Targeting protein processing in Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is an aggressive hematologic malignancy of which 40% of the cases have mutations in the Fms-like membrane receptor tyrosine kinase 3 (FLT3). This receptor in synthesized and processed in the endoplasmic reticulum (ER), and controls cell survival. Mutations in FLT3 prevent its correct processing and it is aberrantly retained in this organelle. This has led us to hypothesize that there are differences in the normal functioning of the ER in FLT3 wild-type or mutated cell lines. Since the ER is responsible for the folding and protein processing in the cell, we blocked these processes using reducing agents (dithiothreitol and 2-mercaptoethanol) and an N-glycosylation inhibitor (tunicamycin), on two wild-type and FLT3 mutant AML cell lines. After 48 hours of treatment, wild-type cells were severely affected by protein folding inhibitors. On the contrary, they were less sensitive to glycosylation inhibition compared to mutant lines. 24 hours exposure to a low dose of tunicamycin affected cellular levels of glycosylated proteins and FLT3 signaling, in addition to potentiation of the antitumor effect of kinase inhibitors (midostaurin and SGI) only in mutated cell lines. We could verify by western blot that doses of tunicamycin that are nontoxic to wild type cells trigger endoplasmic reticulum stress-induced death in AML mutant cell lines, supported by an increased expression of BiP and CHOP. Further studies on basal endoplasmic reticulum protein levels revealed differences in those involved in the process of protein folding (PDI, ERO, calnexin), and found increased in mutant lines. This could explain their higher tolerance to reducing agents. Current AML treatments are mainly based on the administration of FLT3 inhibitors, but in many cases patients suffer a relapse. Our data suggest that wild-type and mutant cell lines differ in ER biology, and that the process of glycosylation and protein folding could be therapeutic targets for AML treatment.