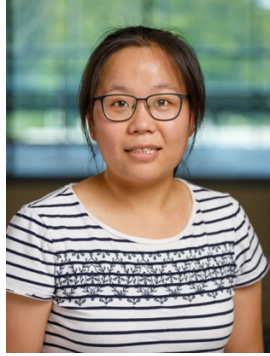




Doctoral Dissertation Defense Announcement

“GATA2-TGF- β 1-TAL1 axis is essential for human NK cell development”



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Committee in Charge:

Subramaniam Malarkannan, PhD (Mentor)

Joseph T. Barbieri, PhD

Scott Terhune, PhD

Sridhar Rao, MD, PhD

Wane Yokoyama, MD (External, WashU)

Date: Wednesday, Sep 4, 2024

Time: 9:00 AM (CST)

Defense Location: VBRI seminar room

Zoom: contact danwang@mcw.edu for zoom link.

Graduate Studies:

IDP program 1st year curriculum

Cellular & Molecular Immunology

Immunology Journal Club

Introduction to Biostatistics

Tumor Immunology

Immunology Seminar Course

Career development (Summer class)

Reading and Research

Ethics and Integrity in Science

Research Ethics Discussion Series

Doctoral Dissertation

Dissertation

“GATA2-TAL1-TGF- β 1 axis is essential for human NK cell development”

Natural killer (NK) cells are the major innate cytotoxic lymphocytes that mediate rapid and robust effector responses against tumors and pathogens without prior sensitization. However, the transcriptional mechanisms involved in human NK cell development and functions are yet to be fully defined. The transcription factor (TF) GATA2 is essential for regulating the survival, proliferation, and differentiation of hematopoietic stem cells (HSCs) and NK cell development. GATA2 forms a chromatin-bound heptad complex with other TFs (TAL1, FLI1, RUNX1, LYL1, LMO2, and ERG), to regulate hundreds of target genes in HSCs, which are lineage and stage-specific. Heterozygous GATA2 pathogenic variants cause monocytopenia with atypical mycobacterial infection; dendritic cells, monocytes, B and NK lymphocyte deficiency (DCML), familial myelodysplastic syndrome/acute myeloid leukemia (MDS/AML); and Emberger syndrome (consists of MDS, lymphedema, and warts from human papillomavirus infection). GATA2 mutation results in the reduction of CD56^{Bright} NK cells with/without reduced CD56^{Dim} NK cells. Variants, including T354M in the second DNA-binding zinc finger domain, impair human NK cell development and function.

TAL1, a class II basic helix-loop-helix TF, plays an essential role in early hematopoiesis along with GATA2. Germline deletion of *Tal1* in mice leads to failure to develop HSCs. TAL1 forms an obligate heterodimer with E proteins, including E2A and HEB. TAL1 is silenced before the CD4-CD8⁻ T cell progenitors commit to the double-positive stage, where HEB or E2A support T cell commitment. ID2, an essential TF for NK cells, blocks HEB DNA binding, blocks transcription of T cell-associated genes, and promotes NK cell commitment. Id2 disrupts assembly of an E2a-TAL1 transcriptional complex and modulates chromatin accessibility of select promoter regions in mice, thus implicating an essential role for TAL1 in NK cell development. Irrespective of these findings, the role of TAL1 or GATA2-TAL1 complex in NK cell development and function is unknown.

TGF- β 1 regulates HSC quiescence and self-renewal and homeostasis of the immune system, especially within the BM niche. Importantly, human primary NK cells produce significant amounts of TGF- β 1 compared to other lymphocytes. TGF- β 1 stimulates the proliferation of myeloid-biased HSCs but inhibits the growth of lymphoid-biased HSCs. TGF- β 1 sustains the stemness of CD34⁺ HSCs by pausing the commitment into NKPs. TGF- β 1 also functions as a checkpoint to maintain NK cell immaturity at the CD56^{Bright} stage. However, it is unknown how TGF- β 1 transcriptionally regulates human NK cell development and whether there are any links between GATA2 and TGF- β 1.

In this study, we identified a GATA2-TAL1-TGF- β 1 axis that is essential for human NK cell development and maturation. We found that primary human NK cells express high levels of TAL1, and TAL1 and its target genes were significantly

upregulated in NK cells from patients with *GATA2* pathogenic variants and *GATA2* variant-expressing cells. *GATA2* repressed *TAL1* transcription, whereas *GATA2*^{T354M} failed to repress *TAL1*. In contrast, *GATA2*^{T354M} substantially reduced the expression of *TGFB1* and known TGF- β 1 target genes in human NK cells. Mechanistically, we identified two GATA motifs in the proximal promoter region of the *TGFB1* gene. *In vitro*, luciferase reporter assay demonstrated *GATA2* regulation of the *TGFB1* promoter. ATAC-seq and CUT&Tag assays using human primary NK cells revealed *GATA2* occupied the *TGFB1* promoter, and occupancy was associated with H3K4me3, an active promoter mark. Thus, *GATA2*-mediated regulation of *TGFB1* at an early commitment stage permits an orderly and successful transition of early NK cell developmental stages. TGF- β 1 stimulated *TAL1* degradation, suggesting that a decrease in TGF- β 1 with a concomitant increase in *TAL1* formed the mechanistic basis for defective NK cell development in patients with the *GATA2*^{T354M} variant. The interplay between *GATA2*, TGF- β 1, and *TAL1* provides an important missing link required for early human NK cell development.

Dandan Wang
Curriculum Vitae
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Education

Medical College of Wisconsin Wisconsin, WI, US	Ph. D. in Immunology	08/2018-current
Shanxi Medical University Shanxi, Taiyuan, China	MBBS. & MMed. in Internal Medicine	09/2009-07/2016

Research Experience

Graduate Student Research Assistant Subramaniam Malarkannan lab, Medical College of Wisconsin	2018-current
Visiting Research Trainee Margret Wong-Riley lab, Medical College of Wisconsin	2017-2018
Master Student in Clinical Research Jing Yang lab, Shanxi Medical University	2014-2016
Medical Student Research volunteer Jin-shun Qi lab, Shanxi Medical University	2011-2016

Awards and Honors

2023	American Society of Hematology (ASH) Abstract Achievement Award
2023	Versiti-BRI Education Committee Award
2023	Versiti-BRI Immunology Program Trainee Award
2023	MCW Grad Student Association Symposium Oral Presentation Award
2023	DOM Heath Foundation Travel Award
2023	MCW Graduate Student Travel Award
2023	Veristi-BRI Immunology Program Trainee Award
2022	MCW Center for Immunology Fall Travel Award, Medical College of Wisconsin
2022	American Society of Hematology (ASH) Abstract Achievement Award
2022	BizTimes Notable Women in Healthcare
2022	American Society of Hematology (ASH) Abstract Achievement Award
2015	Shanxi Province Academic Fellowship for Postgraduates, China
2010	National Endeavor Fellowship (Top 5%), China
2009-2016	Shanxi Medical University Scholarships, China

Grant

2022-2024	Predocorial fellowship from American Society of Hematology (ASH) Graduate Hematology Award (\$80,000)
2022	MCW Center for Immunology, Growth and Research in Immunology Training (GRIT) Award (\$15,000)

EDITORIAL BOARDS/JOURNAL REVIEWS:

Peer Review for Journals: EMBO, Frontiers in Immunology, Journal of Scientific Report, Frontiers in Tumor Immunology

Junior editor: *Critical Rev Immunology*

Publications:

Research articles

1. GATA2-TAL1-TGF- β 1 axis is essential for human NK cell development (2024). **Dandan Wang**, Fangfei Zhang, Chao Yang, Anjali George, Anthony J. Veltri, Monica S. Thakar, James W. Verbsky, Kasiani C. Myers, Emery H. Bresnick, Barbara L. Kee, Sridhar Rao, and Subramaniam Malarkannan. *Blood* (2024, Under Review).
2. Genetic and Immunological Predispositions to Bispecific CAR T cell Therapy. Tyce Kearn*, **Dandan Wang***, Robert Burns, Bryon Johnson, Ao Mei, Sridhar Rao, Dina Schneider, Boro Dropulic, John M. Astle, Parameswaran Hari, Nirav N. Shah, Subramaniam Malarkannan. *Cellular & Molecular Immunology* (2024, Submitted). *Co-first author.
3. Transcriptomic-Based Microenvironment Classification Reveals Precision Medicine Strategies for PDAC (2024). George B, Kudryashova O, Kravets A, Thalji S, Malarkannan S, Kurzrock R, Chernyavskaya E, Gusakova M, Kravchenko D, Tychinin D, Savin E, Alekseeva L, Butusova A, Bagaev A, Shin N, Brown JH, Sethi I, **Wang D**, Taylor B, McFall T, Kamgar M, Hall WA, Erickson B, Christians KK, Evans DB, Tsai S. *Gastroenterology* (2024 May;166(5):859-871.e3. doi: 10.1053/j.gastro.2024.01.028. Epub ahead of print. PMID: 38280684).
4. CD36 Restricts Lipid-Associated Macrophages Accumulation in White Adipose Tissues During Atherogenesis (2024). Jue Zhang, Jackie Chang, Vaya Chen, Mirza Beg, Lance Vick, Dandan Wang, Ankan Gupta, Yaxin Wang, Ziyu Zhang, Wen Dai, Mindy Kim, Shan Song, Duane Pereira, Ze Zheng, Komal Sodhi, Joseph I Shapiro, Roy L Silverstein, Subramaniam Malarkannan, and Yiliang Chen. *Front. Cardiovasc. Med.* (11 - 2024 | doi: 10.3389/fcvm.2024.1436865)
5. MyD88 is an essential regulator of Ly49H-mediated signaling and proliferation in NK cells (2021). Dixon KJ, Siebert JR, **Wang D**, Abel AM, Johnson KE, Riese MJ, Terhune SS, Tarakanova VL, Thakar MS, and Malarkannan S. *Molecular Immunology* (2021 Sep;137:94-104. doi: 10.1016/j.molimm.2021.07.001. Epub 2021 Jul 6. PMID: 34242922).
6. Sarcoma IL-12 overexpression facilitates NK cell immunomodulation (2021). Rademacher MJ, Cruz A, Faber M, Oldham RAA, **Wang D**, Medin JA, Schloemer NJ. *Sci Rep.* (2021 Apr 15;11(1):8321. doi: 10.1038/s41598-021-87700-2. PMID: 33859303).

Research articles in preparation

1. Human primary NK cell mRNA delivering by lipid nanoparticle for future cell therapy potential. **Dandan Wang***, Katherine Badior*, Subramaniam Malarkannan#, Christian Kastrup# (In preparation).
2. Context-dependent transcriptomic alterations dictate the outcome of hematopoiesis in GATA2-ZF1 or ZF2 mutated patients. **Dandan Wang**, Kasiani Myers, and Subramaniam Malarkannan (In preparation).
3. Single cell transcriptome reveals the age-dependent NK cell ontology. **Dandan Wang**, James Verbsky, and Subramaniam Malarkannan (In preparation).
4. SBRT facilitates immune promoting TME in PDAC patients. **Dandan Wang**, Susan Tsai, Matthew Riese, William Hall, and Subramaniam Malarkannan (In preparation).

Review articles

1. Transcriptional regulation of NK cell development and function (2020). **Wang D** and Malarkannan S. *Cancers* (2022 May 25;14(1):57. doi: 10.1186/s13073-022-01059-1. PMID: 35610660).
2. Role of GATA2 in Human NK Cell Development. (2021). **Wang D**, Hashemi E, Thakar MS, and Malarkannan S. *Critical Reviews in Immunology* (2021;41(2):21-33. doi: 0.1615/CritRevImmunol.2021037643. PMID: 34348000).

3. Implications of a 'third signal' in NK cells (2021). Khalil M, **Wang D**, Hashemi E, Terhune SS, Malarkannan S. *Cells* (2021 Jul 31;10(8):1955. doi: 10.3390/cells10081955. PMID: 34440725).
4. Developmental and functional defects of NK cells in Fanconi Anemia patients (2021). Hashemi E, **Wang D**, Thakar MS, and Malarkannan S. *Critical Reviews in Immunology* (2021;41(2):35-44. doi: 10.1615/CritRevImmunol.2021037644. PMID: 34348001).

Methodology articles

1. Methods to analyze the developmental stages of Murine and Human Primary NK cells using Monocle and SCENIC analyses. (2022) **Wang D**, Khalil M, Mei A, Hashemi E, Malarkannan S. *Methods Mol Biol.* (2022;2463:81-102. doi: 10.1007/978-1-0716-2160-8_7. PMID: 35344169).
2. Methods to infect and define the single-cell transcriptomes of MCMV-specific murine NK cells. (2022) Khalil M, **Wang D**, Mei A, Hashemi E, Terhune S, Malarkannan S. *Methods Mol Biol.* (2022;2463:195-204. doi: 10.1007/978-1-0716-2160-8_14. PMID: 35344176).
3. Methods for isolating and defining single-cell of tissue-resident NK cells. (2022) Hashemi E, Khalil M, Mei A, **Wang D**, Malarkannan S. *Methods Mol Biol.* (2022;2463:103-116. doi: 10.1007/978-1-0716-2160-8_8. PMID: 35344170).
4. Isolation of Innate Lymphoid Cells from Murine Intestinal Lamina Propria. (2022) Mei A, Hashemi E, Khalil M, **Wang D**, Malarkannan S. *Methods Mol Biol.* (2022;2463:3-9. doi: 10.1007/978-1-0716-2160-8_1. PMID: 35344163).

Research articles prior to Ph.D. training

1. Uncovering a critical period of synaptic imbalance during postnatal development of the rat visual cortex: role of brain-derived neurotrophic factor. Zhang, H*. Mu, L*. **Wang, D***. Xia, D*. Salmon, A.; Liu, Q. Wong-Riley, M.T.T. *J Physiol.* (2018 Sep;596(18):4511-4536. doi: 10.1113/JP275814. PMID: 30055019). *Co-first author.
2. Desipramine improves depression-like behavior and working memory by up-regulating p-creb in alzheimer's disease associated mice. (2016) **Wang D**, Li Jia, Yu Li-Peng, Wu Mei-Na, Sun Li-Na, Qi Jin-Shun. *J Integr Neurosci.* (2016, 1-14, doi: 10.1142/S021963521650014X, PMID: 27338163)
3. Neck Circumference-a new predicting measurement of BMC, (2016) **Wang D**, Liu Yun-Feng, Lan Li-Zhen, Xu Lin-Xin, Yang Lu-Yang, Yang-Jing, *Chin J Osteoporosis & Bone Miner Res.* (2017, 9-25, doi: 10.3969/j.issn.1674-2591)
4. High-intensity treadmill running impairs cognitive behavior and hippocampal synaptic plasticity of rats via activation of inflammatory response (2017). Sun LN, Li XL, Wang F, Zhang J, **Wang D**, Yuan L, Wu MN, Wang ZJ, Qi JS. *J Neurosci Res.* (2017 Aug;95(8):1611-1620. doi: 10.1002/jnr.23996. Epub 2016 Dec 5. PMID: 27918079).

Presentations in Regional, National, and International forums

Dec. 2023: The 65th American Society of Hematology Annual Meeting & Exposition, San Diego, California

Oral presentation: A novel GATA2-TAL1 axis is essential for human NK cell development and function.

Apr 2023: The 6th Annual GSA Symposium, Milwaukee, Wisconsin

Oral presentation: A novel GATA2-TGF- β 1 axis is essential for human NK cell development.

Mar 2024: 2024 MCW Cancer Center Trainee Symposium, Milwaukee, Wisconsin

Poster presentation: A novel GATA2-TGF- β 1-TAL1 axis is essential for human NK cell development and function.

Apr. 2024: Versiti-BRI scientific retreat, Milwaukee, Wisconsin

Poster presentation: A novel GATA2-TGF- β 1-TAL1 axis is essential for human NK cell development and function.

Sep 2023: Versiti-BRI SAB poster event, Milwaukee, Wisconsin

Poster presentation: A novel GATA2-TGF- β 1-TAL1 axis is essential for human NK cell development and function.

May 2023: The 106th Annual Meeting of American Association of Immunologists

Poster Presentation: GATA2-TGF- β 1 axis regulates human NK cell development

Dec. 2022: The 64th American Society of Hematology Annual Meeting & Exposition, New Orleans, Louisiana, US.

Poster presentation: A novel GATA2-TGF- β 1 axis is essential for human NK cell development.

Sep. 2016: The 17th International Congress of Endocrinology and the 15th annual meeting of Chinese society of Endocrinology, Beijing, China.

Poster presentation: Neck Circumference - a new predicting measurement of BMC.

Oct. 2014: The 24th National Congress and Physiology Academic Conference, Chinese Physiological Society, Shanghai, China.

Poster presentation: Antidepressant Desipramine Prevents Ab-Induced Cognitive Deficit, Mental Depression and p-CREB Reduction in Mice.