DEVELOPMENT AND ESTABLISHMENT OF DENATURING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (DENATURING HPLC) FOR DETECTION OF COMMON POLYMORPHISMS IN THE MULTI-DRUG RESISTANCE (MDR 1) GENE AMONG MALAY PATIENTS WITH LEUKEMIA

Research Center: Human Genome Center, School of Medical Sciences, USM

Current status of project: Ongoing

Researchers:

1. Badrul Hisham Yahaya (post-graduate student)
2. Dr Narazah Mohd Yusoff (Main supervisor)
3. Dr Rosline Hassan (Co-supervisor)

Introduction:

The major problem in the treatment of leukemia patients is the emergence of leukemic blast cells that are resistant to anticancer drugs which eventually will lead to treatment failure. The mechanism of multi drug resistance (MDR) may arise from alterations at any step in the cell-killing process. This is due to the expression of the MDR gene (*mdr1*) and its product P-glycoprotein (P-gp) on the cell surface membrane. In recent years, more than 20 single nucleotide polymorphisms (SNPs) have been identified in the MDR1 gene leading to functional alterations and phenotypic variation in P-gp expression. Many techniques have been used in order to detect the presence of these mutations or SNPs in the MDR1 gene and the latest technique is the fully automated system called denaturing high performance liquid chromatography (DHPLC). Here there is sizing of DNA by ion paired reverse phase HPLC and the detection of sequence variants by DHPLC. DHPLC has recently been described as a feasible method for screening DNA mutations. Although this method is widely used in detection of SNPs and mutations, there is still lack of data reporting the use of this method to screen SNPs or mutations in MDR1 gene. Even though the MDR gene has been studied in western country, data of MDR1 gene in leukemias is scarce especially so in the Malaysian population. In this study DHPLC will be employed to detect mutations in the MDR gene in patients with leukemia.

Objectives:

1. To develop the denaturing HPLC technique for screening known polymorphisms and mutations in multi-drug resistance (MDR1) gene in Malay patients with leukemia.
2. To screen the distribution of common polymorphisms located in exon 12, 21 and 26 of the MDR1 gene in combination with PCR-RFLP based assays among Malay patients with leukemia.
3. To establish the denaturing HPLC technique as a feasible and cost-effective method for mutational screening of the MDR1 gene.

Methodology:

A total of 101 Malay patients with leukemia in the stage of diagnosis, who were admitted in Hospital Universiti Sains Malaysia (HUSM) were enrolled in this study. There were 35 (34.7%) diagnosed as AML, 48 (47.5%) as ALL and 18 (17.8%) as CML. A number of 5 (10.4%) of ALL patients were diagnosed as relapse while the rest of the classifications were based on morphology of blast cell, cytochemical staining and immunophenotype. Age of the patients ranged from 1 to 75 years. There were 58 (57.4%) males and 43 (42.6%) females. Genomic DNA was extracted using commercial kit [QIAamp DNA Mini Kit (QIAGEN, Germany)]. PCR amplification (exon 12, exon 21 and exon 26) was carried out using Ampli*Taq* Gold polymerase (ABI Systems) and amplicons were screened for any mutations using dHPLC (VarianIns, USA). Samples showed heteroduplex mutations were screened for any common mutation using RFLP assays.

Expected Outcome:

1) Development of the dHPLC technique for screening polymorphisms and mutations in the MDR1 gene in leukemia patients.
2) Determination of novel mutations by comparing the profile of the samples and control and confirmation by using sequencing technique.
3) Establishment of this method to be used to determine any polymorphisms or mutations or novel mutations in whole MDR1 gene exon in leukemia patients.
4) To use these markers for patients treatment and prognosis assessment-stratification
5) Genotype –phenotype correlation of different SNPs or mutations