Acute myeloid leukemia (AML) is the most common and most deadly type of leukemia in adults, affecting approximately 3 people per 100,000. There are about 13,000 new cases of AML every year in the United States alone. AML is cancer of the myeloid line of blood and has an unfavorable prognosis. In normal bone marrow, myeloid progenitors develop into distinct blood types including erythrocytes, granulocytes, macrophages, and platelets.

AML is typically treated with a cocktail of chemotherapeutic drugs, most often involving the pharmaceutical agent cytosine arabinoside (Ara-C). Ara-C is a deoxycytidine analog that becomes incorporated into growing DNA daughter strands, interfering with DNA replication.

Figure 2: Ara-C is an anti-metabolic agent that locks cells in S phase, when Ara-C is incorporated into newly synthesized DNA instead of cytidine. Because the DNA structure is modified, DNA polymerase cannot properly synthesize DNA and topoisomerase 1 cannot properly unwind the DNA for replication.

Treatment with Ara-C will almost always cause remission in AML patients. However, developed resistance to Ara-C becomes a problem for many patients suffering from the disease, and many relapse within a few years of remission. As the second round of therapy is almost always ineffective for relapsed patients, AML patients who have a relapse of AML typically don’t survive more than five years following diagnosis.

We are using an in vitro system to model the Ara-C resistance in AML cell lines. The AML cell lines were generated by crossing mice of the C57BL/6 and C3H/HeJ strains. The BH2 strain spontaneously developed AML, so cells were isolated from two different mouse strains (B117 and B140) and propagated in culture. Resistant cell lines (B117H and B140H) were created by exposing the parental cell lines to increasing concentrations of Ara-C. They can tolerate up to 1000 times higher Ara-C concentrations than their parental lines.

Figure 3: Ara-C enters the cells via the SLC29A1 transporter and is phosphorylated 3 times before it is incorporated into DNA.

Methods and Results

Gene Expression Microarray

RNA was isolated from each cell line (B117P, B117H, B140P, B140H) at three different time points during normal cell maintenance. It was used in a gene microarray expression experiment using Affymetrix Mouse Genome 430 2.0 Array chips. The experiment identified the down regulation of Dck as a common feature in the acquisition of Ara-C resistance in both sets of cells.

Table 1: The table shows the changes when comparing Ara-C resistant cell lines with Ara-C sensitive parental lines. This indicates that an important component of developing Ara-C resistance in the B117H and B140H cells involves disabling of the dNTP salvage pathway. This would require an increased dependence on the de novo synthesis pathway, which is less efficient because it relies on rate-limiting enzymes.

Chemotherapy Drug Assays

Drug assays were conducted on the four cell lines using a variety of drugs and combinations of drugs including Ara-C, Decitabine, Daunorubicin, CPEC, 5-fluoro-2-deoxyuridine, and SCH20d99. These drugs are nucleoside analogs or other chemotherapeutic drugs that interfere with DNA synthesis. Increasing concentrations of the drug were added to 96 well flat plates to determine the inhibitory concentration of 50% (IC\textsubscript{50}) value of the cells in response to the different chemotherapy drugs.

Figure 4: qPCR was used to verify Dck expression levels of the marine AML cell lines.

dNTP Salvage Pathway

The dNTP salvage pathway provides a mechanism for cells to synthesize dNTPs in the presence of Ara-C. This pathway is critical for normal cell function and is upregulated in AML cells resistant to Ara-C.

MTS Assay

The MTS (Promega, Madison, WI, USA) tetrazolium assay was used to determine cytotoxic response of AML cell lines, according to the manufacturer’s instructions. The wells containing no cells were used as blank controls, and the wells containing cells but no drugs served as the cell control. After three days of incubation, MTS solution was added to each well and incubated at 37°C for three hours. The intensity of the produced brown formazan, directly proportional to the number of metabolically active cells, was measured by reading absorbance at 490 nm and 650 nm using an ELISA reader.

Figure 5: Ara-C Resistant Cells Also Resistant to Decitabine

Figure 6: Ara-C Resistant B140H Cells Also Resistant to 5-Fluoro-2-deoxyuridine (FdUdR)

Conclusion

B117P and B140P cell lines appear to be two distinct subtypes of AML, with B117P being a less mature form.

Dck is dramatically down-regulated in B117H cells, and the cause of down-regulation may be due to alternative splicing. B140H cells are also highly resistant to FdUdR, suggesting the B140H cells have a mutated SLC29A1 transporter.

Since similar mutations in DCK and SLC29A1 have been found in human AML, the B117 and B140 cells may be effective tools to evaluate drug combinations for treating human disease.

References

8. Cytidine nucleoside analog increases markers of apoptosis, arrests cells in G1, and hypomethylates DNA. ARA-C resistant cell lines B117H and B140H are also highly resistant to Decitabine

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