Abstract

Changes in intracellular activation-related gene expression and induction of Akt contribute to acquired resistance towards nelarabine in CCRF-CEM cell line

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Introduction and Objectives:

Drug resistance is a significant problem in treatment with nelarabine, and its resolution requires elucidation of the underlying mechanisms. In this study, we established nelarabine-resistant sub-clones of the human T-cell lymphoblastic leukemia cell line CCRF-CEM to investigate the factors related to the acquisition of nelarabine resistance *in vitro* to identify strategies to overcome resistance to nelarabine, focusing on intracellular activation-related genes, changes in apoptosis, and signaling pathways.

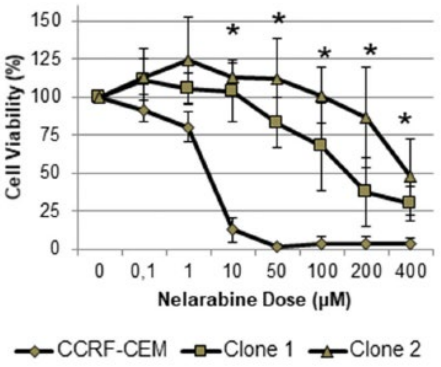

Figure 1: MTT assay showing the viability of CCRF-CEM, Clone 1, and Clone 2

Materials and Methodology:

We established two nelarabine-resistant subclones of the human T-cell lymphoblastic leukemia cell line CCRF-CEM, Clone 1 and Clone 2, by serial incubation with an increasing dose of nelarabine followed by limiting dilutions for cloning (Figure 1). Expression of genes and protein related to nelarabine intracellular activation (ENT1, DCK, DGUOK, RRM1, NT5C2, SAMHD1) were investigated. Apoptosis induction after various concentrations of nelarabine was investigated by examining caspase activation, PARP cleavage, and mRNA expression of BCL2, BCL2L1 (Bcl-xL), BAD, and BAX. Changes in signaling pathways in nelarabine-resistant subclones were investigated by western blotting of p-Akt, Akt, p-ERK, and ERK. Therapies with PI3K/Akt and MEK/ERK inhibitors and other classes of chemotherapy were done to see its effect on resistant subclones.

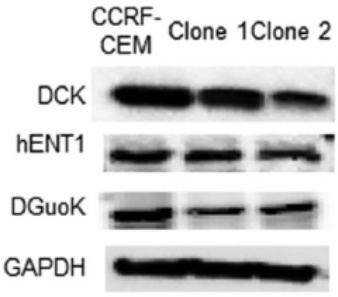

Figure 2: Western blot image

Results:

The resistant subclones showed changes in the expression of several genes related to nelarabine intracellular activation, which are downregulation of ENT1, DCK, DGUOK, and upregulation of SAMHD1 (Figure 2). Resistant clones did not show caspase 3 activation and PARP cleavage after treatment with 10 μM nelarabine, indicating

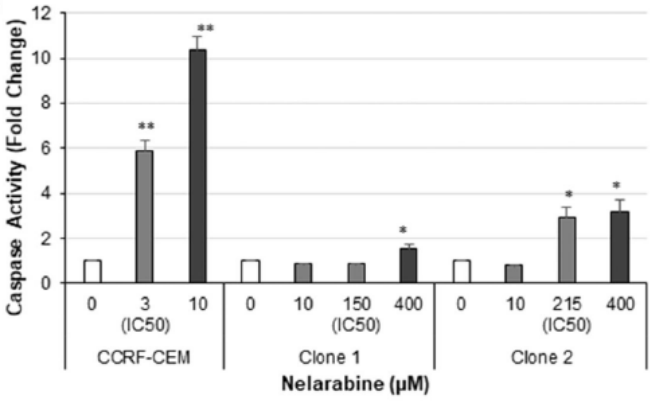

Figure 3: Caspase 3/7 assay after treatment with nelarabine

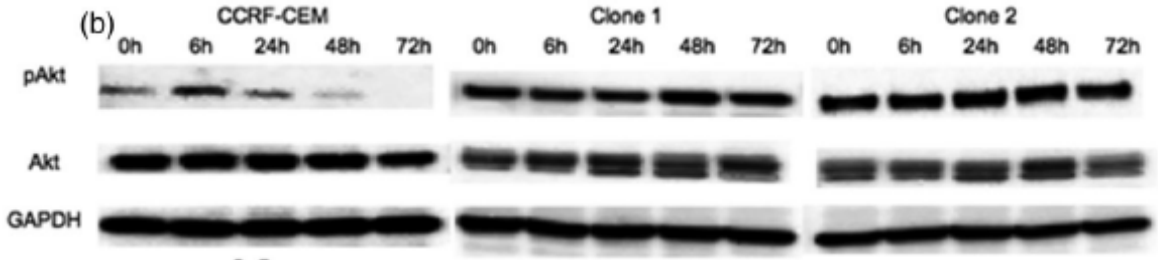

Figure 4: Representative western blot image and p-Akt/Akt quantitative ratio analysis

apoptosis inhibition (Figure 3). There was no significant difference in the expression of BCL2, BCL2L1 (Bcl-xL), BAD, and BAX in basal conditions; however, cells responded differently after nelarabine treatment. Clone 1 showed no changes in the Bcl2 family. Upregulation of BCL2L1 (Bcl-xL) was observed in Clone 2 after 10 μM nelarabine treatment, and upregulations of BAX and BAD were seen after treatment with high dose nelarabine. Upregulation of p-Akt in untreated cells and upon nelarabine treatment were observed in resistant clones, notably in Clone 2 (Figure 4). Compared to the nelarabine-only treatment, combined therapy increased drug toxicity, reduced p-Akt levels, and induced cell death in resistant clones. We found that p-ERK was upregulated after treatment with nelarabine, although not statistically significant. However, we observed no effect on cell viability after treating the cells with the MEK/ERK inhibitors individually or simultaneously with nelarabine (Figure 5). Cross-resistance was observed with ara-C and not with vincristine, daunorubicin, or etoposide treatment (Figure 6).

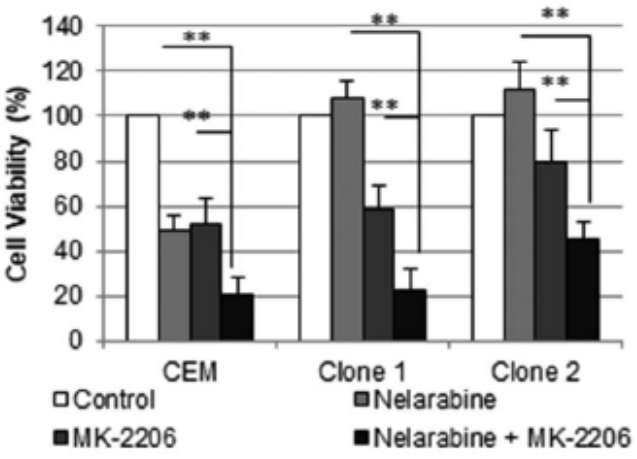

Figure 5: Cell viability with nelarabine, with or without MK-2206

Conclusion and Analysis:

We established nelarabine-resistant subclones from the CCRF-CEM cell line. Cell models showed that nelarabine resistance involved changes in the expression of genes related to nelarabine activation, inhibition of apoptosis accompanied by changes in the response of Bcl2 family genes, and Akt induction. The use of other chemotherapeutic agents or combination therapy with PI3K/Akt pathway inhibitors may be used as a method to overcome drug resistance during treatment.

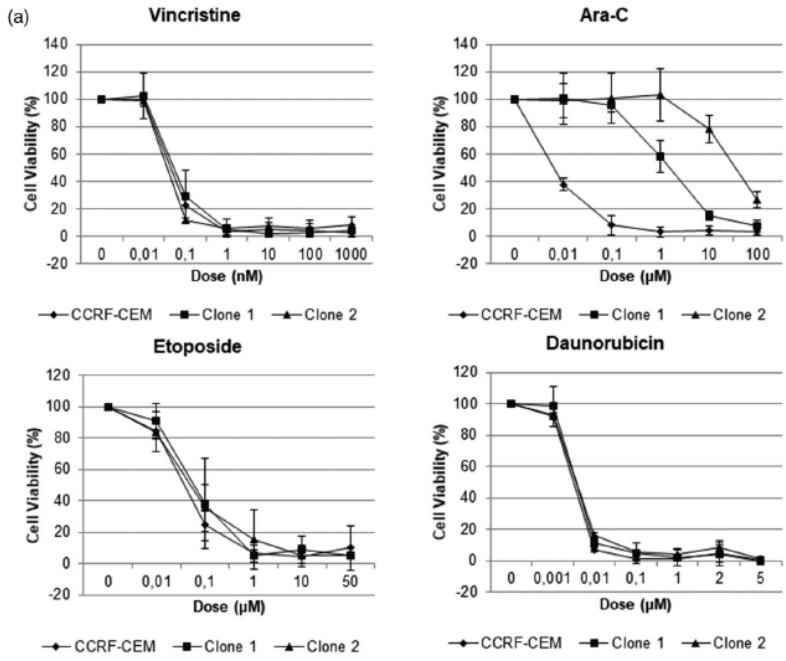

Figure 6: Cell viabilities to various chemotherapeutic agents