



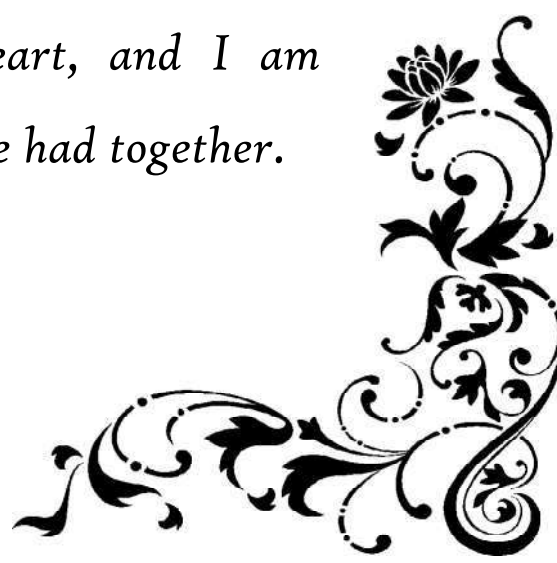
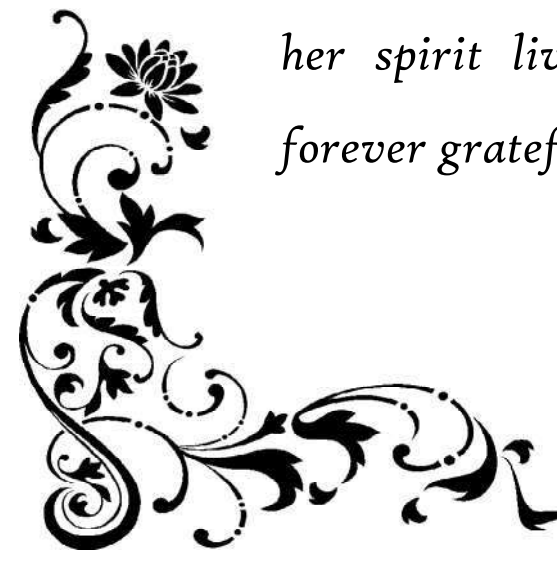
Dedication

I dedicate this thesis to;

✦ *My Parents, my pillars of strength, who have always believed in me and pushed me to pursue my dreams. Without their love, patience, and sacrifices, I wouldn't be where I am today.*

✦ *My Brothers, who have cheered me on every step of the way, I am thankful for their love and words of encouragement.*

✦ *My Grandmother, who is my angel up above, her spirit lives on in my heart, and I am forever grateful for the time we had together.*



SCIENTIFIC PUBLICATIONS FROM THE THESIS

Hemaya, D., & Baruah, S. Folate and Succinate Induce Modulation on NK92 Cell Activation Status in Tumor Microenvironment Mimetic Co-Culture Setting.
Submitted (under review).

SCIENTIFIC PRESENTATIONS FROM THE THESIS

1. **Hemaya, D** and Baruah, S, Investigation into the role of folate and succinate in regulation of mTOR pathway-related genes and functional activation of NK cells, *National Seminar on Biological Sciences: A North East India Perspective*, Department of Biotechnology and Bioinformatics, North-Eastern Hill University, Shillong-793022, Meghalaya, India. 28th February-1st March, 2023. (Oral presentation)

2. **Hemaya, D** and Baruah, S, Folate availability effect on the NK-92 cell activation status, *48th Annual Conference of the Indian Immunology Society (Immunocon 2022)*, Department of Molecular and Human Genetics, Banaras Hindu University, Varanasi, India 8th – 9th July, 2022 (Virtual). (Oral presentation)

3. **Hemaya, D** and Baruah, S, The effect of folate on NK92 cell activation status, *National Seminar on Advances in Basic and Translational Research in Biology (ABTRiB)*, Silver Jubilee Year Celebration (1997-2022), Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur-784028, Napaam, India 11th – 12th March, 2022. (Oral presentation)

DECLARATION

I hereby declare that the thesis entitled “**Study the Effect of Folate and Succinate on Immunometabolism Profiles of NK92 cell line in a Tumor Microenvironment (TME) Mimetic Culture System.**” submitted to the School of Sciences, Tezpur University, is an authentic work carried out by me under the supervision of Prof. Shashi Baruah, at the Department of Molecular Biology and Biotechnology (MBBT), Tezpur University, Assam-784028, India. This work is original and has not been submitted earlier in part or full in any other university or institute for any other degree, diploma or any other similar title or recognition.



Place: Tezpur University
Date: 24.04.2024

{ **Ms. Dalal Hemaya** }

Dept. of MBBT
School of Sciences
Tezpur University
Napaam, 784028,
Assam, India.



TEZPUR UNIVERSITY

(A Central University by an Act of Parliament)

Napaam, Tezpur-784028

District: Sonitpur, Assam, India

CERTIFICATE OF SUPERVISOR

This is to certify that the thesis entitled “**Study the Effect of Folate and Succinate on Immunometabolism Profiles of NK92 cell line in a Tumor Microenvironment (TME) Mimetic Culture System.**” submitted to the School of Sciences, Tezpur University in requirement of partial fulfilment for the award of the degree of Doctor of Philosophy in Molecular Biology and Biotechnology is a record of research work carried out by **Ms. Dalal Hemaya** under my supervision and guidance.

All help received by her from various sources has been duly acknowledged. No part of this thesis has been submitted elsewhere for award of any other degree.

(Signature of Supervisor)

Date: 24.04.2024

Prof. Shashi Baruah



TEZPUR UNIVERSITY

(A Central University by an Act of Parliament)

Napaam, Tezpur-784028

District: Sonitpur, Assam, India

CERTIFICATE OF THE EXTERNAL EXAMINER AND ODEC

This is to certify that the thesis entitled “**Study the Effect of Folate and Succinate on Immunometabolism Profiles of NK92 cell line in a Tumor Microenvironment (TME) Mimetic Culture System**” submitted by **Ms. Dalal Hemaya** in the Department of Molecular Biology and Biotechnology (MBBT) under the School of Sciences, Tezpur University in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy in Molecular Biology and Biotechnology has been examined by us and found to be satisfactory.

The Oral Defence Evaluation Committee (ODEC) recommends the award of the degree of Doctor of Philosophy in Molecular Biology and Biotechnology.

Signature of:

Principle supervisor

Date:

External examiner

Date:

* Acknowledgements *

First and foremost, all praises and heartfelt gratitude to my Lord, *the Almighty Allah*, who has always blessed me with His guidance and divine intervention throughout my life journey and academic pursuits. His mighty strength has been the driving force behind my achievements, and I would not have been able to reach this milestone without His blessings.

Therewith, my heart is filled with gratitude for the mercy of all mankind, *Prophet Mohammad*, peace and blessings of Allah be upon him, who is my mentor throughout my life.

I would also like to extend my gratitude and deepest appreciation to my first and forever divine blessings, *my Dearest Parents*. Since the day I opened my eyes to this world, they have been my constant source of unconditional love, unwavering support, and endless inspiration and motivation. Their faith in me and their prayers have given me the strength and determination to overcome all obstacles and pursue my endeavours. Additionally, I am thankful to *my amazing Brothers*, who have been cheering me on every step of the way and filling my life with their love and words of encouragement.

I am deeply grateful to *Prof. Ghalia Abou-Alchamat*, my local supervisor at Damascus University, Syria, who has already provided me with a solid foundation of knowledge and skills that have been instrumental in my current academic program. I am immensely thankful for her support and belief in my potential, which has been a source of inspiration and motivation throughout the dissertation project. I would also like to acknowledge and thank my parent university, *Damascus University*, and *the Ministry of Higher Education and Scientific Research in Syria*, for giving me the opportunity to pursue my PhD studies in India. Their support has been indispensable in nurturing my intellectual growth and expanding my knowledge and skills.

Furthermore, I would like to thank my supervisor at Tezpur University, **Prof. Shashi Baruah**, for guiding this dissertation, and the members of my doctoral committee, Dr. Suman Dasgupta, Dr. Robin Doley, and Dr. Suvendru Kumar Ray, at Tezpur University, for sharing their expertise and research advice during committee meetings.

I would like to express my appreciation to the **Vice-Chancellor of Tezpur University** for their financial support, which was crucial in enabling me to continue my PhD lab work.

I extend my thanks to the **Indian Council for Cultural Relations (ICCR)** for granting me their generous scholarship during my PhD studies in India.

I would also like to express my gratitude to **Prof. Prasanta Kumar Das**, the director of the International Students Office at Tezpur University, for his significant help and cooperation whenever I needed it.

Last but not least, I would like to acknowledge my lab mates; Ms. **Mayuri Bora**, Ms. **Bhaswattee Das**, Mr. **Gurumayum Chourajit Sharma** and Ms. **Sushmita Singha** for their kind encouragement and invaluable assistance, and for providing such a comfortable and enjoyable environment in the lab over the last five years.

Lastly, I would like to express my gratitude to all of Allah's creatures, including animals and plants, which have been a divine source of comfort and peace of mind during difficult times. They have shared my sorrows and happy moments, and I appreciate Allah's blessings for allowing me to find solace in His meaningful creations.

Again and always, I want to thank The Almighty Allah for allowing me to be a part of discovering His creation. My studies in biology have only strengthened my faith, and I am grateful for the opportunity to explore the wonders of His creation.

Dalal Hemaya
ダラル

November, 2023

LIST OF FIGURES

Chapter II	Review of Literature	Page No.
Figure 1	Scheme explains the major metabolic pathways characterized in immune cells.	9
Figure 2	Distinctive Metabolic Features of Immune Cell Subtypes.	19
Figure 3	mTOR signaling pathway in innate immune cells.	23
Figure 4	The Metabolic Signaling Pathway in Cancer Cells.	27
Figure 5	Folate-Mediated One-Carbon Metabolism (FOCM).	30
Figure 6	Immunometabolic Regulation of NK Cells.	43 - 44
<hr/>		
Chapter III	Material and Methods	
Figure 7	Representative flow plots showed the employed gating strategy.	51
<hr/>		
Chapter IV	Results	
Figure 8	Morphological Changes in NK92 Cell Culture under Different Folate Treatments after 72hrs.	59
Figure 9	Representative flow cytometry plots showed the effect of folate treatments on the viability of NK92 cells in mono-culture after 72hrs.	60
Figure 10	Symbol graph represents the difference in mono-cultured NK92 cell viability between the selected folate treatments after 72hrs.	61
Figure 11	Column graphs representing the effect of folate treatments on the expression levels (represented by MFI) of A). CD56 and B). IFN γ in mono-cultured NK92 cells after 24 hrs, 48 hrs and 72 hrs.	63
Figure 12	Violin plots depicting the effect of folate treatments on the percentage (%) of positive cells expressing A). CD56 and B). IFN γ , in mono-cultured NK92 cells after 24 hrs, 48 hrs and 72hrs.	64
Figure 13	Box plots showing the effect of folate treatments after 72 hrs on the relative gene expression of NK cell function-related genes including <i>IFNγ</i> , <i>GZMB</i> and <i>PRF1</i> .	65
Figure 14	Box plots showing the effect of folate treatments after 72 hrs on the relative gene expression of folate metabolism-related genes including <i>RFC</i> , <i>DHFR</i> and <i>MTHFR</i> .	66

Figure 15	Column graphs represent the effect of folate treatments on the expression levels (represented by MFI) of HIF1 α in mono-cultured NK92 cells for 24 hrs, 48 hrs and 72 hrs.	68
Figure 16	Violin plots represent the effect of folate treatments on the percentage (%) of positive cells expressing HIF1 α in mono-cultured NK92 cells for 24 hrs, 48 hrs and 72 hrs.	69
Figure 17	Box plots showing the effect of folate treatments after 72 hrs on the relative gene expression of mTOR pathway-related genes including <i>mTOR</i> , <i>Raptor</i> , <i>Rictor</i> , <i>HIF1α</i> , <i>AMPKα1</i> and <i>SREBP1</i> .	70
Figure 18	Box plots showing the effect of folate treatments after 72 hrs on the relative gene expression of mTOR pathway-related genes including <i>MYC</i> , <i>RPS6KB1</i> , <i>RPS6KB2</i> , <i>ATF3</i> and <i>ATF4</i> .	71
Figure 19	Bar plots showing the effect of folate treatments on the protein levels (represented by the arbitrary units; AU) of SREBP1, and the phosphorylated proteins including p-P70S6K-Thr389, p-Raptor-Ser863, and p-Raptor-Ser792 in mono-cultured NK92 cells after 72hrs.	72
Figure 20	Symbol graph depicting the effect of succinate treatments on the viability of mono-cultured NK92 cells after 72hrs	73
Figure 21	Column graphs showing the effect of succinate treatments on the expression levels (represented by MFI) of A). CD56 and B). IFN γ in mono-cultured NK92 cells after 24 hrs, 48 hrs and 72 hrs.	75
Figure 22	Violin plots depicting the effect of succinate treatments on the percentage (%) of positive cells expressing A). CD56 and B). IFN γ , in mono-cultured NK92 cells after 24 hrs, 48 hrs and 72hrs.	76
Figure 23	Box plots showing the effect of succinate treatments after 72 hrs on the relative gene expression of NK cell function-related genes including <i>IFNγ</i> , <i>GZMB</i> and <i>PRF1</i> .	77
Figure 24	Box plots showing the effect of succinate treatments after 72 hrs on the relative gene expression of folate metabolism-related genes including <i>RFC</i> , <i>DHFR</i> and <i>MTHFR</i>	78
Figure 25	Column graphs represent the effect of succinate treatments on the expression levels (represented by MFI) of HIF1 α in mono-cultured NK92 cells for 24 hrs, 48 hrs and 72 hrs.	80

Figure 26	Violin plots represent the effect of succinate treatments on the percentage (%) of positive cells expressing HIF1 α in mono-cultured NK92 cells for 24 hrs, 48 hrs and 72hrs.	81
Figure 27	Box plots showing the effect of succinate treatments after 72 hrs on the relative gene expression of mTOR pathway-related genes including <i>mTOR</i> , <i>Raptor</i> , <i>Rictor</i> , <i>HIF1α</i> , <i>AMPKα1</i> and <i>SREBP1</i> .	82
Figure 28	Box plots showing the effect of succinate treatments after 72 hrs on the relative gene expression of mTOR pathway-related genes including <i>MYC</i> , <i>RPS6KB1</i> , <i>RPS6KB2</i> , <i>ATF3</i> and <i>ATF4</i> .	83
Figure 29	Bar plots showing the effect of succinate treatments on the protein levels (represented by the arbitrary units; AU) of SREBP1, and the phosphorylated proteins including p-P70S6K-Thr389, p-Raptor-Ser863, and p-Raptor-Ser792 in mono-cultured NK92 cells after 72hrs.	84
Figure 30	Representative Western Blot analysis of SREBP1 and the phosphorylated proteins in the mTOR pathway	85
Figure 31	Symbol graph showing the effect of folate treatments on the viability of co-cultured NK92 cells with MDA-MB-231 after 72hrs.	86
Figure 32	The effect of folate treatments on the activation status of co-cultured NK92 cells after 72 hrs.	88
Figure 33	The effect of folate treatments on HIF1 α in the co-cultured NK92 cells with MDAMB231 breast cancer cells after 72hrs.	89
Figure 34	Column graphs representing the effect of folate treatments on the production of cytokines in the co-cultured system of NK92 cells with MDAMB231 cancer cells.	91
Figure 35	Column graphs representing the effect of folate treatments on the production of chemokines in the co-cultured system of NK92 cells with MDAMB231 cancer cells.	92
Figure 36	Symbol graph showing the effect of succinate treatments on the viability of co-cultured NK92 cells with MDAMB231 after 72hrs.	93
Figure 37	The effect of succinate treatments on the activation status of co-cultured NK92 cells after 72 hrs.	95
Figure 38	The effect of succinate treatments on HIF1 α in the co-cultured NK92 cells with MDAMB231 breast cancer cells after 72hrs.	96

Figure 39	Column graphs representing the effect of succinate treatments on the production of cytokines in the co-cultured system of NK92 cells with MDAMB231 cancer cells.	97
Figure 40	Column graphs representing the effect of succinate treatments on the production of chemokines in the co-cultured system of NK92 cells with MDAMB231 cancer cells.	98
Figure 41	Symbol graph showing the effect of supplementation with 50 μ M succinate in different folate treatments on the viability of co-cultured NK92 cells with MDAMB231 after 72hrs.	99
Figure 42	The effect of folate treatments in the presence of 50 μ M succinate on the activation status of co-cultured NK92 cells after 72 hrs.	101
Figure 43	The effect of folate treatments in the presence of 50 μ M succinate HIF1 α in the co-cultured NK92 cells with MDAMB231 breast cancer cells after 72hrs.	102
Figure 44	Column graphs representing the effect of folate treatments in the presence of 50 μ M succinate on the production of cytokines in the co-cultured system of NK92 cells with MDAMB231 cancer cells.	103
Figure 45	Column graphs representing the effect of folate treatments in the presence of 50 μ M succinate on the production of chemokines in the co-cultured system of NK92 cells with MDAMB231 cancer cells.	104
Figure 46	Differences in the effect of folate treatments on the activation status of NK92 cells between mono-culture and co-culture systems after 72 hrs.	106
Figure 47	Differences in the effect of folate treatments on HIF1 α between mono-culture and co-culture systems after 72hrs.	107
Figure 48	Differences in the effect of succinate treatments on the activation status of NK92 cells between mono-culture and co-culture systems after 72 hrs.	109
Figure 49	Differences in the effect of succinate treatments on HIF1 α between mono-culture and co-culture systems after 72hrs.	110
Figure 50	Differences in the effect of folate treatments on the activation status of NK92 cells between mono-culture and co-culture systems (with and without 50 μ M succinate intervention) after 72 hrs.	112
Figure 51	Differences in the effect of folate treatments on HIF1 α between mono-culture and co-culture systems (with and without 50 μ M succinate intervention) after 72 hrs.	113

Figure 52	Column graphs representing the differences in the effect of folate treatments on the production of cytokines between co-culture systems; without and with 50µM succinate intervention.	115
Figure 53	Column graphs representing the differences in the effect of folate treatments on the production of chemokines between co-culture systems without and with 50µM succinate intervention.	116

Chapter VI Summary and Conclusion

Figure 54	Schematic diagram illustrates the methodology used to investigate the potential impacts of folate availability and exogenous succinate supplementation on NK92 cells.	133
Figure 55	A graphical model represents the key findings of Folate availability on NK92 Cells in mono-culture setup after 72hrs.	136
Figure 56	A graphical model represents the findings for the effect of Folate availability on the activation status of NK92 cells and the cytokines /chemokines production in the co-culture system with the breast cancer cell line, MDAMB231.	137
Figure 57	A graphical model represents the findings of Succinate treatments on NK92 cells in mono-culture setup after 72hrs.	138
Figure 58	A graphical model represents the findings of the effect of Succinate treatments on the activation status of NK92 cells and the cytokines /chemokines production in the co-culture system with the breast cancer cell line, MDAMB231.	139
Figure 59	A graphical model represents the key findings for the influence of supplementation with 50µM of Succinate in different Folate availability on the activation status of NK92 cells and the cytokines /chemokines production in the co-culture system with the breast cancer cell line, MDAMB231.	140

LIST OF ABBREVIATIONS

Abbreviations	Full forms
μg	Microgram.
μL	Microlitre.
μM	Micromolar.
ng	Nanogram.
kDa	kilo Dalton.
mM	milli Molar.
hrs	hours.
°C	degree Celsius.
ATCC	American Type Culture Collection.
BSA	Bovine Serum Albumin.
FBS	Fetal Bovine Serum.
mRNA	(messenger) Ribonucleic acid.
RPMI-1640	Rosewell Park Memorial Institute 1640 Medium.
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction.
cDNA	Complementary DNA.
Ct	Threshold cycle.
GAPDH	Glyceraldehyde3-phosphatedehydrogenase.
PBS	Phosphate-Buffered Saline.
MFI	Mean Fluorescence Intensity.
SDS-PAGE	Sodium dodecyl sulphate - polyacrylamide gel electrophoresis.
PVDF	Polyvinylidene difluoride.

TME	Tumor Microenvironment.
NK cells	Natural Killer cells.
FA	Folic acid.
5-MTHFA	5-methyl tetrahydrofolic acid.
IP10	Interferon gamma-induced protein 10.
MCP1	Monocyte chemoattractant protein 1.
MIG	Monokine Induced by Gamma interferon (CXCL9 chemokine).
RANTES	Regulated and normal T cell expressed and secreted.
HIF1 α	HIF1A hypoxia inducible factor-1 subunit alpha.
IFN- γ	Interferon-gamma.
ILs	Interleukins.
CDs	Cluster of Differentiation.
CCLs	C-C Motif Chemokine Ligands.
CCRs	C-C Chemokine Receptors.
CXCLs	(C-X-C motif) Ligands.
CXCRs	CXC Chemokine Receptors.
ATP	Adenosine Triphosphate.
mTOR	mammalian Target of Rapamycin kinase.
TCA	Tricarboxylic acid cycle.
OxPhos	Oxidative Phosphorylation.
