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Exploring the Mechanism of Ara-C Resistance in Acute Myeloid Leukemia

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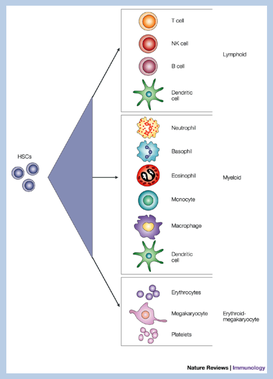
Introduction



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 .

Figure 1: Haematopoietic cell classifications. Nature Reviews Immunology 2002.

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.

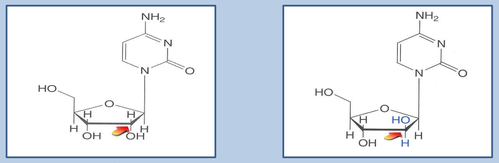
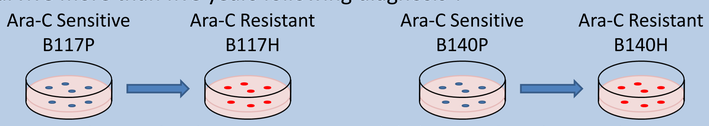


Figure 2: Ara-C is an anti-metabolic agent that locks cells in S phase, when Ara-C is incorporated into newly synthesized DNA instead of cytidine. Because the DNA structure is modified, DNA polymerase cannot properly synthesize DNA and topoisomerase 1 cannot properly unwind the DNA for replication.

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[6]. 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[1].



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/6J and C3H/HeJ strains. The BHX2 strain spontaneously developed AML, so cells were isolated from two different mice (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.

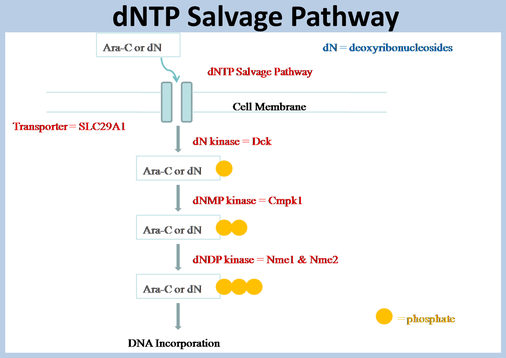


Figure 3: Ara-C enters the cells via the SLC29A1 transporter and is phosphorylated 3 times before it is incorporated into DNA.

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.

Probe ID	Gene ID	Description	Fold Change B117H vs. B117P	Fold Change B140H vs. B140P
1439012_a_at	*Dck*	deoxycytidine kinase	-273.157	-8.264
1449176_a_at	*Dck*	deoxycytidine kinase	-92.503	-7.488
1415673_at	*Psph*	phosphoserine phosphatase	-4.571	-2.606
1424254_at	*Ifitm1*	interferon induced transmembrane protein 1	-4.456	-2.788
1420498_a_at	*Dab2*	disabled homolog 2 (Drosophila)	-4.338	-3.251
1423805_at	*Dab2*	disabled homolog 2 (Drosophila)	-3.632	-2.108
1429693_at	*Dab2*	disabled homolog 2 (Drosophila)	-3.566	-2.480
1455991_at	*Ccbl2*	cysteine conjugate-beta lyase 2	-2.176	-3.308
1433521_at	*Ankrd13c*	ankyrin repeat domain 13C	2.055	2.021
1428114_at	*Slc14a1*	solute carrier family 14 (urea transporter), member 1	2.692	2.135
1433939_at	*Aff3*	AF4/FMR2 family, member 3	2.737	2.180

Table 1: Genes that have 2.0+ fold changes when comparing Ara-C resistant cells lines to their 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.

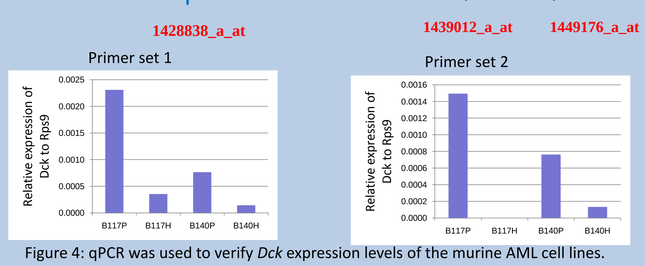
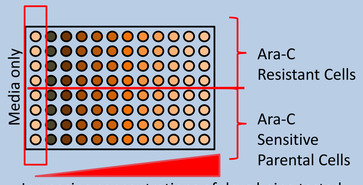


Figure 4: qPCR was used to verify *Dck* expression levels of the murine AML cell lines.

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 SCH23099. 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_{50}) value of the cells in response to the different chemotherapy drugs.



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 instruction. 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.

Ara-C Resistant Cells Also Resistant to Decitabine

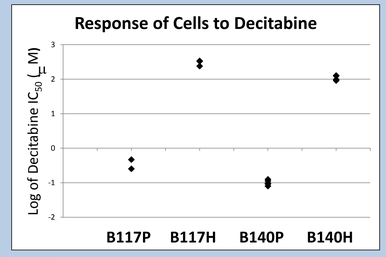


Figure 5.

Decitabine[3]

- Cytidine nucleoside analog
- Increases markers of apoptosis, arrests cells in G1
- Hypomethylates DNA
- Ara-C resistant cell lines B117H and B140H are also highly resistant to Decitabine

Ara-C Resistant B140H Cells Also Resistant to FdUrD

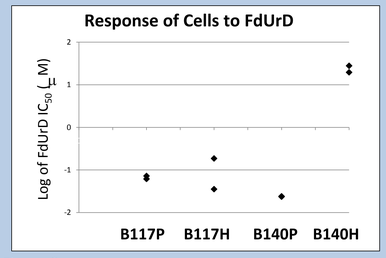


Figure 6.

5-Fluoro-2-deoxyuridine (FdUrD)[4]

- FdUrD is a uridine analog
- Inhibits thymidylate synthesis
- Enters cells via the Slc29a1 transporter
- B140H cells are also highly resistant to FdUrD

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 FdUrD, 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[8]

B117H Cells Depend on an Alternatively Spliced Form of *Dck* for Ara-C Resistance

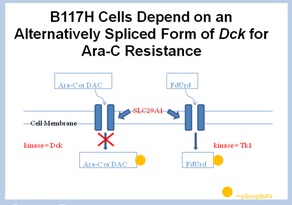


Figure 7.

B140H Cells Depend on a Mutated Form of *Slc29a1* for Ara-C Resistance

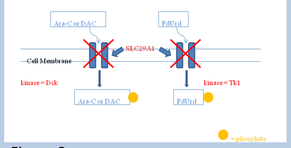


Figure 8.

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Acknowledgements

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