

**Apoptosis regulators as targets for induction of apoptosis of
Imatinib resistant chronic myeloid
leukemia cells**

**Thesis submitted for the partial fulfillment of the requirements
for the degree Doctor of Philosophy in Science**

By

Tamalika Paul

Department of LIFE SCIENCES

Faculty of Natural and Mathematical Sciences

Presidency University

Kolkata, India

Year of Submission:2022

**Apoptosis regulators as targets for induction of apoptosis of
Imatinib resistant chronic myeloid
leukemia cells**

**Thesis submitted for the partial fulfillment of the requirements
for the degree Doctor of Philosophy in Science**

By

Tamalika Paul

Under the Supervision of

Dr. Nabendu Biswas

Department of LIFE SCIENCES

Faculty of Natural and Mathematical Sciences

Presidency University

Kolkata, India

Year of Submission:2022

Thesis Title: Apoptosis regulators as targets for induction of apoptosis of Imatinib resistant chronic myeloid leukemia cells.

Name of Candidate: Tamalika Paul

Registration Number: R-17RS18210108

Date of Registration: 14th August, 2018

Department: Department of LIFE SCIENCES, Presidency University, Kolkata

Tamalika Paul
28/07/2022

Signature of the candidate with date

Declaration

I hereby declare that this thesis contains original research work carried out by me under the guidance of Dr. Nabendu Biswas, Assistant Professor and Department of Life Sciences, Presidency University, Kolkata, India as part of PhD programme.

All information in this document have been obtained and presented in accordance with academic rules and ethical conduct.

I also declare that, as required by these rules and conduct, I have fully cited and referenced all materials and results that are not original to this work.

I also declare that, this work has not been submitted for any degree either in part or in full to any other institute or University before.

Tamaleka Paul
28/07/2022
Signature of the candidate with date

Dedication

I would like to dedicate this thesis to my parents and Ph.D supervisor. Their continuous supports have allowed me to have a key to unlock the mysteries of our world and beyond



PRESIDENCY UNIVERSITY
KOLKATA

Presidency University

Hindoo College (1817-1855), Presidency College (1855-2010)

Certificate

This is to certify that the thesis entitled "Apoptosis regulators as targets for induction of apoptosis of Imatinib resistant chronic myeloid leukemia cells" submitted by Smt Tamalika Paul who got her name registered for PhD programme under my supervision (Registration Number R-17RS18210108) on 14th August, 2018 and that neither her thesis nor any part of the thesis has been submitted for any degree/diploma or any other academic award anywhere before.

Nabendu Biswas
Signature of Supervisor with

date and official stamp

Dr. Nabendu Biswas

Asst. Professor, Dept. of Life Sciences
Presidency University, Kolkata

27/7/2022



Acknowledgement

Firstly, I would like to express my sincere gratitude to my advisor Dr. Nabendu Biswas for the continuous support of my Ph.D study and related research, for his patience, motivation and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my Ph.D study.

Besides my advisor, I would like to thank Head of the department of Life Sciences and all faculty members in Presidency University, Kolkata.

I thank my labmates (Samraj Sinha, Rajdeep Roy, Anupam Banerjee, Rittika Roy Chowdhury, Annesha Dutta, Rupak Dey Sarkar) for the stimulating discussions, for working together and for all the fun we have had in last 5 years.

Last but not the least, I would like to thank my family: my parents and elder sister for supporting me in all possible way throughout myPh.D period.

Jamaleka Paul
28/07/2022

Signature of candidate with date

List of figures

Chapter 1:

| | |
|---|-------|
| Figure 1: Imatinib resistant K562 cells show resistance against TRAIL induced Apoptosis..... | 50 |
| Figure 2: Hydroxychavicol sensitizes imatinib-resistant CML cell lines to TRAIL-induced apoptosis..... | 51 |
| Figure 3: Hydroxychavicol and TRAIL combinatory treatment enhanced cleavage of Pro caspase 8, pro caspase 3 and PARP..... | 52 |
| Figure 4: Involvement of TRAIL mediated extrinsic apoptotic pathway in HCH and TRAIL combinatory treatment in K562(R) cells..... | 53 |
| Figure 5: Hydroxychavicol did not alter DR4 and DR5 and DCR1, DCR2 expression at RNA level and Protein level..... | 54-55 |
| Figure 6: Hydroxychavicol decreased XIAP and FLIP at the protein level..... | 57 |
| Figure 7: Hydroxychavicol mediated FLIP and XIAP is occurred via proteosomal mediated pathway..... | 58 |
| Figure 8: Knockdown of XIAP and FLIP involved in TRAIL mediated apoptosis..... | 59 |
| Figure 9: HCH-mediated TRAIL sensitization of K562(R) cell is ROS dependent..... | 60 |
| Figure 10: Hydroxychavicol induces intracellular ROS level K562(R) cells..... | 62 |
| Figure 11: ROS plays a key role in HCH-mediated XIAP and FLIP downregulation..... | 64 |
| Figure12: ROS is involved in Hydroxychavicol mediated TRAIL induced apoptosis..... | 65 |

Chapter 2

| | |
|---|-------|
| Figure 1: ROS downregulates XIAP and FLIP at protein level in dose and time dependant manner..... | 75 |
| Figure 2: H ₂ O ₂ did not cause cell cytotoxicity..... | 76 |
| Figure 3: H ₂ O ₂ do not downregulate XIAP and FLIP at RNA level..... | 77 |
| Figure 4: H ₂ O ₂ solely involved in XIAP and FLIP downregulation | 77 |
| Figure 5: XIAP and cFLIP degradation is proteasomal degradation..... | 79 |
| Figure 6: XIAP and cFLIP degradation is not lysosomal dependant..... | 80 |
| Figure 7: Involvement of JNK and ERK in the regulation of FLIP and XIAP respectively in H ₂ O ₂ treated K562(R) cells..... | 82 |
| Figure 8: H ₂ O ₂ increases JNK and ERK activation by phosphorylation dependant manner..... | 84 |
| Figure 9: Inhibition of ERK and JNK by siRNA reverses degradation of XIAP and FLIP respectively..... | 86 |
| Figure 10: Degradation of FLIP and XIAP is JNK and ERK mediated respectively..... | 87-88 |

| | |
|--|----------------|
| Figure 11: ERK mediated XIAP regulation in presence of H ₂ O ₂ in K562(R) cells, involves pAkt and pPI3K..... | 90-91 |
| Figure 12: Role of ERK in PI3K and Akt phosphorylation..... | 93 |
| Figure 13: JNK signaling pathway is not involved in pAkt and p PI3K dephosphorylation.. | 94 |
| Figure14: H ₂ O ₂ activated ERK, which inhibited PI3K, Akt by dephosphorylation, thus binding of Akt to XIAP was inhibited, thus degradation and ubiquitination of XIAP was occurred..... | 95 |
| Figure15: Degradation of FLIP is mediated by increased expression of ITCH..... | 96 |
| Figure16: ITCH is responsible for JNK mediated FLIP ubiquitination and degradation..... | 98 |
| Fig 17 | 99 |
| Figure 18: ROS decreases anti-apoptotic protein XIAP and FLIP by activation of ubiquitin proteasomal pathway..... | 100 |
| Chapter 3 | |
| Figure 1: CML cell lines show resistance against TRAIL-induced apoptosis | 106 |
| Figure 2: Hydroxychavicol sensitizes CML cell lines to TRAIL-induced apoptosis | 107 |
| Figure 3: Hydroxychavicol-mediated TRAIL sensitization of K562(R) cell is ROS dependent | 108 |
| Figure 4: Hydroxychavicol decreased XIAP and FLIP at the protein level..... | 109 |
| Figure 5: Downregulation of antiapoptotic proteins XIAP and FLIP happens in a ROS-dependent manner | 111-112 |
| Figure 6: H ₂ O ₂ do not alter XIAP and FLIP at mRNA level in imatinib sensitive K562 cells | 113 |
| Figure 7: H ₂ O ₂ mediated XIAP and FLIP degradation is lysosomal dependant pathway..... | 114-115 |
| Figure 8: H ₂ O ₂ does not cause significant apoptosis as compare to control in imatinib sensitive K562 cells | 116 |
| Figure 9: MAPK signaling pathway regulates Hydroxychavicol mediated TRAIL induced apoptosis | 117 |

Discussion and Conclusion

Figure 1: Apoptosis regulators (antiapoptotic protein XIAP, FLIP) are targeted by Hydroxychavicol (HCH) via intracellular ROS generation to induce apoptosis in imatinib resistant CML cell.....**125**

References..... **130-147**

Annexures