a mechanism involved in beige cell fate determination. Moreover, our discoveries provide a promising strategy for guiding the development of novel PPARγ agonists for the treatment of obesity and related metabolic disorders.



Selected publications

1. Shu YX, Yang NF, Cheng N, Zou ZY, ZhangWL, Bei YC, Shi Q, Qin MH, Zhu WG* and Shen PP*. Intervening pyruvate carboxylase stunts tumor growth by strengthening anti-tumor actions of tumor-associated macrophages. Signal Transduction and Targeted Therapy.2022,7:34.
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5. Bei YC, Cheng N, Chen T, Shu YX, Yang Y, Yang NF, Zhou XY, Liu BR, Wei J, Liu Q, Zheng W, Zhang WL, Su HF, Zhu WG, Ji JG, Shen PP*. CDK5 Inhibition Abrogates TNBC Stem cell Property and Enhances Anti-PD-1 Therapy. Advanced Science. 2020; 7: 2001417.
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8. Niu ZY, Shi Q, Zhang WL, Chen B, Wang QS, Zhao XY, Chen JJ, Shu YX, Cheng N, Feng XJ, Ji JG, Shen PP*. Caspase-1 cleaves PPARγ for potentiating the pro-tumor action of TAMs. Nature Communications. 2017, 8:766.

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Mitotic regulators in cancer and brain development

Our lab is interested in the molecular mechanism involved in both cell division and human diseases, currently focusing on cancer and brain development using human cells and animal models (zebrafish and mouse).

During cell division, proper chromosomes segregation must be achieved otherwise it can result in unequal distribution of chromosomes to daughter cells. Spindle microtubules must attach to a single region of each chromosome, termed the "centromere" in most eukaryotes. The kinetochore is a complex of proteins that is located at the centromere (Figure 1). Defects in the centromere-kinetochore function as well as the spindle check point function, lead to aneuploidy, cancer, and abnormal brain development, and are often associated with a poor prognosis. Therefore, it is highly important to study the temporal-special regulation and the structure of centromere and kinetochore protein(s) to understand chromosome instability (CIN) in cancer and brain development.

Depletion of TSG101 showed synthetic dosage lethality (SDL) in MAD2-overexpressing cells

The spindle assembly checkpoint (SAC) is a surveillance mechanism and its activation is a fundamental step that ensures faithful chromosome segregation stability. Mitotic arrest deficiency 2 (MAD2), a pivotal component of the SAC, and its overexpression also resulted in many types of cancer. However, research into the treatment of MAD2-overexpressing cancer and the cell death mechanism of these cells is still under development. Our lab found that depletion of TSG101 showed lethality in MAD2-overexpressing human cells in a p53-independent but AIFM1- and caspase-dependent manner (Figure 2a-2c), proposing that the TSG101 can be a potential therapeutic target in MAD2-overexpressing tumors. For convenience and simplicity, the following sentence refers to this cell death as MOID (*MAD2-Overexpressing Interphase cell Death*).

MOID is p53-independent but AIFM1- and caspase-dependent cell death

Because we observed the MOID also in HeLa cells of which the p53 gene is inactivated by the human papillomavirus (HPV) E6 protein, we hypothesized that the MOID is p53-independent cell death. In addition, we confirmed that MOID occurs in cells that lack the p53 gene (i.e., non-small-cell lung carcinoma A549 p53KO; Figure 2d). We performed siRNA knockdown of p53 or AIFM1 under the MOID-inducing condition and found that p53 knockdown in p53 gene-active cells (293T and human skeletal muscle primary cells [HSkMC]) did not affect the MOID but AIFM1 knockdown significantly suppressed the MOID regardless p53 status in cells (Figures 2e-2g and data not shown). These data suggest that DNA double-strand breaks are occurring regardless of p53 status in four different types of cells we examined (i.e., HeLa, 293T, A549 cells, and human primary skeletal muscle cells [HSkMCs]), suggesting that this interphase cell death is p53-independent but AIFM1-dependent.

Mitochondria release apoptosis-inducing factor (apoptosis inducing factor mitochondria associated 1: AIFM1) is thought to regulate both caspase-dependent and independent cell death. To further verify the mechanism of cell death, we examined the activity of caspase. The result of our FAM-FLICA caspase assay revealed the activation of caspase(s) in the MOID (data not shown), suggesting that this interphase MOID is caspase-dependent.

Although the aforestated discoveries have been made, assays to assess the long-term viability of cancer cells in ex-vivo and in vivo (animal body level) remains to be performed. We will further clarify the in-depth mechanism of MOID applying the mutational study of TSG101. This mechanism involves how MAD2, AIFM1, and other molecules behave during MOID and their cell cycle machinery.

Figure Legends

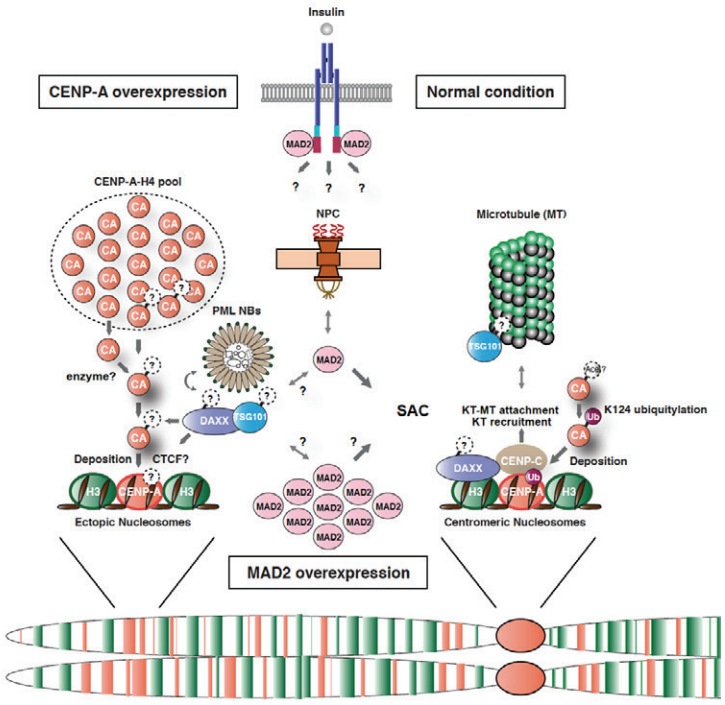

Figure 1. Inter-organelle signaling of MAD2 involved in MAD2 and CENP-A overexpressions.

MAD1 (not shown) and MAD2 associate with nuclear pore complexes (NPCs) in interphase, and nuclear pores and kinetochores (KT) both emit spindle checkpoint signals. Regulation of downstream factors of insulin receptor (IR) and its link to MAD2 overexpressing cancer is not yet clear. CENP-A ubiquitylation is required for CENP-A deposition into centromeric nucleosomes, while CENP-A overexpression leads to CENP-A deposition into ectopic nucleosomes through DAXX.

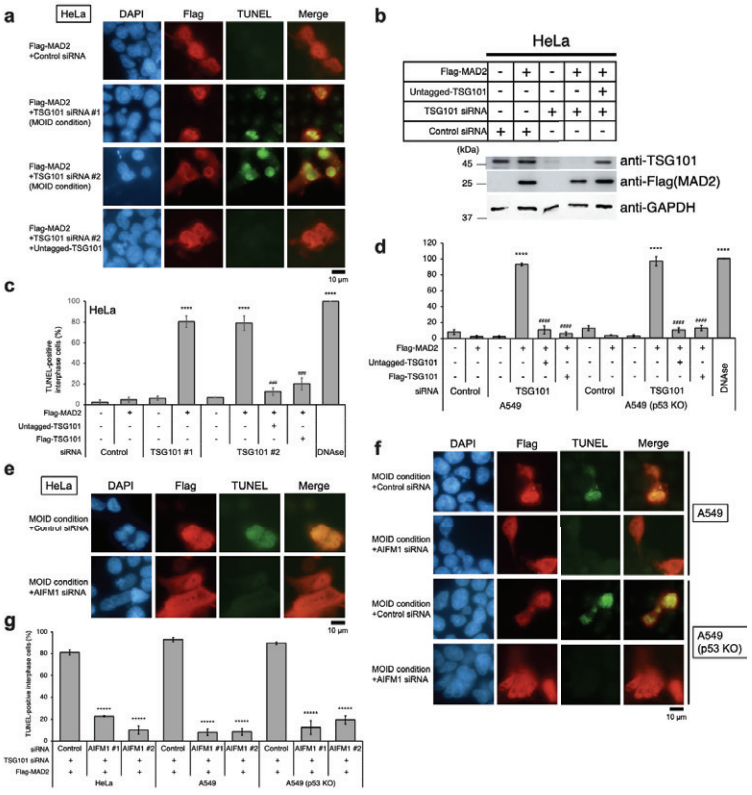

Figure 2. Depletion of TSG101 showed synthetic dosage lethality (SDL) in MAD2-overexpressing cells.

(a) Fluorescence images of TUNEL assay. HeLa cells were transfected with indicated siRNAs and/or overexpression plasmid vectors. Cells were cultured for 96 h at 37°C. DNA fragmentation was detected by the TUNEL assay, and samples underwent indirect fluorescence microscopy using anti-Flag as a primary antibody to sort out Flag-MAD2 overexpressing cells.

(b) Western blot analysis of total lysates of HeLa cells transfected with indicated siRNAs and/or overexpression plasmid vectors. Cells were cultured for 96 h, collected, lysed, and immunoblotted with indicated antibodies. GAPDH protein was used as a loading control.

(c) A histogram summarizing TUNEL assay results of (A). Cells treated with DNAse (10 U/ml, 10 min) after fixation is shownn as a control to verify TUNEL positivity. The mean percentages (± SD) of TUNEL-positive cells were calculated. (****) $P < 0.0001$ compared with 1st column from left (student's t-test). (###) $P < 0.001$ compared with the 4th column from left (student's t-test).

(d) A histogram summarizing TUNEL assay results (Images are not shown). A549 or A549 p53 KO cells were transfected with indicated siRNAs and/or overexpression plasmid vectors. Cells were cultured for 96 h at 37°C. DNA fragmentation was detected by the TUNEL assay as described in Figure 1c. Cells treated with DNAse (10 U/ml, 10 min) after fixation is shown as a control to verify TUNEL positivity. The mean percentages (± SD) of TUNEL-positive cells were calculated. (****) $P < 0.0001$ compared with 1st column from left in each cell (student's t-test). (####) $P < 0.0001$ and (###) $P < 0.001$ compared with 4th column from left in each cell (student's t-test).

(e) Fluorescence images of TUNEL assay. HeLa cells were transfected with indicated siRNAs and/or overexpression plasmid vectors. TUNEL assay was performed as (a).

(f) Fluorescence images of TUNEL assay. A549 cells or A549 p53 cells were transfected with indicated siRNAs and/or overexpression plasmid vectors. TUNEL assay was performed as (a).

(g) A histogram summarizing TUNEL assay results of (e) and (f). HeLa, A549, or A549 p53 KO cells were transfected with indicated siRNAs and/or overexpression plasmid vectors. Cells were cultured for 96 h at 37°C. DNA fragmentation was detected by the TUNEL assay as described in Figure 1c. The mean percentages (± SD) of TUNEL-positive cells were calculated. (****) $P < 0.0001$ compared with 1st column from left in each cell (student's t-test).

Selected publications (*Co-corresponding author)

1. Niikura Y#*, Kitagawa K#. E3 Ligase for CENP-A (Part 2). In: Catala A, editor. London, UK: IntechOpen [one book chapter]; 2022.3.6. (# Equal contribution)
2. Niikura Y#*, Kitagawa K#. E3 Ligase for CENP-A (Part 1). In: Catala A, editor. London, UK: IntechOpen [one book chapter]; 2022.1.24. (# Equal contribution)
3. Niikura Y#*, Fang L#, Kitagawa R#, Li P, Xi Y, You J, Gao Y, Kitagawa K*. Mass Spectrometry Analysis to Identify Ubiquitylation of EYFP-tagged CENP-A (EYFP-CENP-A). J. Vis. Exp. 2020(160). Epub 2020/07/01. doi: 10.3791/61138. PubMed PMID: 32597847. (# Equal contribution)
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Group members

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Shengnan Chen

Guoli Zhong

NJU-MARC Core Facilities

After five years in operation, the Core Facilities of MARC have begun to take shape. We have been equipped with more than 24 state of the art instruments and provide over 34000 hours service within or outside MARC research community.

So far, we have set up Microscopy and Imaging Core, Flow Cytometry Core, Molecular and Metabolomics Core, providing a diverse range of resources and services, including high resolution imaging, flow cytometry, protein and gene expression profiling, and metabolic analysis. The featured instruments are listed below and more resources could be found on our website. http://core.nicemice.cn:8081/.

Qiaoli Chen -Director of MARC Core

Heng Wang -Director of Microscopy and Imaging Core

Imaging

► Services

- Live cell imaging
- Optical sectioning of thick biological samples
- 3D reconstruction of images
- 3-D mosaic imaging
- Multi-area time-laps and spectral scanning
- Super-resolution imaging

► Equipment

- Olympus FluoView 1000 confocal
- Zeiss LSM880 with Airyscan
- Leica TCS II sp5 confocal
- GE Healthcare DeltaVision Imaging System
- GE Healthcare DeltaVision OMX 3D-SIM

Mass Spectrometry

► Services

- Quantitative analysis of small molecules
- Identification of unknown metabolites
- Able to analyze various kinds of samples
- Metabolomics study

► Equipment

- Agilent 6550 iFunnel Q-TOF LC/MS System

High Resolution Ultrasound

► Services

- Cardiovascular research
- Oncology study
- Drug metabolism study
- ……

► Equipment

- FUJIFILM Vevo® 3100 LAZR-X system
- FUJIFILM Vevo® 770

Flow Cytometry

► Services

- Cell sorting
- Able to analyze multiple fluorescent probes simultaneously

► Equipment

- BD LSRFortessa™ Flow Cytometer
- BD FACSCalibur Flow Cytometer
- BD FACSAria™ III Cell Sorter

Cellular Metabolism

► Services

- Live cell energy metabolism

► Equipment

- Agilent Seahorse Xfe24 Extracellular Flux Analyzer

Biomolecule Analysis

► Services

- Fast and reliable protein purification
- SPR based molecular interaction

► Equipment

- GE ÄKTA pure protein purification system
- GE Biacore T200 SPR system

Real time qPCR

► Services

- Gene expression detection

► Equipment

- ABI StepOne Plus
- Roche LightCycler 96

Others

► Equipment

- BioTek synergy H1 plate reader
- Beckman OPTIMA XPN-100 centrifuge

New equipment

► ÄKTA pure protein purification system

- Application: Flexible and intuitive chromatography system for fast purification of proteins, peptides, and nucleic acids from microgram to gram levels of target product
- Application sppourted: Affinity chromatography, size exclusion chromatography (SEC, also known as gel filtration), ion exchange chromatography, hydrophobic interaction chromatography, and reversed phase chromatography (RPC)

► Biacore T200

- A versatile and precise surface plasmon resonance (SPR) system that provides you with a wide range of high-quality molecular interaction data
- Comprehensive characterization and comparability — kinetics, affinity, specificity, concentration, immunogenicity, epitope binning, and transition-state thermodynamics.

► Vevo 3100 Imaging System with LAZR-X

- Use photoacoustic imaging technology, which integrates photoacoustic signals and ultrasound anatomical images, and combines the high sensitivity of optical imaging with the high resolution of ultrasound imaging.
- Support 2D and 3D real-time in-vivo imaging, can track rapid changes in the body, and provides co-registration of photoacoustic images and ultrasound images, accurately giving the source of photoacoustic signals in vivo.
- Blood oxygen saturation and hemoglobin content measurements in the body.
- Combined with contrast agents and nanoparticles, it can detect lymph nodes, biomarker molecules and gene expression.
- Applications in the fields of tumor microenvironment, hemodynamics, nanomedical materials, tumor marker molecules, etc., to provide researchers with real-time, high-resolution, and high-sensitivity in vivo images.

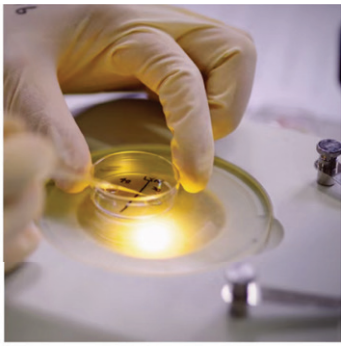
National Resource Center for Mutant Mice of China

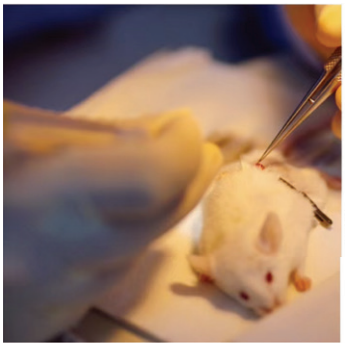
The National Resource Center for Mutant Mice (NRCMM) was established in 2001 by the Ministry of Science and Technology of China and currently co-managed by Nanjing University and GemPharmatech Co. Ltd. The NRCMM is one of the 31 national germplasm centers. The mission of NRCMM is to promote biomedical research and therapeutic development in China by providing valuable mouse model resources and related services.

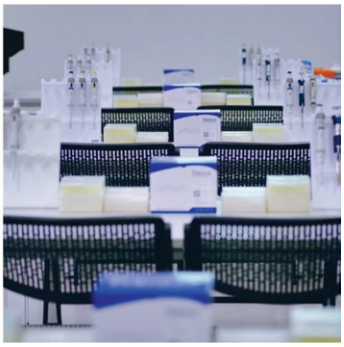
The NRCMM services include repository capabilities, cryopreservation, and the distribution of mutant mice. NRCMM has initiated several projects on standardizing the phenotyping, quality control, cryopreservation and distribution protocols of mutant mice in China. At present, the NRCMM has 26,817 mouse models, becoming the largest resource center in the world . Mouse models from NRCMM have served thousands of research projects by universities, hospitals, research institutes, biotech and pharmaceutical companies.

In 2022, NRCMM launched the "Mouse Models for 2022" project and developed more than 50 new mouse models for supporting the national scientific research projects with professional technology and team.

NRCMM organized the "4th embryo transplantation training course" in Nanjing in September, 2022. More than 30 experimental animal practitioners attended the course. With the combination of theory and practice, the researchers learned the key skills of in-vitro fertilization and embryo transplantation in mice.







NRCMM is also a member of International Mouse Phenotyping Consortium (IMPC), the purpose of which is to create a comprehensive catalog of mouse gene function. In addition, NRCMM hosts education programs for mouse colony management and mouse genetics studies.

Publications in 2022

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Courses and Lecturers

The MARC, as an institute of the NanjingUniversity Medical School, is home to more than100 PhD students. They carry out their dissertation studies under the supervision of MARC group leaders. In addition, MARC group leaders present lectures and laboratory courses at universities in China, in particular, at Nanjing University, and in other countries. In 2021, the following lecture series and courses were given by MARC staff at Nanjing University (unless indicated otherwise).

Progress in Life Sciences

All PIs at MARC

Cell Biology and Molecular Biology

Guoqiang Wan

Hongyu Wang

Genetics

Qing Zhang

Jinzhong Qin

Di Chen

Xin Lou

Mechanism of Development

Jiong Chen

Ying Cao

Zhongzhou Yang

Xin Lou

Information Genomics:

Zhenji Gan

Yohei Niikura

Topics in Genetics I

All PIs at MARC

Topics in Developmental Biology I

All PIs at MARC

Frontier of Cell Biology

Yuanming Cheng(Nanjing University)

Basic Concepts and Frontiers in Immunology

Jianghuai Liu

Yan Li

Huiming Gao

Zhaoyu Lin

Doctoral qualification exam I&II

All PI at MARC

MARC seminar in Genetics

All PIs at MARC

MARC seminar in Developmental Biology

All PIs at MARC

Physiology

Yun Shi

Shuai Chen

Chaojun Li

Guiquan Chen

Qiaoli Chen

PhD Theses

MARC students successfully defended the following PhD theses in 2022

Group Jinzhong Qin

Congcong Wang
The MuvB complex safeguards embryonic stem cell identity through regulation of the cell cycle machinery

Yaru Zhu
Functional study of PRC1 complex in mouse embryonic stem cells

Group Chaojun Li

Tongyu Zhang
Mechanistic study of the metabolic environmental effect on cardiac regeneration in neonatal mice

GroupYun Shi

Qingqing Li
Enhancing GluN2A-type NMDA receptors impairs long-term synaptic plasticity and learning and memory

Guolin Yang
Study on the mechanism of TMEM63B channel as an osmosensor in neural center for thirst in regulating body water homeostasis

Group Zhongzhou Yang

Ke Zhao
LONP1-mediated mitochondrial quality control safeguards metabolic shifts in heart development

Group Jiong Chen

Chen Qu
Rho GTPase regulates mitochondrial dynamics during collective cell migration in Drosophila melanogaster

Group Jianghuai Liu

Limin Yang
IL-6 /ERK signaling pathway participates in type I IFN-programmed, unconventional M2 macrophages polarization

Guiquan Zhang
Design and characterizations of engineered pegRNAs adopting the xrRNA motifs for enhancement of prime editing

Group Minsheng Zhu

YeWang
Mechanisms of Cholestasis-Induced Hepatic Fibrosis Induced by Dysfunctional Motility of the Gallbladder

Yuwei Zhou
Mechanistic study for bitter substances-mediated relaxation of Airway Smooth Muscle

Group Guoqiang Wan

Zhen Chen
Spiral ganglion neuron reprogramming through small molecules and transcription factors

Group Xin Lou

Xue Zhang
Function and mechanism of zinc finger protein Blf and Drl cluster in zebrafish embryonic hematopoiesis

Group Hongyang Wang

Yuting Song
Immunosuppressive microenvironment and its regulatory mechanism in NAFLD-related HCC

Group Xiang Gao

Qiyao Liu
Depletion of GSDMA/ Gsdma1-3 alleviates PMA-induced epidermal hyperplasia through inhibiting EGFR-Stat3/Akt pathway

Xu Li
Apoptotic caspase-7 activation inhibits non-canonical pyroptosisvia GSDMB cleavage

Group Zhenji Gan

Zongchao Sun
The function and mechanistic actions of muscle FNIP1 in vascularization and systemic metabolism

Qiqi Guo
The function and mechanistic actions of mitochondrial protease LONP1 in muscle – adipose crosstalk

Chenyun Ding
The function and mechanistic actions of muscle PA1 in systemic glucose homeostasis

Group Shuai Chen

Qian Du
Rab8a regulates pathological hypertrophy of cardiomyocytes through p38-MAPK/mTOR pathway

Xinyu Yang
Possible roles and mechanisms of an AS160R684X mutation in skeletal muscle metabolic remodeling and disease

Sangsang Zhu
Investigation of roles of the RalGAPα1-RalA axis in the pathogenesis of hypertrophic cardiomyopathy

Group Di Chen

Zi Wang
Molecular Mechanisms of ACS-20/FATP4 in Mediating the Anti-aging Effect of Dietary Restriction in C. elegans

Group Xiaosong Gu

Yisheng Liu
Identification of Neuronal Cells in the Sciatic Nerves of Adult Rats

Qianqian Chen
Transcription factor FOSL1 promotes peripheral nerve regeneration via regulating EPHB2

Group Huiming Gao

Ru Yang
Study on mechanisms of inflammatory neurodegeneration and neuroinflammation-targeting therapeutics for neurodegenerative diseases

Group Guiquan Chen

Yinqian Xia
Study on the role of presenilin enhancer 2 in the fate determinations of hippocampal neural progenitors

2022 Summer Camp

As the primary task for MARC is to excel in scientific research and education, graduate students are the most valuable assets of our institute. To attract more outstanding students to MARC, we held the 13th Summer Camp on June 25th and July 12th this summer.

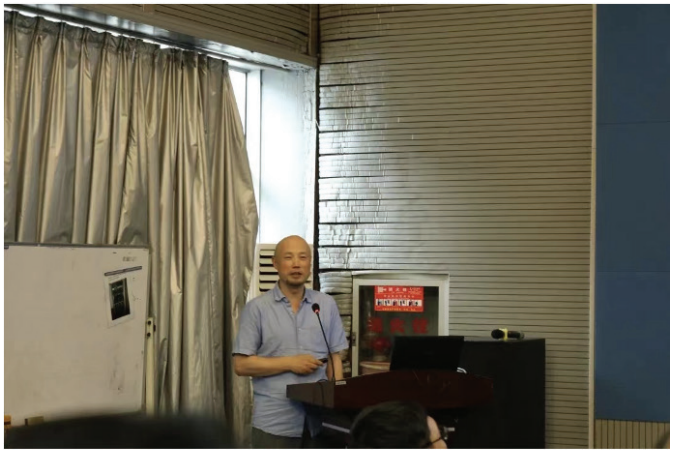
This summer camp was broadcast live at Tencent conference, 64 candidates participated online, allowing productive two-way communications of both enrollments and queries. Sixt excellent undergraduates were awarded "outstanding participants" from a pool of 27 applicants.

Dr Shuai Chen, director of MARC, gave an opening speech .Dr Guoqiang Wan introduced the history and passion of MARC. Three faculty members introduced their research areas at Model Animal Research Center, including areas of metabolism and immunology, neuroscience.

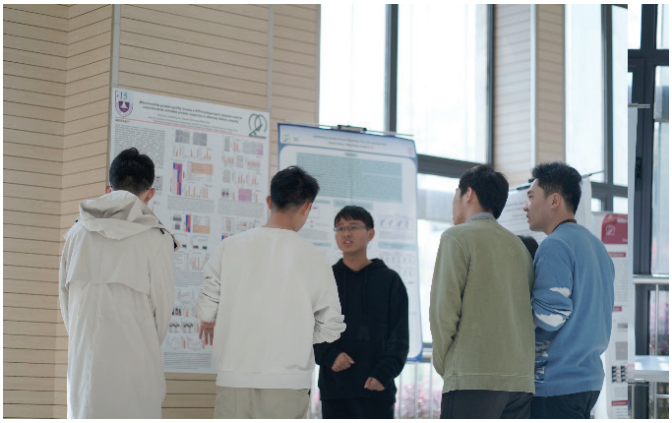
The purpose of the Summer Camp is to train and attract students for future biomedical researches involving model animals both at MARC and at other institutes in China. In addition to scientific courses delivered by our prominent PIs, both PIs and the summer camp students who share same passion continued to change ideas and communicate after the broadcast. Overall, the online Summer Campallowed the participants to experience the strong atmosphere of academic research at MARC and stimulate their enthusiasm for scientific research.

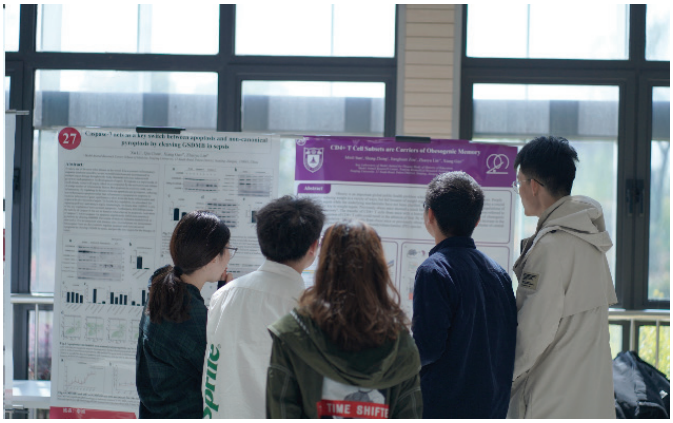
2022 Students Activities

The Student Union, which acts as a bridge between students and teachers, held a lot of academic and extracurricular activities during 2022. In order to promote the communication between each laboratory and stimulate our critical thinking and creativity, we hold the Scientific research exchange meeting between teachers and students monthly. We invite two professors to share their scientific research, new ideas and brain storms with students from different labs. Students can ask questions and communicate with each other at any time. We have a cold meal after the report and talk in a relaxed and pleasant atmosphere.



Except the Scientific research exchange meeting,we also conducted other academic activities, such as Poster Exhibition and select the Star of MARC. During the Poster Exhibition students can show their research to others. All members in MARC voted for top posters in their mind. And finally, authors of the top three were selected as MARC Star Candidates of 2021. Posters ranked third to eighth were selected as outstanding posters. And they all got a award for their excellent research work.





Our extracurricular activities are also rich and colorful in MARC. Besides studying and researching, students of MARC may also found themselves cultivated by a culture promoting humanity, critical thinking, and social well-being here. Thanks to the generous financial support guaranteed by both the MARC and the government, we have adequate resources to enrich our lives here.

Because of the playground, we didn't hold any ball games. Instead, we have more relaxed and interesting activities, such as Singer Contest and Fun Games with Marc Academy. Through all these games and academic activities, we can savage our body and civilize our spirit, so that we can stay in a fuller state of mind for scientific research and live a happier life.

In the future, we will listen to the voice of students more, and continue to improve our planning and organizing ability. On the one hand, we will hold more meaningful academic activities, such as inviting professors from different fields to give lectures and communicate with our students. On the other hand, we will use the limited space to hold more interesting activities, so that students can relax themselves and Full of energy.

Marc Academy

Marc Academy is a newly established teacher-student organization of MARC in 2021. Its purpose is to promote teacher-student exchanges and break the communication barriers of students in various laboratories. At present, Marc College is divided into 4 colleges. The dean of the school is elected spontaneously by students, and competitions between colleges are carried out with the college as a unit. Each college includes students and teachers in each laboratory, and ensures full communication and interaction between teachers and students through activities between each college and within the college. Each activity is sponsored by one of the 4 colleges, and activity funds are obtained through the ranking of each activity to support free activities in the college.

In order to prosper the cultural life of postgraduates, we strive to create a positive, healthy and civilized cultural atmosphere and provide students with a stage to show their talents. We held the first MARC Singer Contest. The students actively signed up to participate, and the beautiful singing and enthusiastic cheers of different styles echoed in the lobby. The first singer competition was held very successfully, which fully motivated the students and added a touch of color to the life of graduate students.

As a traditional MARC activity, table tennis competition is an activity that teachers and students look forward to every year. This year's table tennis competition was held by the MARC Academy. It was the first time everyone won the competition for the Academy.

The early stage of the Fun Games was actively prepared by the Dean of the First Branch Xiang Duan and the students, and it was held on a sunny weekend. The students actively responded to the call of the school, and clamored for the honor of the school.

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NATIONAL RESOURCE CENTER FOR MUTANT MICE