CHAPTER V

DISCUSSION AND CONCLUSION

TEL-AML1 translocation is an important prognostic factor for children with acute lymphoblasctic leukemia (ALL). TEL-AML1 positive patients have distinct clinical characteristics and have a more favorable outcome than TEL-AML1 negative patients. We found the prevalence of t(12;21) in 9 of 39 (23%) cases. Our finding is similar to 24% in Israel [21], 26% in St. Jude [38], 33% in Australia [46], 22% in Italy [48],19% in Taiwan [52], 20% in Japan [84] and 26% in Chicago [85]. The TEL-AML1 expression defines a uniform subgroup of ALL patients characterized by an age of one to ten years who have B-precursor immunophenotype. The TEL-AML1 positive patients had lower WBC counts than TEL-AML1 negative patients at diagnosis and there was no difference in the distribution of males and females. Since the follow up time for these patients was less than one year, it is premature to make a valid prognostic conclusion in our cohort of patients.

The TEL-AML1 product is important in establishing the transformed phenotype in these cases. This product consists of the HLH (Helix-loop-Helix) domain of TEL fused almost to the complete coding region of AML1. Although both genes are the frequent targets of other leukemia-associated translocations, the structure of this product suggests a primary role for altered AML1 activity in leukemogenesis in ALL cases. The mechanism of TEL-AML1 is not clear. However, the TEL-AML1 product may also function in leukemogenesis by abnormally regulating AML1 target genes in lymphoid cells. *AML1* is a member of a family of transcription factors, had been shown to regulate expression of a variety of genes. TEL-AML1 is also a transcription factor and can bind at the same site of AML1 target genes. We hypothesized that target gene expression of TEL-AML1 positive may be different from TEL-AML1 negative ALL. We selected five target genes (*IL-3, TCRγ, CR1, PKC, RAG1)* to study the TEL-AML1 role in their expression. The result did not support this hypothesis. No difference in expression were demonstrated in the relationships between target genes expression and TEL-AML1 expression in B-precursor cell ALL: *IL-3* expression (p=0.30), *TCRγ* expression

(p=1.00), *CR1* expression (p=0.71), *PKC* expression (p=1.00) or in *RAG1* expression (Fisher's exact tests).

We found that *IL-3* can be detected in both TEL-AML1 positive and TEL-AML1 negative. This finding is different from a previous report by Uchida *et al.* [81] who showed that 20 B-precursor ALL patient samples containing TEL-AML1 do not express *IL-3*. In the current study semi-nested RT-PCR was performed that increased the sensitivity of detection and the possibility of detecting *IL-3* expression. Their study did not indicate IL-3 expression, particularly in childhood B-precursor ALL, but the current study is specific in childhood. However, Uchida *et al.* did not study IL-3 expression in TEL-AML1 negative patients. Our study showed that IL-3 was detectable in 24 TEL-AML1 negative patients, but not in 6.

B-precursor cells do not normally express T-cell receptor. Though the samples were sorted with CD19 and CD10 monoclonal antibodies to include especially B-precursor cells so as not to detect *TCRγ* expression, we found that *TCRγ* was detectable in B-precursor ALL patients with or without the TEL-AML1 transcript. A previous report, by Germano G, *et al.*[86] who performed PCR-Gene Scan analysis of TCR gene rearrangement in bone marrow samples from 53 childhood precursor-B ALL patients showed *TCRγ* can rearrange at diagnosis and relapse. This is consistent with our study. The leukemic cells have clonal stability and they are clone-specific cells so B-precursor cells can express *TCRγ*. We found that there was no relationship between *TCRγ* expression and TEL-AML1 expression.

RAG1 expression was detectable in all samples, consistent with the observation that all lymphoid precursors have gene rearrangements. Our experiment found the RAG1 non-specific band that may cause PCR amplification. However, this problem was solved by using Southern blot hybridization analysis.

CR1 expression was shown in vitro reporter assay that TEL-AML1 effect to the promoter of gene and TEL-AML1 disrupts AML1-mediated transactivation on the *CR1*

promoter.[79,80] Our study in patient cells indicated that *CR1* expression can be detectable in the organization of both TEL-AML1 positive, and TEL-AML1 negative groups. It showed the real effect of TEL-AML1 on the *CR1* promoter. In the TEL-AML1 negative cells, it showed both *CR1* expression (n=17) and non- expression (n=13). The relationship between *CR1* expression and TEL-AML1 expression was not significant (p=0.71). However, 3 of 9 TEL-AML1 positive cells did not show *CR1* expression. The result indicates the possibility that TEL-AML1 may repress *CR1* promoter in patient cells.

PKC expression does not have any a relationship (p=1.00) with TEL-AML1 expression. In a previous study, *PKC* was reported to be expressed in relapse ALL patients.[77] So, if patient samples express TEL-AML1, there should not be *PKC* expression. However, in our TEL-AML1 positive cells, both *PKC* express (8 of 9) and does not express (only 1). It may be possible use *PKC* is prognosis marker along with TEL-AML1 in the future to predict relapse.

In summarize, there were no relationship in expression of TEL-AML1 and all target genes. However, we interested in target gene association. We determine the relationship by statistical analysis, which showed that each gene have no association. It may each target gene does not relate by nature or the sample size was too small.

The t(12;21) in our study emphasizes the importance of molecular genetics in the accurate diagnosis and classification of leukemia. In addition, in studying the prevalence of TEL-AML1 in ALL patients for predicting a favorable outcome. Moreover, the study of target gene activation led to understanding of the biological mechanisms of TEL-AML1 gene and its target genes. TEL-AML1 fusion protein is generally known as a transcription factor to the various target genes. Moos, *et al.*[87] used 20 best genes, which were involved in transcription, apoptosis and development, array element discrimination patients, with or without TEL-AML1 translocation by microarray. They indicate that there may be some other properties of samples such that they segregated about prognostic features when using multiple genes in analysis. However, our study used the expression of five target genes in studying TEL-AML1 positive and negative by RT-PCR. Five target

genes were not include in the 20 target genes in their study and it should be noted that 20 genes do not have any AML1 specific binding site on their promoters. The current study was a candidate gene approach to select the best multiple genes to study using other better methods to understand the role of TEL-AML1 and target genes and to use them as standard method in patient diagnosis in the future.

However a major level at which gene expression is controlled in the initiation of transcription. The transcription step is complex, with gene regulation at several different levels, transcriptional regulation emphasis on DNA sequence-specific transcription factors. Many factors are related to the control of gene expression especially in cancer cells, because cancers depend on many mechanisms. There are many factors that control the target gene promoter, for example, other transcriptions; *E2A-PBX1* from t(1;19), *BCR-ABL* from t(9;22). We should remind important other factors and include in next study.