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Apoptosis Signaling in Leukemia and Lymphoma: Understanding Mechanisms of Chemoresistance

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ABSTRACT

Apoptosis (programmed cell death) is a basic physiological process, essential in the balance between life and death of cells of normal tissues in the body. Apoptosis can be considered as cellular “suicide” initiated by the cell itself when infected by a virus or transformed into a cancer cell. Cancer is a genetic disease and in cancer cells the molecules involved in initiation and execution of apoptosis are frequently lost or inactivated. Blockade of apoptosis is associated with resistance to conventional cancer drugs. The importance of intact apoptosis signaling pathways in leukemia and lymphoma was addressed in the current thesis. We found that apoptotic protease activating factor 1 (Apaf-1) is required for second mitochondria activator of caspases (Smac)-dependent potentiation of protein kinase inhibitor staurosporine- and proteasome inhibitor lactacystin-triggered apoptosis in chemoresistant Burkitt lymphoma cell lines Raji and DG-75. Furthermore, the importance of elevated levels of cellular inhibitor of apoptosis 2 (cIAP2), in these cells was examined in cellular extracts from Raji cells overexpressing cytosolic Apaf-1. Subsequently cytochrome c-dependent caspase activation in Raji cells immunodepleted for cIAP2 was assessed. We found immunodepletion of cIAP2 to potentiate caspase activation only in Raji cells stably transfected with cytosolic Apaf-1. To further study the importance of Apaf-1 in response to proteasome inhibitors we used a T cell acute lymphoblastic leukemia (T-ALL) cell line, Jurkat, stably transfected with shRNA against Apaf-1. The clinically relevant proteasome inhibitor bortezomib (Velcade®) failed to induce apoptosis in Jurkat cells without Apaf-1. The bortezomib-induced apoptosis was associated with induction of pro-apoptotic factor Noxa upstream of mitochondria. Moreover, we examined primary leukemic blasts from patients with T-ALL for Apaf-1 protein expression and responsiveness to bortezomib-induced apoptosis ex vivo. The Apaf-1 protein expression varied amongst the different patient samples and although the sample number was low we noted the lowest increase in bortezomib-induced apoptosis in the patient sample completely deficient for Apaf-1. In order to elucidate the importance of other mediators of apoptosis, anti-apoptotic HS-associated protein X-1 (HAX-1) was assessed at the protein and transcript level in malignant lymphomas. We thus determined the mRNA expression of HAX1 in two public transcriptomics databases. HAX-1 protein expression was assessed in a panel of 50 samples from patients with B lymphoma. We found that HAX-1 mRNA and protein was highly expressed in B lymphoma. Furthermore, we found a positive association with the proliferation marker Ki67 at the protein level in diffuse large B cell lymphoma (DLBCL) and Burkitt lymphoma samples, as well as an inverse correlation with Bcl-2 at the protein and transcript level in follicular lymphoma. Finally, the actions of the specific inhibitor of chymotrypsin-like serine proteases, TPCK and the NF-κB inhibitor Bay-11 7082 were elucidated in chemoresistant cell lines Raji and DG-75. Both compounds induced caspase-independent apoptosis as well as a decrease in constitutive NF-κB activity. Moreover, we found that TPCK and Bay-11 7082 reduced protein and mRNA expression of the NF-κB target HAX-1, which may contribute to the sensitization to apoptosis. In summary, these studies contribute to our understanding of the importance of intact apoptotic signaling pathways in sensitivity to apoptosis induced by anti-cancer substances. Studies of different pro- and anti-apoptotic molecules may lead to the identification of novel targets for therapy.

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