EFFECT OF GENETIC ALTERATION OF CYTOSINE ARABINOSESIDE METABOLIZING ENZYMES IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

CHUMPHORN BANKLAUI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (CLINICAL PATHOLOGY) FACULTY OF GRADUATE STUDIES MAHIDOL UNIVERSITY 2010

COPYRIGHT OF MAHIDOL UNIVERSITY
EFFECT OF GENETIC ALTERATION OF CYTOSINE ARABINOSIDE METABOLIZING ENZYMES IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

CHUMPHORN BANKLAUI RACP/M 5036436

M.Sc. (CLINICAL PATHOLOGY)

THESIS ADVISORY COMMITTEE: SUNTAREE APIBAL, M.Sc., SAMART PAKAKASAMA, M.D., SUMALEE JINDADAMRONGWECH Ph.D.

ABSTRACT

The resistance to cytosine arabinoside (AraC) chemotherapeutic agents is the major problem in the treatment of acute leukemia. The objective of this thesis was to evaluate the effect of genetic alteration of two important genes encoded in the AraC metabolizing enzyme; the deoxy cytidine kinase (dCK) gene with two single nucleotide polymorphisms (SNPs) -360 C>G, -201 C>T and the cytidine deaminase (CDA) gene with two SNPs; 79 A>C, 208 G>A. They were evaluated for genotypes using the RFLP and ARMS PCR techniques and for mRNA expression using the quantitative Real time PCR technique.

We performed 94 peripheral blood tests on children with acute lymphoblastic leukemia (ALL) and 100 blood tests on a normal control group from the general population. All genes have 3 possible genotypes; either wild, heterozygous, or variant. The dCK (-360 C>G, -201 C>T) gene in the ALL test group was composed of 72%, 27% and 1% respectively, while the normal control group was composed of 70%, 26% and 4%, respectively. The CDA gene, 79 A>C in the ALL test group was composed of 87%, 13% and 0%, respectively while the normal control group was composed of 81%, 18% and 1%, respectively. The CDA 208 G>A had only one genotype which was 100% wild type. The mRNA expression was studied using a quantitative Real time PCR technique on 44 RNA extracted from bone marrow of recently diagnosed ALL subjects, and calculated using the $2^{-\Delta\Delta CT}$ formula.

We found that there was an association between genotypes and toxicity in ALL patients with heterozygous and variant genotypes (-360 CG/-201 CT, -360 GG/-201 TT) in that there was an AraC related mucositis occurring. However, there was no significant association of AraC toxicity in the allelic genotype of CDA, but this gene was connected to the improvement of patients. It showed that there was an association between Minimal Residual disease (MRD) and mRNA expression. Neither the genes nor the genotypes were significantly associated with mRNA expression. In conclusion, the results of this study indicated that dCK and CDA were important genes in AraC metabolism.

KEY WORDS: ACUTE LYMPHOBLASTIC LEUKEMIA / RFLP-PCR / ARMS-PCR / QUANTITATIVE REAL TIME PCR / mRNA EXPRESSION
EFFECT OF GENETIC ALTERATION OF CYTOSINE ARABINOSIDE METABOLIZING ENZYMES IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

Chumphon Bannakul

Th.(Pathology)

Committee Chair: Sunthi Aboobal, M.Sc., Somasak Ckman, M.D., Sunadee Jindadharavich, Ph.D.

AraC is one of the drugs used to treat acute lymphoblastic leukemia and the problem of drug resistance of AraC is also a significant issue to be addressed. This study aims to assess the impact of genetic changes of the enzymes dCK and CDA associated with the metabolism of the drug AraC in the -360 C>G, -201 C>T and 79 A>C, 208 G>A polymorphisms of the dCK and CDA genes in order to study the genotype and mRNA expression of these genes.

The RFLP and ARMS PCR methods were used to study the genotype. From a total of 94 leukemia patient samples and 100 healthy control samples, the genotype of the dCK gene was wild type in 72%, heterozygous and variant in 27% and 1%, respectively. The CDA 79 A>C gene was wild type in 87%, heterozygous in 13% and variant in 0%, while the CDA 208 G>A gene was wild type in 81%, heterozygous in 18% and variant in 1%. The mRNA expression of dCK and CDA was studied by quantitative Real time PCR on bone marrow RNA samples.

Correlation between genotype and mRNA expression was examined. The results showed that in acute lymphoblastic leukemia patients with genotype heterozygous and variant of dCK (-360 C>G, -201 C>T), mucositis was more frequent. If the genotype variant of CDA 79 A>C was found, MRD and mRNA expression increased. Therefore, the analysis suggested that dCK and CDA may be important factors in the metabolism of AraC.