SUPPRESSION OF *AML1/ETO* VIA siRNA MEDIATED GENE KNOCKDOWN AND ITS EFFECTS ON *FOXO3* AND *c-MYC* EXPRESSION IN AML t (8,21) CELLS

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DECLARATION

Here, I declare that this research has been sent to Universiti Sains Malaysia for degree of Master of Science. It is also not be send to any other Universities. With that, this research might be used for consultation and can be photocopied as reference.

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ONG SI MN

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMNS

AML	Acute Myeloid Leukemia
ALL	Acute Lymphoid Leukemia
AGO2	Argonaute 2
ºC	Degree Celsius
CBFβ	Core binding factor β
cDNA	Complementary DNA
cm^2	centimeter square
CO_2	Carbon Dioxide
CLL	Chronic Lymphoid Leukemia
CML	Chronic Myeloid Leukemia
DMSO	Dimethyl sulfoxide
dsDNA	Double stranded deoxyribonucleic acid
FAB	French-American-British
FBS	Fetal bovine serum
g	Gravity
LAC	Lung adenocarcinoma
µL	Microlitre
mL	Mililitre
msec	Milisecond
mm	Milimeter
mRNA	Messenger Ribonucleic acid
miRNA	Micro Ribonucleic acid
ng	Nanogram
nm	Nanometer
nM	Nanomolar

NPM	Nucleophosmin
NSCLC	Non-small cell lung cancer
OD	Optical density
OGG1	8-Oxyguanine DNA glycosylase
PCR	Polymerase chain reaction
PBS	Phosphate buffered saline
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
RPMI	Roswell Park Memorial Institute
rpm	Revolution per minute
siRNA	small interfering Ribonucleic acid
v/v	Volume/volume
w/v	Weight/volume
SD	Standard deviation
TRAIL	Tumor necrosis factor related apoptosis that induce ligand
WHO	World Health Organization
USA	United States of America
V	Voltage
%	Percentage

ABSTRAK

Akut mieloid leukemia (AML) dengan t (8,21) translocation adalah salah satu kelainan karyotypic yang paling kerap diperhatikan dalam AML yang menghasilkan gen bergabung onko-protein, *AML1/ETO*. siRNA adalah alat penggangguan RNA (RNAi) yang biasa digunakan untuk mendorong pembubaran jangka pendek gen pengekodan protein. Terdapat kajian menunjukkan bahawa pembubaran *AML1/ETO* akan menaikkan atau mengurangkan regulasi dalam pelbagai gen yang bertanggungjawab untuk apoptosis, percambahan, dan pembaharuan diri sel. *FOXO3* bertindak sebagai gen penindas tumor melalui pencetusan apoptosis dengan menaikkan regulasi sementara *c-MYC* adalah proto-onkogene yang didapati dinaikkan regulasi dalam banyak jenis kanser. Walau bagaimanapun, hubungan antara ketiga-tiga gen ini tidak diketahui. Oleh itu, objektif kajian ini adalah untuk mengkaji supresi gen *AML1/ETO* melalui kaedah perencatan gen dengan menggunakan siRNA dan kesannya terhadap ekspresi gen *FOXO3* dan *c-MYC* dalam sel-sel t (8,21) Kasumi-1. Ia telah dihipotesiskan bahawa kaedah perencatan gen menggunakan siRNA adalah cekap dalam supres gen *AML1/ETO* dan terdapat korelasi antara gen *AML1/ETO, FOXO3* dan *c-MYC*. Hipotesis ini diuji secara eksperimen menggunakan perencatan gen *AML1/ETO* melaui electroporasi siRNA diikuti oleh kajian tahap ekspresi gen *FOXO3* dan *c-MYC* menggunakan qPCR. Hasil daripada siRNA yang diantarkan eksperimen supresi *AML1/ETO* dengan

menggunakan siAGF1 menunjukkan bahawa terdapat penurunan sebanyak 52% pada 24 jam (p=0.005), 65% (p=0.002) pada 48 jam dan 85% (p=0.018) pada 72 jam. Sedangkan keputusan tahap ekspresi gen *FOXO3* dikurangkan sebanyak 3% pada 24 jam (p=0.860), 48 jam menunjukkan 50% peningkatan (p=0.153) dan 72 jam menunjukkan 46% (p=0.154). Ekspresi *c-MYC* meningkat 11% pada 24 jam (p=0.821), 48 jam menunjukkan 83% meningkat (p=0.232) manakala 72 jam menunjukkan 25% penurunan ekspresi (p=0.209). Keputusan ekspresi gen setelah perencatan gen tidak menunjukkan korelasi konklusif atau signifikan antara *AML1/ETO*, *FOXO3* dan *c-MYC*. Perencatan gen berpanjangan disarankan untuk mengesahkan lagi hasil carian.

SUPPRESSION OF *AML1/ETO* VIA siRNA MEDIATED GENE KNOCKDOWN AND ITS EFFECTS ON *FOXO3* AND *c-MYC* EXPRESSION IN AML t (8,21) CELLS

ABSTRACT

Acute Myeloid Leukemia (AML) with t (8,21) translocation is one of the most frequent karyotypic abnormalities observed in AML that results in formation of fusion onco-protein, *AML1/ETO*. siRNA is a commonly used RNA interference (RNAi) tool to induce short-term silencing of protein coding genes. There are studies shows that silencing of *AML1/ETO* results in up- or down-regulation of various gene responsible for apoptosis, proliferation, and self-renewal of cells. *FOXO3* act as a tumor suppressor gene by triggering apoptosis with its upregulation while *c-MYC* is a proto-oncogene which is found to be upregulated in many types of cancers. Nonetheless, the correlation between these three genes is not known. Therefore, the objective of this study was to study the suppression of *AML1/ETO* gene via siRNA mediated knockdown and its effect on *FOXO3* and *c-MYC* gene expression in AML t (8,21) positive Kasumi-1 cell. It was hypothesized that siRNA mediated knockdown is efficient in suppressing *AML1/ETO* gene and there is a correlation between *AML1/ETO*, *FOXO3* and *c-MYC* gene. This hypothesis was experimentally tested using a siRNA

mediated gene knockdown by electroporation of *AML1/ETO* followed by the study of gene expression level of *FOXO3* and *c-MYC* using qPCR. The result of siRNA mediated *AML1/ETO* gene knockdown experiments using siAGF1 reveal that there is a knockdown of 52% at 24 hours (p=0.005), 65% (p=0.002) at 48 hours and 85% (p=0.018) at 72 hours. Whereas the result of gene expression level of *FOXO3* reduced by 3 % at 24 hours (p=0.860), 48 hours showed a 50% of increase (p=0.153) and 72 hours showed a 46% (p=0.154) of decrease in expression. Expression of *c-MYC* increased by 11% at 24 hours (p=0.821), 48 hours showed 83% of increased (p=0.232) while 72 hours showed 25% of decreased in expression (p=0.209). The results of gene expression after gene knockdown does not show conclusive or significant correlation between *AML1/ETO*, *FOXO3* and *c-MYC* which a prolong knockdown is suggested to further verify the finding.

CHAPTER 1 INTRODUCTION

Leukemia is a disease involving bone marrow and blood which consists of four types of leukemia namely Chronic Myeloid Leukemia (CML), Chronic Lymphocytic Leukemia (CLL), Acute Lymphoblastic Leukemia (ALL) as well as Acute Myeloid Leukemia (AML). Among the four types of leukemia, The most commonly occur acute leukemia among adults is AML and the occurrence will increase with age (Deschler and Lubbert, 2006).

As stated by French-American-British (FAB) classification, t (8,21) (q22; q22) is the most general karyotypic abnormalities encounter in M2 subtype of AML (LeBeau and Rowley, 1984). Runt-related transcription factor RUNX1/AML1 which is a key regulator that involve in process of hematopoiesis is the most common target of chromosomal translocations occur in Acute Myeloid Leukemia. During the translocation, *AML1* gene located at chromosome 21 will fuses with ETO gene which is located at chromosome 8 resulting in the construction of *AML1/ETO* fusion protein (Sweetser et al., 2005).

Sweetser *et al*,. at 2005 suggest that cells which loss the expression of *AML1/ETO* will caused the increased in proliferation and cell survival thus giving a good prognosis for AML patients (Sweetser et al., 2005). However, *AML1/ETO* gene singly may be insufficient to induce leukemia (Rhoades et al., 2000) . There is a lack of study regarding the roles of *AML1/ETO* involve in process of inducing leukemogenesis (Heidenreich et al., 2003) . According to Heidenreich *et al*., 2003, an approach which is through the selective depletion of *AML1/ETO* gene in leukemic cells can give a rough idea on the role of the gene and it can also study the effects of *AML1/ETO* depletion on gene expression (Heidenreich et al., 2003) .

A study by Spirin *et al*., in 2014 that used RNA-interference (RNAi) specifically short hairpin RNA (shRNA) as a method for explanation regarding the functional consequences on the suppression of *AML1/ETO* in AML t (8,21) Kasumi-1 cell line shows a notable decrease in expression of KIT accompanied by growth inhibition and increase in apoptosis of the leukemic cells (Spirin et al., 2014).

FOXO transcription factors functions in regulation of growth, cells differentiation, cells survival, stress, cell cycle, metabolism, and tumor suppression pathways. The function of *FOXO* factors involve in development of tumor was primarily proposed through studies that three of the *FOXO* genes were found at chromosomal breakpoints in various category of cancer which are rhabdomyosarcomas for *FOXO1*, and Acute Myeloid Leukemia for *FOXO3* and *FOXO4* (Barr, 2001).

While in some studies, *FOXO3* was found to be removed in carcinogen-induced human lung adenocarcinoma (LAC) of mice and in human non-small cell lung cancer (NSCLC) cell lines. The findings from this study show that the loss of *FOXO3* causes the pathogenesis of NSCLC (Herzog et al., 2009, Blake et al.). *FOXO3* gene was recognize as one of the novel target for deletion in human lung adenocarcinoma. Homozygous or bi-allelic removal of *FOXO3* was identified mostly in early-stage LAC among smokers which made up of 24.2% (Mikse et al., 2010). Inactivation of *FOXOs* occurs in most of human cancers, due to over activation of PI3K/Akt pathway and the latter is caused by mutations that occur in RAS, PTEN or PI3K (Dansen and Burgering, 2008). These studies suggest that *FOXOs* can act as a tumor suppressors factor that may be use as therapeutic purposes (Jin et al., 2004).

Posttranslational modifications (PTMs) play a crucial part in regulating the activity of *FOXO* transcription factors which resulting in changes of the subcellular localization of these

proteins (Brunet et al., 1999) . Based on a study by Chapuis *et al*., on 2010 the importance of PTMs on *FOXO3* activity and the involvement of *FOXO3* in tumor-associated chromosomal aberrations in AML had assumed that the loss of *FOXO3* tumor suppressive function may represent a common feature in AML cells and could contribute to leukemogenesis. The killing of leukemic cells can be induced through restoring of *FOXO3* tumor suppressive functions and could thus represent an attractive perspective in AML therapy (Chapuis et al., 2010).

The *c-MYC* gene has appear as central oncogenic switch in various types of cancers. The discovery that human cancers that often show altered expression of human *c-MYC* gene had drawn attention to the importance on the function of this gene in development of human cancers (Evan et al., 1992). The ability of *c-MYC* protein as a transcription factors that are able to regulate gene expression both positively and negatively plays a major part in cell growth and proliferation (Adhikary and Eilers, 2005, Eilers and Eisenman, 2008). The most common events related with cancer are the amplification, mutation, or activation of *MYC* oncogene family (Eilers and Eisenman, 2008). In AML, activation of the *c-MYC* gene is commonly occur and the activation has play a major part in triggering the event of leukemia (Hoffman et al., 2002, Renneville et al., 2008).

RNAi has been proven to be a very useful research tool that allows a much more rapid characterization on the function of certain known genes (Mocellin and Provenzano, 2004). Currently, RNA interference is a popular method in gene silencing or gene knockdown where it is easy to perform, relatively nontoxic, affordable, and it is highly specific. RNA interference (RNAi) which consists of siRNA, miRNA and shRNA has known as a natural mechanism for gene expression silencing over the past decades. siRNA technology is

successfully used in genomic studies to study between the genes functions and their interaction, and also serve as a method in discovery for new pharmaceutical preparations (Rulina et al., 2010). RNA interference is a promising option as a base for new biomedical approach against different diseases which also include tumors (Rulina et al., 2010).

However, RNAi has its own limitations despite the facts that significant efforts have been made as compared to previous practice done. Not all sequence can achieve its target, with most of the researchers only reports about one in three of a success rate. In addition, although the effects of RNAi are generally assumed to be highly sequence-specific, there are some doubts that still remain as to whether some of the effects observed are "off target"(Mocellin and Provenzano, 2004).

As all three *AML1/ETO*, *FOXO3* and *c-MYC* plays a role in affecting Acute Myeloid Leukemia individually, however, there is a lack of study in understanding the correlation between these three genes during the process of leukemogenesis in Acute Myeloid Leukemia. Therefore, a study on the relationship between *AML1/ETO*, *FOXO3* and *c-MYC* can be an interesting knowledge gap to fill. Besides that, this study also aims to experiment on the efficiency of siRNA in suppressing *AML1/ETO* gene.

1.1 Problem Statement:

AML1/ETO, FOXO3 and *c-MYC* genes are involved in the progression of cancer respectively, however the correlation between these three genes in AML are not well established. Hence, the study serves to explore the association between *AML1/ETO, FOXO3* and *c-MYC* genes in AML cells.

1.2 Objectives:

1.2.1 General objective:

To study the expression among *AML1/ETO, FOXO3* and *c-MYC* genes in AML t (8,21) cells

1.2.2 Specific objectives:

i) To suppress *AML1/ETO* gene in Kasumi-1 cell line using siRNA

ii) To measure the gene expression level of *FOXO3* and *c-MYC* after suppression of *AML1/ETO* in Kasumi-1 cells using Real Time PCR

1.3 Hypothesis

1.3.1 Hypothesis 1:

siRNA is efficient in suppressing *AML1/ETO* gene in AML t (8,21) cells.

1.3.2 Hypothesis 2:

There is correlation between *AML1/ETO, FOXO3* and *c-MYC* genes in AML t (8,21) cells.

CHAPTER 2 LITERATURE REVIEW

2.1 Acute Myeloid Leukemia (AML)

Leukemia is one of the well-known oncological disease that is distinguished by an increased in morphologically immature blood cells which are known as blasts, where the blast cells are unable to perform normal functions of mature cells and instead it will exclude normal precursors because of the active proliferation (Rulina et al., 2010). In clinical definition, leukemias that start acutely and display rapid progression are known as acute leukemias. Delayed in or lack of treatment will cause death within a few months' time. Acute leukemia is a form of heterogenous group of malignant diseases of the hematopoietic system characterized by the existence of large number of blast cells in blood of the patient which will cause specific damage to the blood marrow (Rulina et al., 2010).

AML is one of the cancerous disease of blood which commonly occur among the adults that arise from the transformation and differentiation of hematopoietic cells of the myeloid cell lines (Rubnitz et al., 2008). AML can be distinguished by a rise in numbers of myeloid cells in bone marrow and halt in the progression of the precursor cells which usually results in insufficiency of hematopoietic cells such as thrombocytopenia, anemia or granulocytopenia, with or without the occurrence of leukocytosis (Lowenberg et al., 1999). In the absence of treatment within one year, the failure of myeloid precursor cells to differentiate will lead to fatal infection, bleeding or organ infiltration (Estey and Döhner, 2006).

AML patients with cytogenetic abnormalities of t (8,21) will present with specific biological and clinical characteristics and the criteria of diagnosis varies from different AML patients where the biological features that the leukemia showed are atypical in other AML subtype

and the prognosis after an intensive chemotherapy shows significant improvement in these patients compared to the majority of AML patients (Campo et al., 2011).

2.1.1 AML Classification

Two different classification systems that are frequently used in staging Acute Myeloid Leukemia which are the French-American-British (FAB) classification system (Table 2.1) that define a specific immunotypes based on the cells morphology observed and the World Health Organization (WHO) classification system(Table 2.2) that analyze the chromosome translocation and evidence of dysplasia (Rulina et al., 2010).

In FAB classifications, AML is subdivides into eight different subclasses which is M0-M7 and the patients will have different prognoses and receive different treatment depending on the subclasses (Bennett et al., 1976). While WHO classification of AML was established take into consideration of the FAB system. WHO classification is more conveniently use for clinical application because it take into account the most significant prognostic signs of the disease found in AML (Vardiman et al., 2002).

Table 2.1. French-American-British (FAB) classification of AML. (Tenen, 2003)

FAB subtype	Description	Comments
M0	Undifferentiated	Myeloperoxidase negative; myeloid marker positive
M1	Myeloblastic without maturation	Some evidence of granulocytic differentiation
M2	Myeloblastic with maturation	Maturation at or beyond the promyelocytic stage of differentiation; can be divided into those with t (8,21) AML1-ETO fusion and those without
M3	Promyelocytic	APL; most cases have t (5,17) PML-RARα or another translocation involving RARα
M4	Myelomonocytic	
$M4_{EO}$	Myelomonocytic with bone-marrow eosinophilia	Characterized by inversion of chromosome 16 involving CBFβ, which normally forms a heterodimer with AML1
M5	Monocytic	
M6	Erythroleukaemia	
M7	Megakaryoblastic	GATA1 mutations in those associated with Down's syndrome

Table 2.2. WHO classification of Acute Myeloid Leukemia. (Arber et al., 2016)

Acute myeloid leukemia (AML) and related neoplasms
AML with recurrent genetic abnormalities
AML with t (8,21) (q22; q22.1); RUX1-RUNX1T1
AML with inv (16) (p13.1q22) or t (16;16) (p13.1; q22); CBFB-MYH11
AML with PML-RARA
AML with t (9,11) (p21.3; q23.3); MLLT3-KMT2A
AML with t (6;9) (p23; q34.1); DEK-NUP214
AML with inv (3) (q21.3q26.2) or t (3;3) (q21.3; q26.2); GATA2, MECOM
AML (megakaryoblastic) with t (1;22) (p13.3; q13.3); RBM15-MKL1
Provisional entity: AML with mutated RUNX1
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, NOS
AML with minimal differentiation
AML without maturation
AML with maturation
AML myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis (TAM)
Myeloid leukemia associated with Down syndrome

2.1.2 Clinical and Hematological Characterization of AML

The appearance and development of leukemias during the initial stage is generally asymptomatic. Usually, the patients will feel themselves in good health until the spread of cancer cells occur throughout the hematopoietic system. Acute leukemia can be diagnosed through morphological examination of bone marrow or peripheral blood of patient for presence of blast tumor cells. The morphology of AML cells in bone marrow and peripheral blood shown in Figure 2.1 is characterized by large myeloblasts with plenty of basophilic cytoplasm and often containing azurophilic granules. Replacing normal hematopoietic stem cells with neoplastic cells will cause a decrease in range of blood cells such as anemia, thrombocytopenia, and neutropenia, the replacement will also leads to the manifestation of frequent infectious disease and mucosal ulcerations (Vorob'ev and Lorie, 2002).

In acute myeloid leukemia, presence of blast cells and a "leukemic gap'' can be seen in the peripheral blood leukocytosis. The ''leukemic gap'' is characterized by a drastic elevation in the number of blast cells and unique mature elements that exist along with the lack of transitional maturing forms. While in few cases of AML, the leukocyte number will remain the same but the blast cells will consistently present and if the blast cells are incapable of leaving the bone marrow, the number of blast cells will be rather high (Vorob'ev and Lorie, 2002). According to Rubnitz *et al*., on 2008, AML can be diagnosed depend on the existence of over 30% of myeloblasts present in the bone marrow (Rubnitz et al., 2008).

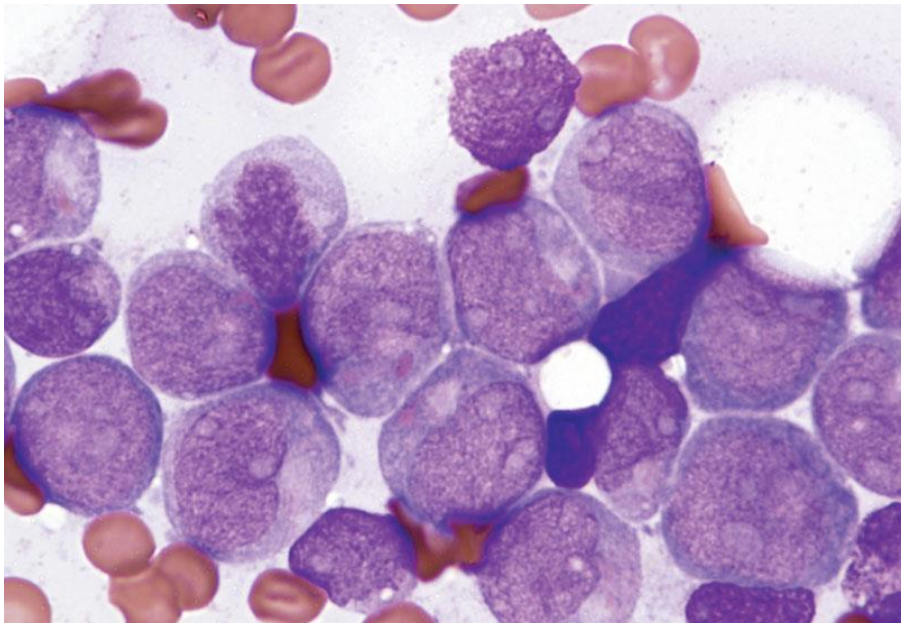

Figure 2.1. Acute Myeloid Leukemia with t (8,21) (q22; q22). Bone marrow blasts showing abundant granular cytoplasm with perinuclear clearing and large orange-pink granules. (1000x magnification)

2.1.3 Molecular Genetics of AML t (8,21)

Separation of cooperating mutations of different genes that results in the manifestation of AML will increase the cells proliferative ability and survival which have an impact on the normal differentiation and apoptosis process of cells precursors that incudes myeloid, erythroid, megakaryocytic and also monocytic lineage. Acute leukemias are made up of clonal cell line that arise from either a partially differentiated or a very early cell precursor towards different hematopoietic series in a single mutant hematopoietic cell. The clinical course of acute leukemia, therapy programs and its effectiveness can be determine through the relation between a definite hematopoietic series of blast cells and their level of differentiation (Grimwade et al., 1998).

Among the types of mutations involve in AML, Class II mutations are the mutations that affect the genes of transcription factors and cause alterations in the functions or activity of the genes and affects cell differentiation. The most frequent class II mutation that occurs in AML is the translocation of t (8;21) (q22; q22) and inversion (16) (p13; q22) and this translocation results in the formation of fused proteins AML1-ETO and CBFβ/MYH11 on the functional level (Wakita et al., 2011). Class II mutation is inadequate for cancer transformation therefore class I mutations which is a secondary genetic rearrangement is required to obtain the tumor phenotype. Class I mutation happens in the tyrosine kinases gene which cause the essential activation and thus cell proliferation and cell survival. Among the tyrosine kinases genes, tyrosine kinases *KIT* and *FLT3* genes are the genes that the AML mutations most commonly occur (Kelly and Gilliland, 2002).

2.1.4 Epidemiology of AML in Malaysia

According to forecast made by the World Health Organization (WHO), oncologic diseases may surpass cardiovascular diseases and become the leading killer disease in near future (Rulina et al., 2010). Cancer has become one of the major cause of death in Malaysia nowadays. As reported by the Malaysian National Cancer Registry Report (MNCR), there are a total of 103,507 new cancer cases were diagnosed in Malaysia during the duration of 2007 to 2011, of which 46,794 or 45.2% were reported in males and 56, 713 or 54.8% were reported in females. As per the report, the chances of males getting cancer was 1 in 10 while there is a risk of 1 in 9 for females.

Among the cancers reported shown in Table 2.3, from 2007 to 2011 leukemia is the sixth most common cancers occur in Malaysia population with the number of 4, 573 out of total cases of 103,507 or 4.4% of which 2, 549 or 5.4% were reported in males and 2, 024 or 3.65% were reported in females. It is the most common cancer among 0-14 years old with Malay had the highest rate followed by Chinese and Indian in both sexes. The lifetime risk for males was 1 in 275 and 1 in 348 for females.

According to the data collected by the Malaysian National Cancer Registry, AML is commonly occurring among elderly people as compared to other types of leukemia and as shown in Figure 2.2 and Figure 2.3, the average age of incidence of AML among females and males are between 60 to 75 years old. The statistical data also showed that AML is more commonly occur in males compare to females with males has a prevalence rate of 7 cases in every 100,000 people while females has a prevalence rate of 6 cases in every 100,000 people in population.

Table 2.3 Ten most common cancers in Malaysia population. (Adapted from Malaysian National Cancer Registry Report (MNCR) 2007-2011).

ICD-10	**Sites**	**Number**	**%**
C50	Breast	18,343	17.7
C18-C21	Colorectal	13,693	13.2
C33-C34	Trachea, Bronchus, Lung	10,608	10.2
C81-C85, C96	Lymphoma	5,374	5.2
C11	Nasopharynx	5,090	4.9
C91-C95	Leukemia	4,573	4.4
C53	Cervix Uteri	4,352	4.2
C22	Liver	4,128	4.0
C56	Ovary	3,472	3.4
C16	Stomach	3,461	3.3
	Others	30,413	29.4
	Total	**103,507**	**100.0**

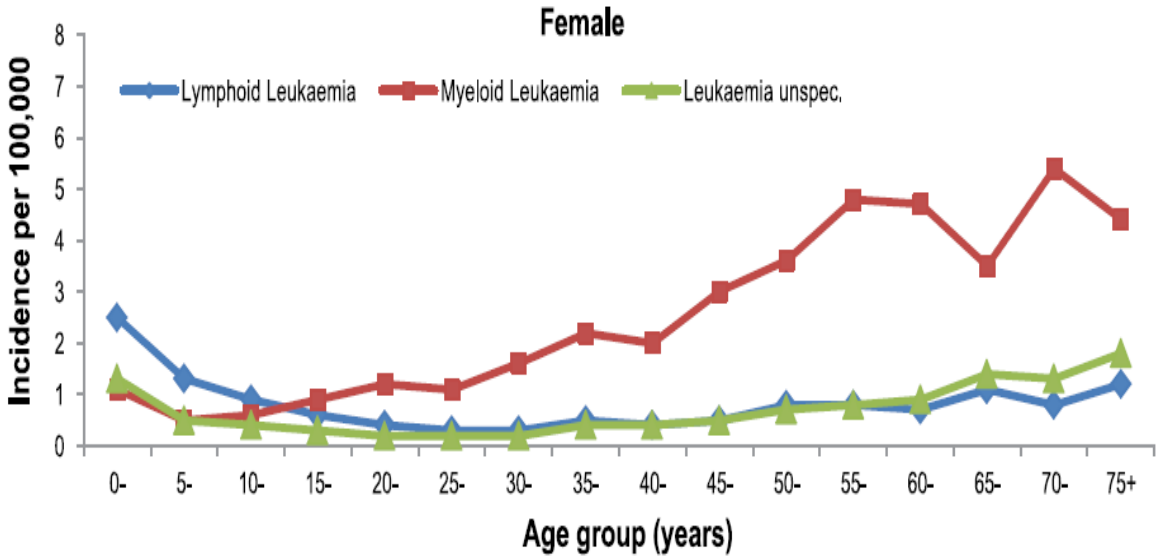

Figure 2.2 Leukemia types: Age-specific incidence rate, females, Malaysia, 2007-2011(Adapted from Malaysian National Cancer Registry Report (MNCR) 2007-2011).

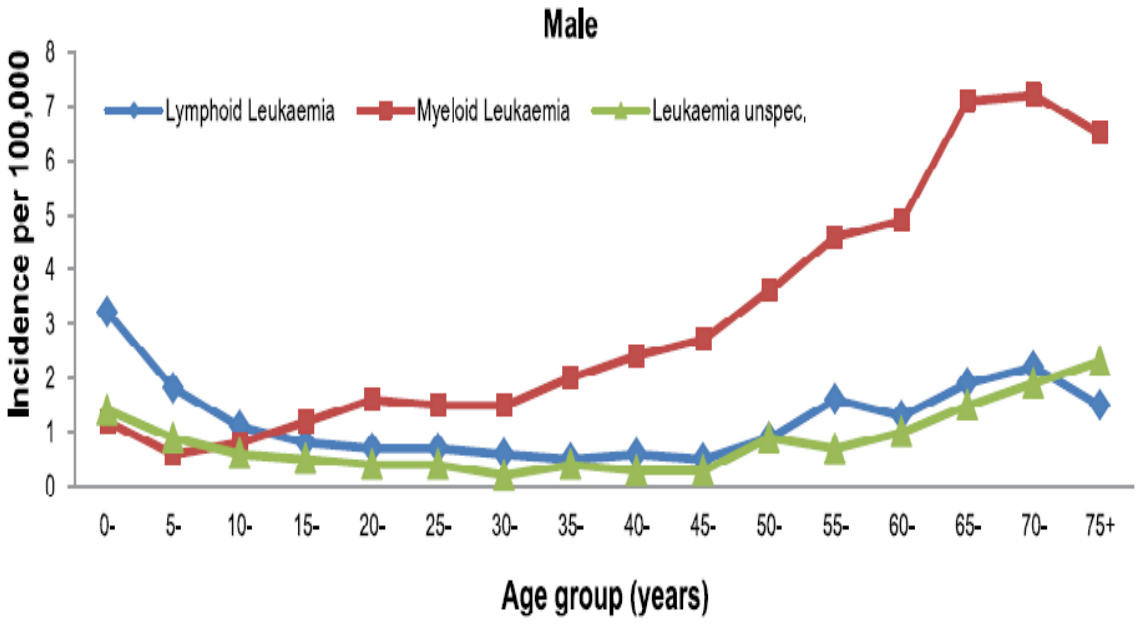

Figure 2.3 Leukemia types: Age-specific incidence rate, males, Malaysia, 2007-2011(Adapted from Malaysian National Cancer Registry Report (MNCR) 2007-2011).

2.2 *AML1/ETO* Gene

There are around 40% of M2 AML cases is associated with t (8,21) karyotypic abnormalities (Bitter et al., 1987) and this translocation has the most frequent chromosomal anomaly that can be found in 18-20% of leukemia cases (Look, 1997, Mitelman and Heim, 1992). The combination of AML1 gene that located at chromosome 21q22 with ETO gene located at chromosome 8q22 results in formation of AML1/ETO chimeric protein as shown in Figure 2.4 which carries a transcriptional activity (Rulina et al., 2010). This fusion protein of AML1-ETO encodes an initial 177 of AML1 amino acids that links to the ETO sequences (Erickson et al., 1992, Miyoshi et al., 1991). *AML1/ETO* is identify in blood and also in marrow sample of AML patient who achieve long-term complete remissions after treated with chemotherapy or hematopoietic stem cell transplantation (Miyamoto et al., 1995).

AML1/ETO fusion protein solely is inadequate in causing leukemia, however the downregulation in the expression of enzyme 8- oxoguanine DNA glycosylase (OGG1) which is involved in the DNA repair might cause the addition of genetic abnormalities that leads to development of AML (Liddiard et al., 2010). There are some experimental and clinical studies that draw attention to the importance of secondary mutations in AML1-ETO that mediates the development of leukemia. For instance, expression of *AML1/ETO* gene in mice will require an exposure to mutagen such as N-ethyl-N-nitrosourea or through a co-expression of constitutively active tyrosine such as TEL-PDGFRβ fusion for the development of AML (Higuchi et al., 2002, Grisolano et al., 2003).

Similarly, the clinical data also show high prevalence of secondary genetic alteration that will affects the tyrosine kinase signal transduction pathways in AML patient present with t (8,21) translocation. Based on the observation done by Wang *et al*., in 2005, approximately

50% of the involved AML patients that present with t (8,21) had point mutations in the tyrosine kinase gene *C-KIT* receptor (Wang et al., 2005). The hyperexpression of tyrosine kinase KIT receptor only occur in approximately 5% of AML patient but with the presence of *AML1/ETO* which results from the t(8,21) translocation frequency of mutation has shown an increased up to 30% (Peterson and Zhang, 2004) . The association between the two oncogenes was known for a certain of time. It was assumed that the presence of two activated oncogenes in cells for example transcription factor AML1-ETO and tyrosine kinase c-kit will function to promote their malignization.

Besides that, Wang *et al*., also discovered that majority of leukemia with t (8,21) translocation are more likely to overexpress c-Kit despite the mutational status. While in other study by Schessl *et al*., at the same year of 2005 found that more than one third of AML patient with t (8,21) translocation to conceal activated mutations in either *FLT3, C-KIT* or *NRAS* (Schessl et al., 2005). Furthermore, the study also showed *AML1/ETO* will cooperate with mutated *FLT3* to trigger the development of leukemia in transplanted mice and this emphasizes the importance of cooperation between expression of AML1/ETO and abnormal tyrosine kinase signaling in the development of leukemia (Schessl et al., 2005).

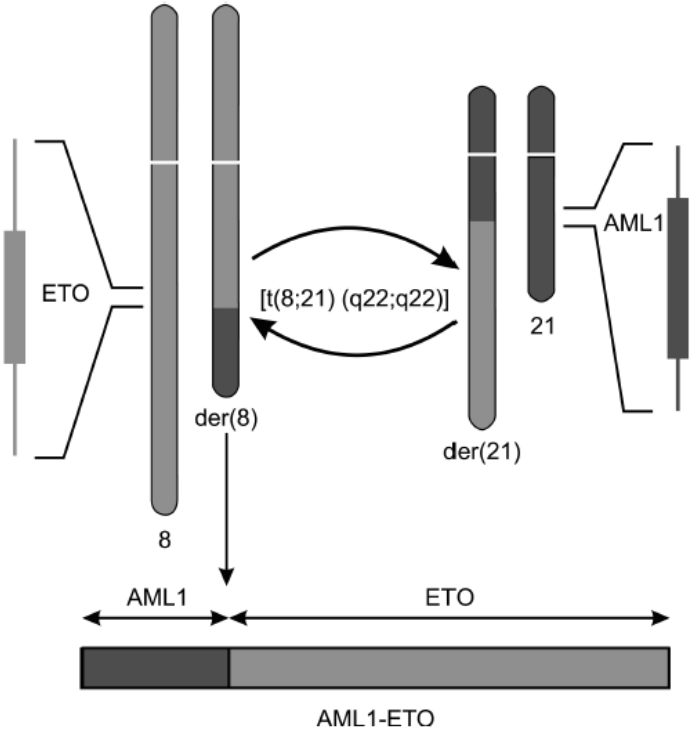

Figure 2.4. Translocation of t (8,21) (q22; q22) resulting in formation of fused gene *AML1/ETO*. (Modified from Rulina *et al*., 2010).

2.2.1 Classical Model of Leukemogenesis by *AML1/ETO*

In a corresponding model, RUNX 1 will commonly functioned as transcriptional activator that upregulate target genes from specific lineage such as myeloperoxidase to promotes differentiation of granulocytes. RUNX1 will go through a metamorphosis process of switching from activator to suppressor which will downregulate all of the target genes that involved in differentiation of cells from granulocytic lineage through the combination of RUNX 1 with ETO which result from the t (8,21) translocation. (Elagib and Goldfarb, 2007)

2.3 A Model Cell Line for AML t (8,21)

The Kasumi-1 cells is a human cells line that was obtained from peripheral blood sample of a 7-year old Japanese boy present with AML that has relapsed after a bone marrow transplant. The Japanese boy was diagnosed with M2 subtype AML at Matsuyama Red Cross Hospital (Matsuyama, Japan). The first incident of leukemia relapsed occurred after a complete remission that was achieved through chemotherapy. On the following relapsed, a bone marrow transplant was done on this boy at Hiroshima Red Cross Hospital (Hiroshima, Japan) collected from his HLA-matched sibling at the second complete remission after introduced by mitxantrone and cytosine arabinoside. The relapsed occurred 98 days after the transplantation where the engraftment was achieved and further application of chemotherapy failed. The patient died due to disease progression.

During the whole clinical course, there was no leukemic cells with cancer formation observed outside the marrow cavity. The blood sample was collected when the patient's leukocyte count was 99,800/μL with 93% blasts using a heparinized syringe. The Kasumi-1 cells were