Expression of Transcription Factor LYL1 In Acute Leukemia

Thesis submitted for the partial fulfillment of M.D degree in clinical pathology

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Abstract

The LYL1 gene identified by non-random chromosomal translocation t(7;19)(q35;p13) associated with T-cell acute lymphoblastic leukemia (T-ALL), was mapped to the short arm of chromosome 19 (19p13) by in situ hybridization.

LYL1 is required for fetal and adult hematopoietic stem cell function and B-cell differentiation. LYL1 may play a very important role in leukemogenesis.

Over expression of LYL1 is implicated in the pathogenesis of T-ALL as well as myeloid malignancies. The LYL1 protein is a transcription factor (TF), structurally and functionally similar to another bHLH protein TAL1/SCL which is also implicated in T-ALL. Their expression may have clinical relevance and important role as risk and prognostic factors in acute leukemia. They may be useful as predictive test for treatment outcome in acute leukemia patients.

Key Words:

LYL1, Acute leukemia

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List of Abbreviations

Abbreviation	The Full Term
aa	Amino acid
TF	Transcription factor
LYL1	Lymphocyte derived sequence 1
ALL	Acute lymphoblastic leukemia
AML	Acute myeloblastic leukemia
DBDs	DNA binding domains
ANLL	Acute non lymphocytic leukemia
GTFs	general transcription factors
APL	Acute promyelocytic leukemia
Ara-C	Cytosine arabinoside
Arg	Arginine
Asp	Aspartate
ATL	Adult T cell leukemia/ lymphoma
Bcl-2	B cell lymphoma-2
BCR	Breakpoint cluster region
bFGF	Basic fibroblast growth factor
BM	Bone marrow
BMSCs	Bone marrow stromal cells
bp	Base pair
C-ALL	Common acute lymphoblastic leukemia
cAMP	Cyclic adenosine monophosphate
CBC	Complete blood count
CBFbeta	Core binding factor beta
CD	Cluster of differentiation
CEPs	Circulating endothelial progenitor cells
CI	Confidence interval
SRY	sex- determining region Y

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Introduction

Leukemias, myelodysplastic syndromes (MDS), lymphomas and multiple myeloma (MM) are complex diseases with a wide range of clinical, morphologic, biologic, cytogenetic, molecular and immunophenotypic features ***(Estey, 2001)***. With this multitude of disease-associated variables, it is not surprising that response to treatment differs considerably among patients ***(Bennett, 2000)***. Although significant progress has been made in the management of these disorders, the majority of patients with leukemia or lymphoma who fail front-line therapies or who relapse after an initial response, die from progressive disease. No effective therapy has been developed for the majority of patients with advanced myeloproliferative diseases (MPD). As the relative ineffectiveness and toxicities of traditional cytotoxic therapies become more appreciated, the search for therapeutic advances is increasingly focused on affecting the critical steps involved in the development and mutation of malignant clones ***(Fragoso et al., 2005)***.

The Lymphoplastic Leukemia derived sequence 1 (LYL1) gene, encodes a basic helix loop helix (bHLH) transcription factor which is important for many cell decisions as proliferation & differentiation. ***(Ferrando et al ,2004).***

Aberrant gene expression is the major mechanism in oncogenesis. In cases with T-ALL it has been found that LYL1can be aberrantly expressed as a result of chromosome translocation involving 19p13 the site at which LYL1 resides or in the face of cytogenetically normal chromosome 19. It was originally identified at the breakpoint of chromosomal translocation t(7;19)(q35;p13) found in some cases of T-

ALL. The translocation brings the LYL1 gene under the control of the T cell antigen receptor beta gene (TCR-beta) resulting in ectopic expression of LYL1 ***(Ferrando et al, 2002).***

T-ALL which express LYL1 tend to have a more immature phenotype with high level of expression of genes such as BCL2, cyclin D and CD34 an expression pattern found in many cases of Acute myeloid leukemia(AML) ***(Asnafi et al, 2004).***

Recently a high level of LYL1expression was observed in AML and Myelodysplastic syndromes(MDS)(CMML or RAEB) in comparison with normal bone marrows, The exact mechanism by which LYL1 is highly expressed in AML is not known, may be relevant in leukomogenesis as well as transformation from chronic myeloproliferative disorder to acute leukemia ***(Meng et al, 2005).***

Aberrant expression of LYL1 in MDS and AML plays a role in development and phenotype of the disease, by altering the differentiation potential of the cells, increasing the growth rate and decreasing the drug sensitivity. These observations indicate that LYL1 or its downstream molecules are potential targets in the treatment of AML or MDS***(Meng et al, 2005).***

Aim of the Work

The present work aims to study the expression of LYL1 on the transcriptional level in leukemic blast cells isolated from newly diagnosed AML and ALL patients and to examine their prognostic significance.

Transcription Factors in Hematopoiesis

Definition:

Transcription factor is a protein that binds to specific sequence of DNA and thereby controls the transfer of genetic information from DNA to RNA. Transcription factors perform this function alone, or with other proteins in a complex ,by promoting, or blocking the recruitment of RNA polymerase(the enzyme which activates the transcription of genetic information from DNA to RNA) to specific genes ***(Gilbert.,2008).***

A defining feature of transcription factors is that they contain one or more DNA binding domains (DBDs) which attach to specific sequences of DNA adjacent to the genes that they regulate ***(Ptashne et al.,1997).***

Additional proteins such as co activators, chromatin remodelers, histone acetylases, deacetylases, kinases and methylases, while also playing crucial roles in gene regulation, lack DNA binding domains, and therefore are not classified as transcription factors ***(Brivanlou et al.,2002).***

Conservation in different organisms

Transcription factors are essential for the regulation of gene expression and consequently are found in all living organisms. The number of transcription factors found within an organism increases with the genome size and the larger genomes tend to have more transcription factors per gene ***(Van.,2003).***

There are approximately 2600 proteins in the human genome that contain DNA-binding domains and most of these are presumed to function as transcription factors ***(Babu et al., 2004).***

Approximately 10 % of genes in the genome code for transcription factors which makes this family the single largest family of human proteins. Genes are flanked by several binding sites for distinct transcription factors and efficient expression of each of these genes requires the cooperative action of several different transcription factors. Hence the combinatorial use of a subset of the approximately 2000 human transcription factors easily accounts for the unique regulation of each gene in the human genome during development ***(Brivanlou et al.,2002).***

Mechanism

Transcription factors bind to either enhancer or promoter regions of DNA adjacent to the genes that they regulate. Depending on the transcription factor, the transcription of the adjacent gene is either up or down regulated. Transcription factors use a variety of mechanisms for the regulation of gene expression ***(Gill., 2001).***

These mechanisms includes :

- Stabilize or block the binding of RNA polymerase to DNA.
- Catalyze the acylation or deacylation of DNA . The transcription factor can either do this directly or recruit other proteins with this catalytic activity. More especially many transcription factors use one or the other of two opposing mechanisms to regulate transcription:

1. Histone acetyltransferse (HAT) activity- acetylate DNA which weakens the association of DNA with histones which make the DNA more accessible to transcription and upregulate transcription .
2. Histone deacetylase (HDAC) activity- deacetylate DNA which

strengthens the association of DNA with histones which make the DNA less accessible to transcription and down regulate transcription ***(Narlikar et al., 2002).***

- Recruit co activator or corepressor proteins to the transcription factor DNA complex ***(Xul et al., 1999).***

Biological roles

Transcription factors are one of the groups of proteins that read and interpret the genetic **(blue print)** in the DNA. They bind DNA and help initiate a program of increased or decreased gene transcription. They are vital for many important cellular processes. Below are some of important functions and biological roles transcription factors are involved in:

- *Basal transcription regulation*

In eukaryotes, an important class of transcription factors called general transcription factors (GTFs) are necessary for transcription to occur ***(Shilatifard et al., 2003).***

Many of these GTFs do not actually bind DNA but are part of the large transcription pre-initiation complex that interacts with RNA polymerase directly ***(Reese., 2003).***

The most common GTFs are TF11A, TF11B, TF11D, TF11E, TF11F, TF11H. The pre-initiation complex binds to promoter regions of DNA upstream to the gene they regulate ***(Thomas et al., 2006).***

- *Differential enhancement of transcription*

Other transcription factors differentially regulate the expression of various genes by binding to enhancer regions of DNA adjacent to regulated genes.

These transcription factors are critical to make sure that genes are expressed in the right place at the right time and in the right amount depending on the changing requirements of the organism ***(Gilbert., 2008).***

- *Development*

Many transcription factors in multicellular organisms are involved in development ***(Lobe., 1992).*** Responding to stimuli, these transcription factors turn on\off the transcription of the appropriate genes which in turn allows for changes in cell morphology or activities needed for cell fate determination and cellular differentiation. The Hox transcription factor family, for example, is important for proper body pattern formation in organisms as diverse as fruit flies to humans ***(Lemons et al., 2006).***

Another example is the transcription factor encoded by the sex-determining region Y(SRY) gene which plays a major role in determining gender in humans ***(Ottolenghi et al., 2007).***

- *Response to intercellular signals*

Cells can communicate with each other by releasing molecules that produce signaling cascades within another receptive cell. If the signal requires up regulation or down regulation of genes in the recipient cell, often transcription factors will be downstream in the signaling cascade ***(Pawson., 1993).***

Estrogen signaling is an example of a fairly short signaling cascade that involves the estrogen receptor transcription factor : estrogen is secreted by tissues as the ovaries and placenta, crosses the cell membrane of the recipient cell, and is bound by the estrogen receptor in the cell's cytoplasm. The estrogen receptor then goes to the cell's nucleus and binds to its DNA binding sites, changing the transcriptional regulation of the associated genes ***(Osborne et al.,***