CURRICULUM VITAE

Chuandong Fan

Current position

Research scientist and lab manager in Yungki Park's lab
Institute for Myelin and Glia Exploration
Department of Biochemistry, University at Buffalo
B4-136, 4th floor, Park lab, CBLS building
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Email:chuandon@buffalo.edu
chuandong.fan@gmail.com
Immigration status: Citizen of USA since 2019. The former nationality is China

Professional Experience

Mar, 2022-present - Research scientist in Yungki Park's lab
Jan 4, 2016-Feb, 2022 - Research technician in Yungki Park's lab
Institute for Myelin and Glia Exploration, Department of Biochemistry, University at Buffalo, Buffalo, NY 14203, USA

I studied ubiquitination, phosphorylation and sumoylation of transcription factor Myrf in oligodentrocyte differentiation. Besides, I investigated the interacting proteins of Myrf using TurboID and mass-spectrometry, as well as co-immunoprecipitation by which I proved Myrf protein complex is a homotrimer.

Further, we analyzed Olig2 and Sox10 gene enhancers which are important in oligodentrocyte differentiation by silencing them with CRISPRi. We generated doxycycline inducible expression Oli-neu cell lines with CRISPR/Cas9 system and piggibac and sleeping beauty transposon/transposase system. DNA segment harboring sgRNA targeting enhancers, GFP and puromycin resistance gene was inserted into the genome of Oli-neu cells with hypBase. The positive cells are selected by puromycin. KRAB-dCas9 with doxycycline inducible promoter, blasticidin resistance gene, and rtTA gene were integrated into the genome of Oli-neu cells with SB100X, and the cells were selected with blasticidin. The effect of enhancer silencing was quantified with RT-qPCR of expressions of Olig2 or Sox10.

We also had generated inducible expression Oli-neu cell lines with inducible expression of shRNA and sleeping beauty transposon transposase system. Positive cells are selected with blasticidin.

Nov 27, 2014 – Jan 3, 2016

I took a break for 1 year to focus on personal development. I improved my English by reading, writing, and speaking. I read the English version books of biology, cell biology, molecular biology, biochemistry, immunobiology, pathology, physiology, and latest biomedical literatures, to get ready for the future career.

Nov 13, 2009 – Nov 26, 2014 Post-doctoral research

Post-doctoral research associate in Dr Xinjiang Wang's lab
Department of Pharmacology and Therapeutics
Roswell Park Cancer Institute

My research in this period focused on the mechanism of how the ubiquitin E3 ligases regulate the oncoprotein and tumor suppressor through ubiquitination in cancer cells.

(1) Ubiquitin-dependent regulation of phospho-AKT dynamics by the ubiquitin E3 ligase, NEDD4-1, in the IGF-1 response

(2) Ubiquitination of the Thoc1 Encoded Ribonucleoprotein by NEDD4-1 E3 Ubiquitin Protein Ligase

(3) Ubiquitination of MDM2 by NEDD4-1 E3 Ubiquitin Protein Ligase

(4) Role of NEDD4-1 in glucose metabolism in mammal cells

(5) Novel chemotherapeutic drug development for Glioblastoma, lymphoma and pancreatic cancer in in vitro models

(6) Novel mechanism of action of a potential anti-cancer drug FL118 induces p53-dependent senescence by targeting MdmX degradation in colorectal cancer cells

Graduate student, School of Life Science (supervisor: Prof. Junying Miao**),**
2003-2009 Shandong University

(1) Investigation of the mechanisms by which the complexes of copper and salicylaldehyde pyrazole hydrazone derivatives induces apoptosis in human umbilical vein endothelial cells and lung cancer cells;

(2) Investigation of the autophagy induced by pyrazole hydrazide derivatives in human lung cancer cells;

(3) Study on mechanisms underlying chloroquine inhibits cell growth and induces cell death in A549 lung cancer cells;

(4) Preliminary study of ion sensors for Cu^{2+}, Fe^{3+}, Cr^{3+}, Hg^{2+}, H^{+}, Cl^{-} and cysteine in living cells;

(5) Other duties:
Repairing some of the experimental apparatus in the lab;
In charge of lab safety works

1992-2003 Shandong non-metallic geological engineering exploration academy (www.sinoma-shdd.cn), China National Materials Group Corporation (www.sinoma.cn), at Jinan city, China
Geophysical exploration for non-metallic minerals and for foundation of buildings
●Engineer, August 1998- August 2003
●Assistant engineer, July 1992- July 1998

1988-1992 Jilin University, Jilin, China
●Mathematical model designing in geophysics
●Computational software designing using FORTRAN 77 language

Education

Shandong University, Jinan, Shandong, China
Ph.D., 2003-2009, Cell Biology, Supervisor: Prof. Junying Miao
Jilin University, Changchun, Jilin, China
Bachelor's degree in Engineering, 1988-1992, Exploratory Geophysics

Doctoral thesis: “The molecular mechanisms of apoptosis and autophagy in lung cancer cells and vascular endothelial cells induced by novel copper-chelator complexes and pyrazole hydrazide derivatives”.

Honors and Awards

Achievements in scientific research scholarship, 2008, by Shandong University

Working skills

Cell biology techniques

●Cell types that I had cultured:
►Primary cells: HUVEC, MEF
►Transformed cell lines: CG4, Oli-neu, HEK293, 293T, 293FT
►Cancer cells: Glioblastoma cells (T98G, A172), lymphoma cells, breast cancer cell MCF-7, prostate cancer cell line PC3, pancreas cancer cells (BxPC-3, PANC-1), Liver cancer cell BEL7402, lung cancer cells (A549, H460, H322, H1299), sarcoma U-2 OS cells, PC12, Hela, hybridoma cells, C2C12, etc.

►Insect cells: SF9

●Cell culture medium formulation for glucose metabolism research
●MTT, CCK-8 assay for cell growth
●Confocal microscopy
●Hoechst 33258, DAPI, acridine orange, neutral red, trypan blue staining
●TUNEL assay for cell apoptosis
●Lactate Dehydrogenase for cell necrosis
●Immunocytochemistry
●C2C12 differentiation to muscle cells
●CG4 and Oli-neu cell differentiation to oligodendrocyte-like cells
●Extraction of Sub-cellular fraction by using differential centrifugation, including membrane, cytoplasm, nuclei soluble part, and nuclei insoluble part
●De-comtamination of micoorganisms from mammalian cells

Biochemistry

●Recombinant Protein extraction and purification from TNT Rabbit Reticulocyte Lysate System (Promega), Wheat Germ Extract (Promega), bacteria, SF9 cells, mammalian cells, and mouse brain and spinal cord
●Western blot (One of my top expertise, with ability to detect protein out of 100 ng of whole cell lysate proteins, experiences of more than 2,000 times)
●UV/Vis spectroscopy
●Glucose and Lactate assay
●Luciferase assay
●Fluorescence spectroscopy
●In vitro and in vivo ubiquitination assay (One of my top expertise, able to catch weak ubiquitination signal)
●Immunoprecipitation (One of my top expertise, able to perform sequential IP)
●TurboID: Proximity labeling of target protein (prey) with biotin by biotin ligase fused to bait protein, then followed by biotin based purification and protein analysis (often Mass spectrometry)
●Mass spectrometry (sample preparation, protein post-translational modification and protein interaction analysis)

Molecular biology techniques

●Construction of plasmid and bacmid vector
●RNA and DNA extraction
●Regular PCR and RT-qPCR
●Gene microarray analysis
●RNA interference with siRNA or shRNA
●Over-expression of genes with transient transfection in mammalian cell
●Generation of inducible expression Oli-neu cell lines with sgRNA and

CRISPR/Cas9 system and transposon system (Regulating elements are integrated into the geneome of cells, and then they are induced to express and target the genes of interest).
●Generation of stable expression cell lines with lentivirus vector

In vivo experiments

●Completion of the IACUC Responsible Care and Use of Laboratory Animals Certification Program training (2016, 2019, 2022)
●Mouse breeding, genotyping, and tissue harvesting
●Immunoprecipitation, western blot, RT-qPCR with mouse tissue

Other skills

●Maintenance, calibration or minor repair of experimental apparatus, including CO_2 incubator, microscope, electrophoresis apparatus, refrigerator, air-conditioner, balance, pipette

●Leadership Experience: having directed small teams of undergraduate students and graduate students doing life sciences research.

Publication list

Posted at google scholar, 1137 citations as of April 5, 2023, link
http://scholar.google.com/citations?user=l6C2U5kAAAAJ&hl=en&oi=ao

C Fan, D Kim, H An, Y Park. Identifying an oligodendrocyte enhancer that regulates Olig2 expression. Human Molecular Genetics, 2023, 32(5):835-846

C Fan, H An, M Sharif, D Kim, Y Park. Functional mechanisms of MYRF DNA-binding domain mutations implicated in birth defects. Journal of Biological Chemistry, 2021, 296: 100612.

D Kim, H An, **C Fan**, Y Park. Identifying oligodendrocyte enhancers governing Plp1 expression. Human Molecular Genetics, 2021, 30 (23), 2225-2239

H An, **C Fan**, M Sharif, D Kim, Y Poitelon, Y Park. Functional mechanism and pathogenic potential of MYRF ICA domain mutations implicated in birth defects. Scientific Reports, 2020, 10 (1), 1-10

D Kim, H An, RS Shearer, M Sharif, **C Fan**, J Choi, S Ryu, Y Park. A principled strategy for mapping enhancers to genes. Scientific reports, 2019, 9 (1), 1-141

J Choi*, **C Fan***, D Kim, M Sharif, H An, Y Park (***co-first author**) Elucidating the

transactivation domain of the pleiotropic transcription factor Myrf. Scientific reports, 2018, 8 (1), 13075

X Ling, W Wu, **C Fan**, C Xu, J Liao, LJ Rich, RY Huang, EA Repasky, ...An ABCG2 non-substrate anticancer agent FL118 targets drug-resistant cancer stem-like cells and overcomes treatment resistance of human pancreatic cancer. 2018, Journal of Experimental & Clinical Cancer Research, 37 (1), 240

Dongkyeong Kim, Jin-ok Choi, **Chuandong Fan**, Randall S. Shearer, Mohamed Sharif, Patrick Busch and Yungki Park. Homo-trimerization is essential for the transcription factor function of Myrf for oligodendrocyte differentiation. Nucleic Acids Res. 2017, 45(9):5112-5125.

Chuandong Fan, Xinjiang Wang. Mdm2 Splice isoforms regulate the p53/Mdm2/Mdm4 regulatory circuit via RING domain-mediated ubiquitination of p53 and Mdm4. Cell Cycle, 2017, 16(7):660-664.

Xin-Peng Chen, **Chuan-Dong Fan**, Le Su, Bao-Xiang Zhao and Jun-Ying Miao. A synthesized butyrolactone derivative in combination with chloroquine can inhibit cancer cell growth and lysosome vacuolation induced by chloroquine in A549 lung cancer cells. RSC Adv., 2016, 6, 54099

W Wu, C Xu, X Ling, **C Fan**, BP Buckley, MV Chernov, L Ellis, F Li, IG Muñoz and X Wang. Targeting RING domains of Mdm2–MdmX E3 complex activates apoptotic arm of the p53 pathway in leukemia/lymphoma cells. Cell Death and Disease (2015) 6, e2035

Chao Xu, **Chuan-Dong Fan** and Xinjiang Wang. Regulation of Mdm2 protein stability and the p53 response by NEDD4-1 E3 ligase. Oncogene, 2015, 34(3):281-9.

Xiang Ling, Chao Xu, **Chuandong Fan**, Kai Zhong, Fengzhi Li, Xinjiang Wang. FL118 Induces p53-Dependent Senescence in Colorectal Cancer Cells by Promoting Degradation of MdmX. Cancer research, 2014, 74 (24), 7487-7497.

F Song, **C Fan**, X Wang, DW Goodrich. The Thoc1 Encoded Ribonucleoprotein Is a Substrate for the NEDD4-1 E3 Ubiquitin Protein Ligase. **PloS one** 2013, 8 (2), e57995.

Fan CD, Lum MA, Xu C, Black JD, Wang X. Ubiquitin-dependent regulation of phospho-AKT dynamics by the ubiquitin E3 ligase, NEDD4-1, in the IGF-1 response. **J Biol Chem**. 2013, 288(3):1674–1684. (**My representative publication**)

ChuanDong Fan, Hua Su, Jing Zhao, BaoXiang Zhao, ShangLi Zhang, JunYing Miao. A novel copper complex of salicylaldehyde pyrazole hydrazone induces

apoptosis through up-regulating integrin beta4 in H322 lung carcinoma cells. **Eur J Med Chem**. 2010, 45(4):1438-46.

Bo Meng, et al. Suvivor stories biological laboratory - incidents notes for safety (In Chinese), 2010, Science press, Beijing, China. (I am one of the authors of chapter 3 in this book)

Chuandong Fan, Jing Zhao, Baoxiang Zhao, Shangli Zhang and Junying Miao. Novel Complex of Copper and a Salicylaldehyde Pyrazole Hydrazone Derivative Induces Apoptosis through Up-Regulating Integrin β4 in Vascular Endothelial Cells. **Chemical Research in Toxicology**. 2009, 22:1517-1525.

Chuan-Dong Fan, Bao-Xiang Zhao, Fang Wei, Gai-Hua Zhang, Wen-Liang Dong and Jun-Ying Miao. Synthesis and discovery of autophagy inducers for A549 and H460 lung cancer cells, novel 1-(20-hydroxy-30-aroxypropyl)-3-aryl-1H-pyrazole-5-carbohydrazide derivatives. **Bioorganic & Medicinal Chemistry Letters**. 2008 Jul 15;18(14):3860-4.

Jin-Hua Zhang, **Chuan-Dong Fan**, Bao-Xiang Zhao, Dong-Soo Shin, Wen-Liang Dong, Yong-Sheng Xie and Jun-Ying Miao. Synthesis and preliminary biological evaluation of novel pyrazolo[1,5-a]pyrazin-4(5H)-one derivatives as potential agents against A549 lung cancer cells. **Bioorganic & Medicinal Chemistry**. 2008 Nov 5; 16(24):10165-71

Yong Xia, **Chuan-Dong Fan**, Bao-Xiang Zhao, Jing Zhao, Dong-Soo Shin, and Jun-Ying Miao. Synthesis and structure-activity relationships of novel 1-arylmethyl-3-aryl-1H-pyrazole-5-carbohydrazide hydrazone derivatives as potential agents against A549 lung cancer cells. **European Journal of Medicinal Chemistry**. 2008 Nov; 43(11):2347-53.

Chuandong Fan， Weiwei Wang，Baoxiang Zhao， Shangli Zhang，Junying Miao. Chloroquine inhibits cell growth and induces cell death in A549 lung cancer cells. **Bioorgnic & Medicinal Chemistry**. 2006, May 1; 14(9):3218-3222.

L Sha, B Zhao, **C Fan**, W Tan, X Li, J Miao. Synthesis and primary evaluation of lung cancer cell growth inhibitory activity of novel 3-arylmethyl-5-aroxymethylbutyrolactones. **CHINESE JOURNAL OF ORGANIC CHEMISTRY**. 2006, 26 (4), 537

Peer review works

I am an active peer reviewer of these journals
BMC Cancer
OncoTargets and Therapy.

Theranostics
PLOS ONE
BMC Molecular and Cell Biology
Current Biomarker Findings
International Journal of Biological Sciences