CHAPTER II

REVIEW AND RELATED LITERATURE

Molecular genetics of ALL

ALL is considered to have a genetic basis; that is, somatically acquired genetic changes contribute in major ways to the pathogenesis of ALL and have important implications for diagnosis and treatment.[13,25-27] Acquired genetic lesions restricted to the leukemia clone include changes in both the number ploidy and structure of the blast cell chromosome.[13,28] The structural aberrations comprise reciprocal translocations, inversions, deletions, gene amplifications and point mutations. Although many of these abnormalities are detectable by routine cytogenetic analysis, others require molecular assay.[29] Molecular identification of the gene located at the sites of these aberrations has led to the isolation and characterization of numerous oncogenes and tumor suppressor genes providing valuable clues to the mechanisms of leukemogenesis.[5,14,30] These leukemogenic genes are normal genes that have become altered by mutations, fusion to other genes, rearrangement, or loss. The genes that are potentially leukemogenic in hematopoietic cells can be conveniently grouped into five families. The first consists of genes that convey growth-stimulating signals from the cell membrane to the nucleus. The second is composed of genes that activate transcription; the protein products of these genes bind to specific DNA sequence near target genes and enhancer the synthesis of messenger RNA. The third family comprises genes involved in tissue differentiation. The fourth consists of genes involved in programmed cell death. The fifth comprises anti-oncogenes that may normally function to suppress tumor development. The products of a gene may have more than one function, depending on the assay used to detect its biological activity.[5]

Molecular assays are used with cytogenetic analysis because the segment of DNA rearranged, lost, or mutated is submicroscopic; evidence of a translocation is seen in about 75% of acute leukemia cases.[13] The most frequent targets of chromosomal

translocations in the acute leukemias are genes that encode transcription factors, emphasizing the critical role of these regulatory proteins in the control of blood cell development.[31] Activation of transcription factor genes by chromosomal translocations takes two main forms.[14] The situation in which a proto-oncogene is juxtaposed to an immunoglobulin or T-cell receptor gene by chromosomal fusion with joining or diversity segments, thereby activating the oncogene. Immunoglobulin or T-cell receptor genes are frequently involved in chromosomal aberrations because they are naturally rearranged to generate active antigen-receptor genes. This process occasionally, in error, leads to an interchromosomal translocation or inversion. On the other hand, typifies the situation in which breakage on each chromosome occurs within introns of genes, producing fusion genes and subsequently leading to expression of the fusion protein with unique properties.[13]

Chromosomal translocations

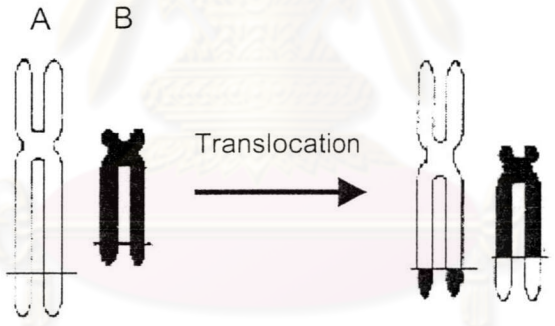

Figure 1 Model of chromosome translocation. A and B represent each chromosome.

Chromosomal translocations occur within a gene on each chromosome involved, creating a fusion gene encoding a chimeric protein. The genes involved often encode transcription factor, indicating that altered transcription plays a major part in tumorigenesis.[14] A chromosome translocation is a condition in which a small piece of one chromosome switches pieces with a small piece of another chromosome. There are carrier of a balanced translocation appears, for almost all purposes are normal, since there is no missing or extra chromosome material. Normally, there are 23 pairs of chromosomes making a total of 46. In each pair, one of the chromosomes comes from

each parent. It turns to make sperm or eggs contribute only 23 chromosomes to the future pregnancy egg or sperm end up. Many pregnancies that result from chromosome imbalances miscarry before the woman is even aware that she has conceived. It usually noted that about one-third will be normal, one third will carry the balanced translocation, and one-third will likely miscarry from an unbalanced chromosome translocation.[32]

The various types of human leukemia are almost certainly determined both by the nature of the oncogenes and anti-oncogenes involved and by the level of differentiation of the hematopoietic stem cell in which the genetic alterations occur. Some genes are found in a wide variety of cancers, whereas others are associated with specific types of leukemia.[29]

Table 1 Genetic abnormalities in leukemia.[5]

Functions	Molecular alteration	Chromosomal abnormality	diseases	Reference
Signal transduction	N-ras mutations	None	AML	Bos et al.
Membrane signal transduction	*C-ABL/BCR*	t(9;22)(q34;q11)	CML,ALL	Konopka et al, Ahuja et al.
Differentiation and gene transcription	*HOX-11/TCR*	t(10;14)(q24;q11)	T-ALL	Kennedy et al.
Tumor supression	*P53*	Del 17	Blast crisis of CML	Ahuja et al.
Gene transcription	*MYC/Ig*	t(8;14)(q24;q23)	Burkitt's lymphoma	Tsujimoto et al.
			Pre-B ALL	Croce et al.
	E2A/PBX	t(1;19)(q23;p13)	Pre-B ALL	Inaba et al.
	SCL/SIL	t(1;14)(p32;q11)	T-ALL	Bernard et al.
	TAL-2/TCR	t(7;9)(q35;p13)	T-ALL	Xia et al.
	TEL-AML1	t(12;21)(p21;q22)	Pre-B ALL	Romana et al.

AML ; Acute myeloid leukemia, ALL ; Acute lymphoblastic leukemia,

CML ; Chronic myeloid leukemia, T-ALL ; T-cell lymphoblastic leukemia

Many translocations have been found in ALL patients; for example, *E2A-PBX1* from t(1;19), *E2A-HLF* from t(17;19), *BCR-ABL* from t(9;22) and the most common translocation in ALL is *TEL-AML1* from t(12;21).[15]

TEL-AML1 translocation

TEL is identified as a region on the short arm of chromosome 12 (12p13) The TEL mRNA sequence (GENBANK accession number U11732) is shown below :

```
1    tcctgatctc tctcgctgtg agacatgtct gagactcctg ctcagtgtag cattaagcag
61   gaacgaattt catatacacc tccagagagc ccagtgccga gttacgcttc ctcgacgcca
121 cttcatgttc cagtgcctcg agcgctcagg atggaggaag actcgatccg cctgcctgcg
181 cacctgcgct tgcagccaat ttactggagc agggatgacg tagcccagtg gctcaagtgg
241 gctgaaaatg agttttcttt aaggccaatt gacagcaaca cgtttgaaat gaatggcaaa
301 gctctcctgc tgctgaccaa agaggacttt cgctatcgat ctcctcattc aggtgatgtg
361 ctctatgaac tccttcagca tattctgaag cagaggaaac ctcggattct tttttcacca
421 ttcttccacc ctggaaactc tatacacaca cagccggagg tcatactgca tcagaaccat
481 gaagaagata actgtgtcca gaggaccccc aggccatccg tggataatgt gcaccataac
541 cctcccacca ttgaactgtt gcaccgctcc aggtcaccta tcacgacaaa tcaccggcct
601 tctcctgacc ccgagcagcg gcccctccgg tccccctgg acaacatgat ccgccgcctc
661 tccccggctg agagagctca gggacccagg ccgcaccagg agaacaacca ccaggagtcc
721 taccctctgt cagtgtctcc catggagaat aatcactgcc cagcgtcctc cgagtcccac
781 ccgaagccat ccagcccccg gcaggagagc acacgcgtga tccagctgat gcccagcccc
841 atcatgcacc ctctgatcct gaacccccgg cactccgtgg atttcaaaca gtccaggctc
901 tccgaggacg ggctgcatag ggaagggaag cccatcaacc tctctcatcg ggaagacctg
961 gcttacatga accacatcat ggtctctgtc tccccgcctg aagagcacgc catgcccatt
1021gggagaatag cagactgtag actgctttgg gattacgtct atcagttgct ttctgacagc
1081cggtacgaaa acttcatccg atgggaggac aaagaatcca aaatattccg gatagtggat
1141cccaacggac tggctcgact gtggggaaac cataagaaca gaacaaacat gacctatgag
1201aaaatgtcca gagccctgcg ccactactac aaactaaaca ttatcaggaa ggagccagga
1261caaaggcttt tgttcaggtt tatgaaaacc ccagatgaaa tcatgagtgg ccgaacagac
1321cgtctggagc acctagagtc ccaggagctg gatgaacaaa tataccaaga agatgaatgc
1381tgaaggaacc aacagtccac ctcagcgggc cagcagccca gggaacccct gcccaccagg
1441attgctggaa gtgtgacgga gcaggcgggc tgaggagagt ggaaaaggaa gcgacccaga
1501aatggcaggg acacttctct tgcagaccaa gagggaccct ggagcacctt agacaaacta
1561cccagcacag gcggggctgg
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The *TEL* gene encodes a new member of the ETS family of transcription factors.[32] The TEL gene (Translocation, Ets, Leukemia, and also known as ETS-Variant Gene 6 or ETV6) is a member of the Ets Family of transcription factors that is characterized by a c-terminal winged helix-loop-helix DNA-binding domain referred to as the ETS domain.[33] The TEL consists of 8 exons. Like other members of this family, TEL functions as a sequence specific DNA-binding transcriptional regulator.[34] TEL also contains on N-terminal helix-loop-helix (HLH) domain called the pointed domain, that is found in only a subset of Ets family members.[35] TEL was first identified as part of the TEL-platelet derived growth factor receptor beta fusion (TEL-PDGFRβ) that is created by the t(5;12)(q33;p13) in chronic myelomonocytic leukemia (CMML).[36] Moreover, TEL is translocated with other genes, such as *TEL-ABL*, *TEL-JAK2*, and *MN1-TEL*.[37,38]

The *AML1* (Acute Myeloid Leukemia 1) gene is also known as *CBFA2* (Core Binding factor Alpha 2). The *AML1* mRNA sequence (GENBANK accession number U19601) is shown below :

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1    atggcttcag acagcatatt tgagtcattt ccttcgtacc cacagtgctt catgagagaa
61   tgcatacttg gaatgaatcc ttctagagac gtccacgatg ccagcacgag ccgccgcttc
121  acgccgcctt ccaccgcgct gagcccaggc aagatgagcg aggcgttgcc gctgggcgcc
181  ccggacgccg gcgctgccct ggccggcaag ctgaggagcg gcgaccgcag catggtggag
241  gtgctggccg accacccggg cgagctggtg cgcaccgaca gccccaactt cctctgctcc
301  gtgctgccta cgcactggcg ctgcaacaag accctgccca tcgctttcaa ggtggtggcc
361  ctaggggatg ttccagatgg cactctggtc actgtgatgg ctggcaatga tgaaaactac
421  tcggctgagc tgagaaatgc taccgcagcc atgaagaacc aggttgcaag atttaatgac
481  ctcaggtttg tcggtcgaag tggaagaggg aaaagcttca ctctgaccat cactgtcttc
541  acaaacccac cgcaagtcgc cacctaccac agagccatca aaatcacagt ggatgggccc
601  cgagaacctc gaagacatcg gcagaaacta gatgatcaga ccaagcccgg gagcttgtcc
661  ttttccgagc ggctcagtga actggagcag ctgcggcgca cagccatgag ggtcagccca
721  caccacccag cccccacgcc caaccctcgt gcctccctga accactccac tgcctttaac
781  cctcagcctc agagtcagat gcaggataca aggcagatcc aaccatcccc accgtggtcc
841  tacgatcagt cctaccaata cctgggatcc attgcctctc cttctgtgca cccagcaacg
901  cccatttcac ctggacgtgc cagcggcatg acaaccctct ctgcagaact ttccagtcga
961  ctctcaacgg cacccgacct gacagcgttc agcgacccgc gccagttccc cgcgctgccc
1021 tccatctccg acccccgcat gcactatcca ggcgccttca cctactcccc gacgccggtc
1081 acctcgggca tcggcatcgg catgtcggcc atgggctcgg ccacgcgcta ccacacctac
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1141ctgccgccgc cctaccccgg ctcatcgcaa gcgcagggag gcccgttcca agccagctcg
1201ccctcctacc acctgtacta cggcgcctcg gccggctcct accagttctc catggtgggc
1261ggcgagcgct cgccgccgcg catcctgccg ccctgcacca acgcctccac cggctccgcg
1321ctgctcaacc ccagcctccc gaaccagagc gacgtggtgg aggccgaggg cagccacagc
1381aactccccca ccaacatggc gccctccgcg cgcctggagg aggccgtgtg gaggccctac
1441tga

AML1 encodes 472 amino acid.[39] AML1 is a region on the long arm of chromosome 21 (21q22) which was identified in 1991 by cloning t(8;21) or AML1-ETO translocation which is translocation about acute myeloid leukemia; subtype M_2 .[40] Cloning of the AML1 gene led to identifying the AML1 transcription factor that was found in both myeloid and lymphoid leukemia. AML1 encodes a nuclear transcription factor (TF) which shows homology in its five parts with the *Drosophila Melanogaster* segmentation gene runt and contains a transactivation domain in the carboxy terminal portion.[41] AML1 consists of 8 exons and is translocated in human leukemia with many genes, for example, *AML1-ETO, AML1-EAP, AML1-MDS1 and AML1-EVI1*.[41] The normal AML1 protein forms the α-subunit of the heterodimeric TF core binding factor (or CBF) whose β-subunit is encoded by the CBFβ gene on chromosome 16q22. CBFβ is rearranged and fused to the MYH11 gene in the AML M4Eo-associated inv(16) aberration. Thus the two most common AML chromosome abnormalities are t(8;21) and inv(16). This suggests that the wild type CBF must exert an important role in the control of myeloid cell growth and differentiation, so AML1 is a regulator of hematopoiesis.[42]

The *TEL-AML1* translocation is the most common genetic alteration found in pediatric B-precursor ALL.[43-45] The TEL-AML1 fusion is created by t(12;21). Because this translocation exchanges chromosome tips, it could be detected by standard karyotyping in less than 0.05% of ALL cases, and is therefore called a cryptic translocation.[46] TEL-AML1 gene translocation was found in subtype B-precursors ALL but not in T-ALL (T-Cell Acute leukemia), AML (acute myeloid leukemia) or Non-Hodgkin's lymphoma.[33] This translocation has been demonstrated in approximatety 20–25% of all childhood ALL patients by molecular technique.[21,43,47-49,50-52] The TEL-AML1 fusion gene disrupts the TEL gene in the region of intron 5 (exon 5), nucleotide 1033 and

most of the AML1 gene (exon 2).[53] The resulting chimeric protein fuses the helix-loop-helix (HLH) domain of TEL, a member of the ETS-like family of transcription factor, to the DNA-binding runt homology domain and its c-terminal transactivation domains of AML1. Expression of this chimeric gene is driven by the ubiquitous *TEL* promoter.[54,55] Both TEL and AML1 are involved in a variety of other leukemia-associated translocations. TEL-AML1 fusion protein functions as a transcription factor.

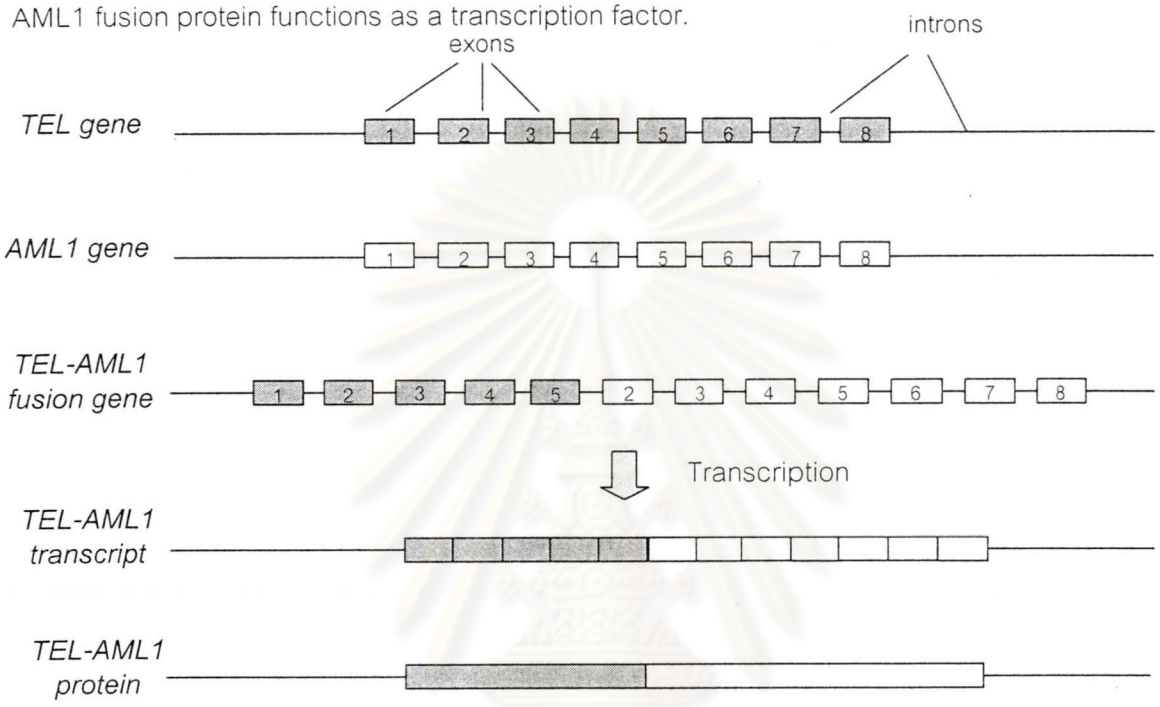

Figure 2 Schematic representation of TEL-AML1 fusion transcript

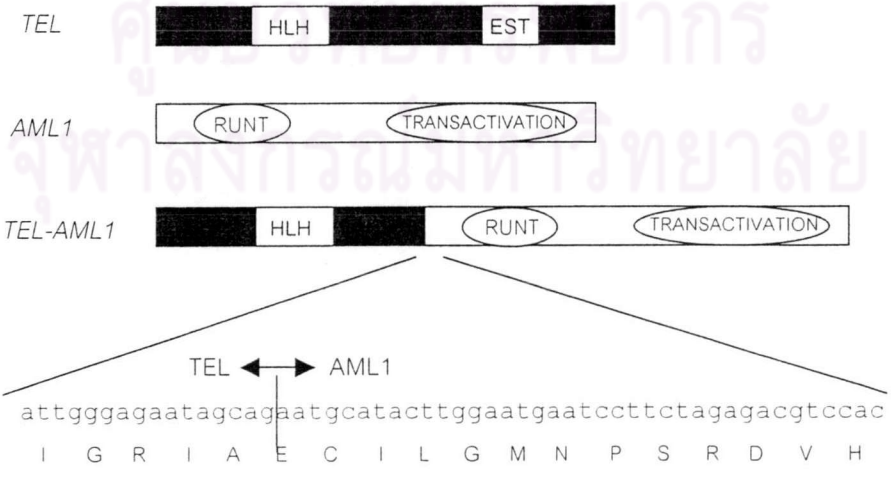

Figure 3 *TEL-AML1* translocation breakpoint

Mechanisms of transformation : TEL-AML1

The structure of TEL-AML1 suggests that it retains the ability to bind to the AML1 DNA target sequence and interact with core binding factor beta (CBFβ).[22] Moreover, this chimeric protein should retain the normal function of the HLH domain of TEL. Thus, the chimeric TEL-AML1 protein is predicted to alter the normal functions of endogeneous AML1 and TEL and to acquire novel functions as a result of the fusion of these different domains into a single molecule.[33] TEL-AML1 can directly repress AML-mediated transcriptional activation. This activity requires both the HLH domain of TEL and the DNA-binding domain of AML1.[19,22] Analysis of the transcriptional regulatory functions of the t(12;21) fusion protein led to the suggestion that TEL acts as a transcriptional repressor.[56] TEL are fused to most of AML1, a RUNT family protein; AML1 is reported to be either an activator or a repressor of transcription.[41] AML1 is one of the most frequently mutated genes in human leukemias. It is targeted directly by the t(8;21), t(16;21) and t(3;21) in various forms of acute myeloid leukemia (AML), as well as by the t(12;21) in B-cell ALL.[41] Each of these translocations results in the formation of a dominant negative fusion protein that interferes with normal transcriptional activation by AML1.[57] AML1 is expressed in hematopoietic tissues and recognizes the DNA sequence TGTGGT. TEL-AML1 protein still binds the enhancer core motif, TGTGGT, and interacts with the AML1 binding protein, core-binding factor beta.[58] The enhancer core motif is required for the tissue-specific transcription of a number of genes, including those encoding the T-cell receptor (TCR) alpha, beta, gamma, and delta enhancers, Myeloperoxidase, Granulocyte macrophage colony-stimulating factor, and Interleukein-3 promoter.[22] The transcription repression mechanism of TEL-AML1 is not clear and although some possible mechanisms are suggested to explain the negative effect of the chimeric TEL-AML1 protein, no direct biochemical evidence has been demonstrated.

Previously, Arthur Zelent *et al.* studied the mechanism of TEL-AML1 and found that interaction between TEL and N-coR, a component of the nuclear receptor core repressor complex with histone deacetylase activity, requires the central region of TEL, which is retained in TEL-AML, and TEL lacking this domain is impaired in transcriptional

repression. It may be concluded that TEL-AML1 recruits N-CoR to AML1 target genes and thus imposes and altered pattern of their expression.[59] Moreover, Hiebert *et al.* reported that the conserved pointed domain of TEL, present in the TEL-AML1 fusion protein, acts as a portable transcriptional repression domain when this TEL domain is active. They showed that the pointed domain of TEL is sufficient for association with the mSin3A corepressor; proteins have a key role in transcriptional repression mediated by histone deacetylation. Evidence showed that TEL-AML1 also interacts physically with mSin3A. Furthermore, both AML1 and TEL sequence contribute to this mSin3A association.[60]

Transcription regulation of gene expression

Primary control of gene regulation occurs at the level of initiation of transcription. Regulation of expression can occur through the core promoter of a gene, at the level of recruitment and processivity of the relevant RNA polymerase. Expression of genes is initiated by the binding of transcription factors to the promoter. Basal levels of transcription can be modulated by the binding of protein factors to other regulator regions occurring in the sequence flanking the gene. The protein factors engaged in regulating gene expression are themselves encoded by distantly located genes. They are required to migrate to their site of action, and so are called *tran*-acting factors. In contrast, the regulatory sequences to which they bind are on the same DNA or RNA molecule as the gene or RNA transcript that is being regulated. Such sequences are said to be *cis*-acting.[61]

A molecular basis of the control of gene expression is the binding of protein factors to regulatory nucleic sequences. There is a variety of transcription factors, such as TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH, etc, which can be complex in structure. The complex of polymerase and general transcription factors is known as the basal transcription apparatus; it constitutes all that is required to initiate transcription. Genes are constitutively expressed at a minimum rate determined by the core promoter unless the rate of transcription is increased or switched off by additional positive or negative

regulatory elements. In addition, general transcription factors or tissue-restricted transcription factors regulate the expression of many genes which encode polypeptide by recognizing and binding specific cis-acting sequence elements.[62]

Target genes

Interleukin-3 (IL-3)

Interleukin-3, also known as multilineage colony-stimulating factor (multi-CSF) in one of the cytokines that acts during the early and late stages of blood cell formation.[63] IL-3 is a member of the family of CSFs and serves as an important growth and differentiation factor for most immature cell lineages in the blood and bone marrow.[64] IL-3 is expressed by mitogen or antigen-activated T lymphocytes and natural killer cells. IL-3 supports the growth and differentiation of early hemopoietic progenitors and acts synergistically with more restricted cytokines to promote erythroid, myeloid, mixed and megakaryocytic colonies.[65]

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1261ctcggccatc caccagaaac aaagtgtcaa ggagaagctg cccgaagccc atgggacaaa
1321ccactgggga ctggaacacc agtaattctg tattgggaag cggcaccaag agatgtgctt
1381ctcagagcct gaggctgaac gtggatgttt agcagcgtga ccggctacca gacaaactct
1441catctgttcc agtggcctcc tggccaccca ccaggaccaa gcagggcggg cagcagaggg
1501ccagggtagt ccaggtgatg gcagatgaga tcccactggg caggaggcct cagtgagctg
1561agtcaggctt ccccttcctg ccacaggggt cctctcacct gctgccatgc ttcccatctc
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1741catgtcagat aaagatcctt ccgacgcctg ccccacacca ccacctcccc ccgccttgcc
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1921ctgcccgtcc tgctcctgct ccaactcctg gtccgccccg gactccaagc tcccatgacc
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2041acacacttaa agcagccacc tttgcctttg ctggtgagta gcttggataa gactggcctg
2101cagcagtgag gggtggtggc tgcctaaggc caaaaggcct catgggcctt tctctccctt
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Figure 4 Partial nucleotide sequence of *Interleukin-3* promoter region (GENBANK accession number AF365976). The TEL-AML1 binding region is indicated by a solid line.

T Cell Receptor Gamma (TCRγ)

The human γ locus is organized similarly to the TCRβ chain locus with two JC clusters containing five J segments and two C segments in total. The γ protein is present on T cells and cooperates with δ proteins that express the CD3 proteins but do not produce αβ receptors. The γ chain is composed of transmembrane glycoproteins with structures similar to the α and β chains. The percentages of T cells expressing the γδ TCRs vary widely, depending on tissue and specificity. T cell receptor provides cell-mediated immune responses to foreign antigens. The unique arrangement of gene segments in the TCR gene clusters reflects the very unusual way in which somatic recombinations are required in T lymphocytes before functional TCR genes can be assembled and then expressed.[66]

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1   caaccagact taagaaagat caatttaatt tagatctact ccttgattct catgaattga
61  ttgaatctct ttagggcaaa taacctggcc tctatagaga aatctggaat gattttctgc
121 actgggagag gaaactttca gatcactaag ggctttgtgg ggctgatatc accagccgag
181 gtactgggca atgtctcctg tggttcaca cttcttagcg atttccttcc ccaacgcaag
241 ctgggctgcc ccgaacttca gcaatgtaga gagagaatgc agtggtggtg gtggaggagg
301 cagagtgggt ggtgggaggg cgctgatgtg gcggatggtg ttttattttc tcaggcttct
361 tttgtttacg tgctctggca aatgtggttg attcatggaa aatgctcaaa gtcaaaccgg
421 cccctactgg ttggggctgt gcatggggtg gctagggtgt tgtaagacaa aacgaggaca
481 gttaaaccac aaccaacttt gctcactttc aagagcccac agctaatgga aataaaatat
541 ccatcttcac acatacaaga ttactatcaa acacactctc cacctgtgg taggtggggc
601 tccctgcatt tgatattcaa ggttcattag ctaccaggtg agttgggaat ctgatgccca
661 ggactgttca gacatggcac cttcggtcca ctcccatgac actgtcctga agaccagagg
721 acatcttgca gcacggagat gatcctaaca ttccacccag tatctgtacc ttccagaggc
781 acctgattca gagacactaa taccttggt ttcatttcct cagtgtaaac agggtgacac
841 aagactcatg aacctattag cacaggtaga tcacagatgt gggcaacagt gatgtgcttc
901 tttctccaca ggaaaccagg gatatagaag ccct
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Figure 5 Partial nucleotide sequence of *T cell receptor gamma* enhancer region (GENBANK accession number S71037). The TEL-AML1 binding region is indicated by a solid line.

Complement receptor type 1 (CR1)

The human Complement receptor type 1 (CR1, C3b/C4b receptor) is a single chain membrane glycoprotein that is a member of the group of regulators of the complement activation system.[67] The human CR1 gene is expressed specially in hematopoietic cells, expressed on erythrocytes, monocytes, neutrophils, B cells, some T cells, follicular dendritic cells and glomerular podocytes. It is capable of binding C4b and C3b fragments of complement, functioning on phagocytic cells to facilitate the ingestion by these cells of particles that have activated complement.[68]

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1501cacggagagc aggcatttca ttagctgacc ttcccacaca cattcttgca aagaggaaaa
1561gttaagcagg gtgtttggag gcgagctgcc atcatccacc gcctttgtct ggaagcgcag
1621ggcctcacac gcgggatcca tcggaagccc gagcattgtc aagctctgct gctgcacctg
1681ggtcagcaag gtgggctctg ccagcgaaac tcgttagaaa caatgcaaat ggggagtaaa
1741catgacctcg cccatgaagg ggaagctgtg gtcaaaagca ttttgtcccg gaaccccgca
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2041ggtgagaggc gggcgggcgt ggggaggcgc ccgggcggac gaggaacccg gggccccgca
2101gagaactcgc gtgcagcgct gagctgcgct gctctgcgcg cccgggtccg aaggcagcgc
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Figure 6 Partial nucleotide sequence of human *complement receptor type 1* promoter region (GENBANK accession number L17390) The TEL-AML1 binding region is indicated by a solid line.

Protein Kinase C (PKC)

Protein Kinase C activation and/or modulation of its isoenzyme expression play key roles in regulating the response of hematopoietic cells to both growth factors and non-physiogical inducers of cell growth and differentiation.[69] Protein kinase C proteins have been shown to be involved in diverse cellular responses of various cell types.[70] Protein kinase C has been shown to be involved in the mitogenic response and in oncogenic cell transformation in many experimental models.[71] Protein kinase C has a

crucial role in signal transduction for a variety of biologically active substances that activate cellular growth regulation, proliferation, differentiation and malignant transformation.[72,73]

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121 agatccttag gacagatgac ggcacctgga gatattttaa taatgtagat accctcttgc
181 tgttcaaact cagaccaaaa gagatggctt tttttccccc agagggtgca caaatacgac
241 agaatttgtg aagacgagtc agaaatgaat gaaatttgga aaaatattga tctactgaaa
301 tccttcctcc ccacactatt agccctatgt tacagttggg gaaacggagt cgttttgcag
361 aggggatgga cagaaggtag ggagttctct tccaacgtgc aggaggcaag caaagccaag
421 catcttctct gtggtggagt tagagacata taaaataaga tcgctcctcc cctacctctg
481 cagaacgtgt gtgtgtatgt gtgtgtaacg tgtgtgcggc cacaagcctt tccgaatgag
541 tgacagcggg agcccatccc tccaggagac gcgtgcagaa tgaccaatgg gatggatggg
601 ggtggatggg taccgtctcc gcgaggccgg ggtggaattc gctgcgcccc acccttcca
661 cccgctcccc ttcgccccgt aggtctttcc actctcgctc ctcccctggg cacatctcct
721 gaacgcagcc ccgggggccg aggacggggt ggggtggggg gcgaggctcg ggtccgacga
```

Figure 7 Partial nucleotide sequence of *Protein kinase C* promoter region (GENBANK accession number X62532) The TEL-AML1 binding region is indicated by a solid line.

Recombination Activating Gene 1 (RAG1)

RAG1 encode a lymphocyte-specific enzyme that catalyzes V(D)J recombination, which is dependent on the recombination activating genes shoes expression is strictly limited to immature lymphoid cells. The gene products of recombination gene, RAG1 is crucial for rearrangement process.[74] The rearrangement of the Ig genes is an important step in the development of B-cells. The development of B and T cells is organized around V(D)J recombination, the assembly of Immunoglobulin and T-cell receptor (TCR) genes from component V(variable), D(diversity) and J(joining) gene segments. Two periods of V(D)J recombination, at the pro- and pre-lymphocyte stages of development, lead to expression of the B-cell receptor (BCR) and T-cell receptor (TCR) on the surface of immature B and T cells, respectively. The essential, lymphocyte-specific components of the V(D)J recombination machinery are encoded by

the recombination activating gene, RAG1. The RAG proteins initiate recombination by recognizing and cleaving chromosomal DNA at specific recombination sites.[75]

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1    attcttagct ctcacctgcc tttctcgcca tgttgcctca aatatgaccc tcaggtaaag
61   gctgcatctc cttggggaaa aacacactga agctcctaga agccacagac cagtttctcc
121  tctctcccag tgttactgca gtggcttctg ccttgtcttt agtgagcagc tgtggcccca
181  gggctgttgt gtggttaagt aggtccaaat gcgaaagagt gggagatgta agaggagcgt
241  cgaatgaggg cagcacagaa cagtgaaatc cttgcacctg cctccttgac agtttttgct
301  gagaatcaga caagcaagga atctctcact tattagctga acacagaaag actttgggga
361  taaagctggt ctgagaatta taaaggggtt ttgaaacaga gacagaggtg tgtatacacc
421  tattcattct tgaattatcc ttagactatt actacaaagt ggctttcatt attctccttt
481  catggtataa tggagtgatt aggaacatga gctttggaat tccaccccac cttcactaag
541  tagtggctgt gtgaccatgg gcaagtcact ccattgctct gaacttctgt tgcttctttt
601  tagacataat attatcaaat gcgtatgact gttgtgaaaa ttaaatgaca cataaaacac
661  ttacaagcct agcccattgc tctcaataat ggggactaat attattaatc ttaggttgca
721  ggtgatgaga ttgaagttcc taaagtttaa gtaatgaatc aaaggcctga gtcaagattt
781  aaatccaggt ctgttgatgt ccatactcga gtaatgttta aacttacagt agttacaagg
841  agcatcaacc ttcctcagca agatttgacc aggttgaaag gttctgagat tgtttggata
901  acatttcaag ataataataa aaaaattcca tagtgttgtt aatatctgat gaacatctac
961  aaagaggcag acatcgtttc aagtattttc ctataataac tgtcattgta taacattatt
1021 gactctttga ggtatatact actagccttt ccttttaca gatgtggaac tgaggcacag
1081 agatatagaa ccctggcaaa agctggggct tatactgact gacaagacca tttccaaccc
1141 aaacattctc agggagggaa ctggcagcca gtgccccagc aaactctcgc acatggttct
1201 acactcaggc ccccctgagc taagcttcct aaagagccag gtggcagctg gagctggggt
1261 ctcctggccc atgattggct gccatcattt gtggttagcc ctccatggtg ggggaggctg
1321 ggaaggacag tggaagctga taaacagctc agcagcatgt tctgagaaac aagagggcaa
1381 ggagagagca gagaacacac tttgccttct ctttggtatt gagtaatatc aaccaaattg
1441 cagacatctc aacactttgg ccaggcagcc tgctgagcaa g
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Figure 8 Partial nucleotide sequence of *Recombination activating gene 1* promoter region (GENBANK accession number AF014844) The TEL-AML1 binding region is indicated by a solid line.

TEL-AML1 and target genes

TEL-AML1 fusion protein is generally known as a transcription factor to the various target genes. The target genes of TEL-AML1 transcription factor have the DNA

sequence TGTGGT on the regulatory region. A number of genes have this region including *Interleukin-3 (IL-3), T-cell receptor gamma (TCRγ), Complement receptor type 1 (CR1), Protein kinase C (PKC), Recombination activating gene 1 (RAG1)*. All of these genes are related with leukemogenesis. Previous studies have shown that the *RAG1* gene was expressed in all precursor-B ALL but not in B-CLL[76] and *RAG1* is related with B-cell development by rearrangement of Ig genes in important steps. *Protein kinase C* was revealed to have a relationship with developing relapses in ALL patients.[77] *T-cell recepter gamma (TCRγ)* is similar to the TCRβ chain locus and it is an enhancer core motif for tissue-specific transcription[22]. Uchida *et al.*[78] demonstrated that TEL-AML1 act as repressors of basal transcription of T-cell receptor beta enhancer in cell lines.[35] However, that study did not report about *T-cell receptor gamma* in the patients. In the same way, reporter gene assays have shown that TEL-AML1 protein inhibits the transactivation activities of AML1 protein on the human *CR1* promoter, even though TEL-AML1 retains the transactivation domain of AML1.[79,80] Finally, fusion protein TEL-AML1 repressor was consisted with human ALL cells that contain TEL-AML1 do not express *IL-3*.[81]

To gain insight into the mechanism of TEL-AML1, we studied the expression of five target genes of TEL-AML1 in pediatric ALL samples from Thai patients with TGTGGT at the regulatory region of the genes, to determine the relationship between target gene expression in TEL-AML1 positive, and TEL-AML1 negative, patients.