STRONG CHILDREN'S RESEARCH CENTER

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ABSTRACT

Title: Effects of MLN4924, an inhibitor of NEDD8-activating enzyme (NAE), on cell cycle, apoptosis, and cell death in Acute Myelogenous Leukemia cell lines

Background: Acute Myelogenous Leukemia (AML) is a hematological cancer that targets myeloid cells in the bone marrow and results in the production of abnormal blood cells. AML is an aggressive cancer with a low five-year survival rate (between 20-30%). Better treatments are needed to improve prognosis and survival rates for individuals with AML. The NEDD8-activating enzyme (NAE) is a key regulator in the NEDD8 conjugation pathway, which plays a vital role in protein degradation, cell cycle regulation, and survival of AML cells. MLN4924 is an inhibitor of NAE. Based on the inhibitory effects of MLN4924, we hypothesize that MLN4924 will induce cell cycle arrest, increase apoptosis, and increase cell death in AML cell lines suggesting a potential clinical effectiveness of this drug in treatment of AML.

Objective: The purpose of this study is to determine the effects of NAE inhibitor, MLN4924, on cell cycle, apoptosis, and cell death of various acute myelogenous leukemia cell lines.

Methods and Results: Three cell lines were used to test the cytotoxic effects of MLN4924. Derived from humans, HL-60 is a promyelocytic leukemia cell line, MV4-11 is an acute monocytic leukemia cell line, and KG1a is a hematopoietic progenitor cell line. These cell lines were treated with different concentrations of MLN4924. After 48 hours incubation, the cells were stained with fluorescent dyes, Hoechest (for cell cycle analysis), FITC-Annexin V (apoptotic cell staining) and PI (dead cell staining) to quantify cell cycle, apoptosis, and cell death via flow cytometry. We found that MLN4924 changed cell cycle and significantly induced AML cell death and apoptosis in a dose-dependent manner in all three cell lines. For HL-60, lower concentrations (125nM and 250nM) induced only a small amount of apoptosis and cell death. However, when MLN4924 concentration was more than 500nM, we saw a significant increase in cell death (Figure 3). Almost all cells were undergoing apoptosis or dead. Similarly, at the lowest concentration used for MV4-11 (62nM), there was only a slight increased cell death rate; when MLN4924 concentration was more than 125nM, cell death and apoptotic rate were markedly raised. At 250nM, almost all MV4-11 cells were undergoing apoptosis or dead. The KG1a cell line appeared to be the most resistant cell line to MLN4924 with minimal changes in cell cycle, apoptosis, and cell death. At 2μM of MLN4924, the highest MLN4924 concentration used in these experiments, there was only a 1.6-fold increase in KG1a cell death. However, the addition of an NFκB inhibitor, IKKB inhibitor IV, significantly boosted the sensitivity of KG1a cells to the cytotoxic effects of MLN4924. A 10.5-fold increase in KG1a cell death was observed with a combination treatment of 10μM of IKKB inhibitor IV with 2μM of MLN4924.

Conclusion: Our results support the hypothesis that MLN4924 increases apoptosis and cell death in AML cell lines. Our results also demonstrate that KG1a cell line showed resistance to MLN4924 as evidenced by minimal changes in cell cycle, apoptosis, and cell death with drug treatment. Future studies are investigating the resistant nature of KG1a cell line and potential role of NFκB. Understanding the role of MLN4924 in AML apoptosis and cell death may provide important insight into AML survival mechanisms and lead to better treatment and improved prognosis of AML.