Abstract:

Apoptosis is a regulated cell death characterized by cell shrinkage, nuclear condensation, DNA fragmentation, membrane blebbing. There are several apoptosis regulators eg: BCL2, Bcl-xL, BAX, Survivin, FLIP, XIAP, cIAP etc. Targeting these apoptosis regulators may be a viable strategy for the treatment of cancer because one of the hallmarks of cancer is the deregulation of apoptosis. In our first study we tried to induce cell death in Imatinib-resistant chronic myelogenous Leukemia cell line K562, by using TRAIL, a well-studied anti-cancer agent. Normally these cells are also resistant to TRAIL. We used Hydroxychavicol, a Piper betel leaf derived polyphenol, to make this Imatinib- resistant K562 cells sensitive to TRAIL. In imatinib resistant K562 cells, XIAP, FLIP have emerged as targets by hydroxychavicol to sensitize the cells to TRAIL mediated apoptosis. Reactive Oxygen Species, particularly H_2O_2 has been shown to be a key player for this TRAIL sensitization by Hydroxychavicol. We found that ROS decreased XIAP, FLIP in imatinib sensitive K562(S) and imatinib resistant K562(R) both. These XIAP and cFLIP downregulation abrogated their inhibitory effect on Caspase activation and removed its break from extrinsic apoptotic pathway activation by TRAIL and this leads to apoptosis of Imatinib-resistant K562 cells and imatinib sensitive K562 cells. In Imatinib-resistant K562 cells, FLIP and XIAP were differentially regulated by JNK and ERK respectively. Akt phosphorylation was decreased by ROS-activated ERK. Dephosphorylation of Akt inhibited its binding to XIAP and that lead to the destabilization of XIAP. On the other hand, ROS-activated JNK increased the expression of an ubiquitin ligase ITCH which degraded FLIP by binding to it and ubiquitination. However, interestingly, when we checked these finding on K562(S) cells, some of the findings were different. In K562(S) cells, ROS degrades XIAP, FLIP by lysosomal degradation pathway. Moreover, JNK alone instead of JNK and ERK played important role in this XIAP and FLIP downregulation. Thus, our findings suggest anti-apoptotic proteins XIAP and FLIP as a viable therapeutic target for Imatinib-resistant CML. We have also identified a novel ROS mediated regulatory pathway of these two proteins which may be further explored for therapeutic targets.