UNIVERSITI TEKNOLOGI MARA

CORRELATION OF THE EXPRESSION WITH FLT3 AND RAC1 WITH THE LEUKEMIC FUSION GENE AML1-ETO IN ACUTE MYELOID LEUKEMIA t(8,21) SUBTYPE M2

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ABSTRACT

Acute Myeloid Leukemia is amongst the most known malignant disease involving accumulation and dysfunction of the cells within the hematopoietic lineage. There are several types of AML according to the FAB and WHO classification; we focused on a specific subtype expressing the leukemic fusion construct AML1-ETO (M2 subtype, FAB classification). The fusion construct, a transcription factor fusion is known to mediate cellular proliferation, apoptosis block, and cellular differentiation of AML M2. Experiments was carried out to unravel the control on FLT3 a receptor tyrosine kinase essential for proper hematopoietic development by AML1-ETO and RAC1 a protein essential for proper actin development and cellular migration. Initially we employ the antisense technology in downregulating the expression of the three genes mentioned. siRNA mediated gene knockdown was first carried on FLT3, RAC1 and AML1-ETO via electroporation respectively in order to observe correlation at transcript levels, measured via quantitative real time PCR. Phenotype changes were then measured, in our case cellular proliferation and overall cellular ROS production. The first study was to see correlation between previously reported ROS production model involving RAC1 and FLT3 in AML M2. Results obtained does not show any particular changes in the level of cellular ROS at maximal transcript downregulation of RAC1 and FLT3 therefore the model reported does not occur in AML M2. However, intriguing results were obtained via downregulation of AML1-ETO and RAC1 where this shows a reduction in FLT3 expression. To understand whether control of FLT3 via AML1-ETO occurs, prolonged AML1-ETO knockdown was implemented and results obtained indicates that the fusion construct activates FLT3 in AML M2 development and that RAC1 may be involved as a cofactor as direct downregulation of RAC1 resulted in the reduction in FLT3 transcript abundance. To validate whether control of FLT3 occur via AML1-ETO sequence alignment study between previously reported AML1-ETO immunoprecipitated DNA sequence and 1000 bp sequence located upstream of the FLT3 transcription start site (TSS) was done, result showed that AML1 binds a particular DNA fragment containing the AML1 binding DNA sequence on -50bp upstream of FLT3 TSS. Therefore, it could be postulated that control of FLT3 occur via synergy between AML1-ETO and RAC1

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INTRODUCTION

1.1 OVERVIEW OF THE STUDY: A BRIEF INSIGHT

Advances in molecular biology research allow us to better understand the molecular events in unveiling the mechanism of leukaemia. Leukaemia is generally understood as the cancer of white blood cells (WBC), where upon acquiring leukemia patients will have an accumulation of WBC inside their bone marrow and peripheral blood. The biology of leukaemogenesis is well understood where this would allow us to better postulate molecular events occurring in specific leukemia subtypes in order to come up with a therapeutic solution towards the disease.

Several factors contributes to leukemia, amongst them are mutation of core binding factor such as transcription effectors, chromosomal translocation leading to fusion genes which will result in translation of abnormal fusion proteins, mutation in the receptors essential for signalling in hematopoietic lineage differentiation, in frame mutation which causes either abnormal protein function or null proteins, chromatin disengagement due to abnormal activity of histone modifying enzyme and dysregulation of pathways essential in maintaining cellular activities.

Our study focused on the screening of possible gene interactions in order to better understand the underlying molecular mechanism of acute myeloid leukemia. We focused on a specific leukaemia, subtype M2 (French American British Classification) to understand the molecular mechanism of the frequently occurring *AML1-ETO* (*AE*) fusion gene resulting from chromosomal translocation involving the longs arms of chromosome 8 and 21. We concentrated on the interaction between the construct AE, FLT3 a receptor tyrosine kinase essential for AML differentiation and RAC1, generally understood to act as actin reorganizer.

AE is a fusion of two critical transcription factors essential in stable hematopoietic development. AML1 is a transcription factor responsible for activating transcription of several genes required for proper hematopietic development while ETO on the other hand, suppresses gene transcription by associating with histone deacetylases. It is crucial in our study to see whether regulation of *FLT3* occurs via