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

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Faculty of Natural and Mathematical Sciences

Presidency University

Kolkata, India

Year of Submission:2022

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Conclusion:

From thesis, it is concluded that XIAP and FLIP could be targeted to induce apoptosis in Imatinib sensitive and Imatinib resistant CML cell by TRAIL. Hydroxychavicol downregulates XIAP and FLIP in a ROS dependent manner.

In Imatinib resistant CML cell, ROS decreases anti-apoptotic protein XIAP and FLIP by activation of ubiquitin proteasomal pathway. However, It is the Lysosomal degradation pathway that plays key role in ROS-dependent XIAP and FLIP downregulation. ROS-activated ERK subsequently decreases Akt phosphorylation which inhibits the binding of Akt to the XIAP and increases its ubiquitin mediated degradation. On the other hand, ROS increases ITCH at protein level by activation of JNK. ITCH, being an E3 ubiquitin ligase, associates with FLIP and degrades it by ubiquitin-proteasome pathway. Thus, we have, for the first time, shown that ERK and PI3K/Akt pathways crosstalk in the regulation of XIAP which could be further another target for CML therapy. We have also shown for the first time that ROS increases expression of ITCH which, by its association with FLIP, degrades it via proteasome pathway. So, In Imatinib resistant CML cell, two component of MAPK pathway i.e JNK and ERK had stimulatory role in ROS mediated FLIP and XIAP proteosomal mediated downregulation therby stimulates TRAIL mediated apoptosis. However, ROS activated JNK signaling pathway has a role in TRAIL mediated apoptosis of Imatinib-sensitive CML cells although ERK signaling pathway has been observed to have no role in TRAIL mediated apoptosis in Imatinib-sensitive CML cells. This finding may be further investigated for a specific targeted therapy for Imatinib-resistant CML cells.

Therapeutic implications of this study:

Resistance to chemotherapy, antibody-based therapy or TRAIL therapy has been fast emerging in various types of cancer including CML where imatinib (1st generation TKI against Bcr-Abl kinase) resistance is extremely prevalent. Inhibitor of apoptotic proteins like XIAP and FLIP

have been shown to play a role in promoting tumorigenesis in ovarian and breast cancer [189]. In the study, shRNA-mediated knockdown of XIAP even sensitized the tumor cells to cisplatin therapy [190]. Most of the anti-cancer drugs ultimately try to upregulate apoptosis in cancer cells and hence modulating IAPs in combination with existing anti-cancer drugs like cisplatin or imatinib or pemetrexed could prove to be fruitful in bypassing the drug resistance. Modulating IAPs is also advantageous as it overcomes the need to target upstream signaling molecules of the apoptotic pathways that might have a role in other important signaling cascades.

Main therapeutic implication from thesis is that downregulation of XIAP and FLIP leads to TRAIL induced apoptosis in imatinib resistant K562 cells. We also have targeted PI3K/Akt for antileukemic treatment in imatinib resistant K562 cells. For first time, our data indicated that ROS downregulates FLIP and XIAP (IAPs) via ITCH and ERK in K562-imatinib resistant cells. Hence, using these insights, XIAP and FLIP can be targeted as a monotherapy or in combination with other therapies to sensitize CML and other related leukemia patients.

Future Direction:

We studied that ROS regulates MAPK kinase signaling pathway in Imatinib sensitive and resistant K562 cells. We studied that the mechanisms by which ROS mediated MAPK kinase signaling pathway regulate antiapoptotic proteins FLIP, XIAP expression. In future, we will find out several signaling pathways other than MAPK pathway that will be regulated by ROS. Then we will study the mechanisms by which these ROS mediated signalling pathways will regulate several proapoptotic proteins and antiapoptotic proteins. We will study the mechanism of differential control of XIAP and FLIP by ROS between Imatinib resistant and sensitive CML cells