Interphase FISH Analysis of 9p21 Deletion in Childhood Acute Lymphoblastic Leukemia

Thesis

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List of Abbreviations

ALL	: Acute lymphoblastic leukemia
AML	: Acute myeloid leukemia
AP	: Acid phosphatase
AT	: Ataxiatelangiectasia
BM	: Bone marrow
CALLA	: Common ALL antigen
CBC	: Complete blood cell count
CD	: Cluster of differentiation
CGH	: Comparative genomic hybridization
CML	: Chronic myelogenous leukemia
CR	: Complete remission
CSF	: Cerebrospinal fluid
DW	: Distilled water
EBV	: Epstien-Barr virus
EDTA	: Ethylene diamine tetra acetic acid
FAB	: French, American, and British
FCL	: Folliclular center lymphoma
FCS	: Fetal calf serum
FISH	: Fluorescence in situ hybridization
FLT3	: FMS-related tyrosine kinase-3
Hb	: Hemoglobin
HM	: Hepatomegaly
HS	: Highly significant
Ig	: Immunoglobulin
IPT	: Immunophenotyping
IR	: Incomplete remission
LDH	: Lactate dehydrogenase
LN	: Lymphadenopathy
LOH	: Loss of heterozygosity
MALT	: Mucosa-associated lymphoid tissue
MPO	: Myeloperoxidase
MRD	: Minimal residual disease
MTAP	: Methylthioadenosine phosphorylase
MTS-2	: Multiple tumor suppressor 2

List of Abbreviations (Cont...)

NB	: Neuroblastoma
NOS	: Not otherwise specified
NS	: Non- significant
PB	: Peripheral blood
S	: Significant
SBB	: Sudan black
SIg	: Surface immunoglobulin
SM	: Splenomegaly
SSC	: Standard saline citrate
TCR	: T cell receptor
TdT	: Terminal deoxynucleotidyl transferase
TF	: Transcription factor
TK	: Tyrosine kinase
TLC	: Total leucocytic count
TSG	: Tumor suppressor genes
WBC	: White blood cell
WCPP	: Whole chromosome painting probe
WHO	: World Health Organization

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بِسْمِ اللّهِ الرّحمَنِ الرّحيمِ

(...رَبِّ أَوزِعنِي أَن أَشكُرَ نِعمَتَكَ الّتِي أَنْعَمْتَ عَلَيَّ و عَلى والِدَيَّ

و أَنْ أَعْمَلَ صَالِحاً تَرْضَاهُ و أَدْخِلْنِي بِرَحْمَتِكَ فِي عِبَادِكَ الصَّالِحِينَ)

صدق الله العظيم

النمل.. اية رقم ١٩

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a malignant disease resulting from the accumulation of genetic alterations of B or T lymphoid precursor cells ***(Meshinchi and Arceci, 2007).*** It is considered the most common cancer among persons under 15 years of age and accounting for > 30% of all childhood malignancies ***(Ries et al., 1999).***

Pediatric ALL is one of the success stories of cancer research and treatment. Not only this fatal disease is now curable in the majority of patients, who have access to appropriate therapy and support ***(Pui, 2004)***, but much of its cellular and molecular biology has been uncovered ***(Cogen and Franco, 2003; Pui et al., 2004).***

A number of clinical and laboratory features evident at diagnosis, have prognostic value for predicting the outcome of patients treated for ALL. The identification of these prognostic factors has provided a mean of stratifying patients into different risk groups and "tailoring" treatment accordingly ***(Carrol, 2003; Bhatias, 2004).***

Molecular characterization of the genetic changes has yielded a wealth of information on the mechanism of leukemogenesis. These findings have also allowed the development of sensitive techniques such as fluorescence in situ hybridization (FISH) for identification of underlying molecular defects, which can be applied to evaluate disease prognosis,

monitor response to treatment and predict minimal residual disease ***(Lee et al., 2007).***

Genetic alterations of tumor suppressor genes (TSG) such as p53, RB gene, p15 and p16 contribute to leukemic transformation of hemopoietic stem cells or their committed progenitors by changing cellular functions ***(Krug et al., 2002; Berlin et al., 2003).***

Alterations of the 9p21 locus have been implicated in many types of cancer including ALL, indicating a role for the tumor suppressor genes CDKN2A (MTS1) and CDKN2B (MTS2), which encode for $p16^{INK4a}$ and $p15^{INK4b}$, respectively. Loss of cell proliferation control and regulation of the cell cycle are known to be critical to cancer development ***(Sarina et al., 2009)***.

Both $p16^{INK4a}$ and $p15^{INK4b}$ tumor suppressor genes counteract the activity of cyclins, cyclin dependent kinase (cyclin D – CDK 4/6 complexes) and other regulatory proteins of the cell cycle such as p53 and pRb. Dysregulation of cell cycle control is crucial in the development and progression of ALL. Thus, deletions of p16 or p15 located at 9p21 may play a role in leukemogenesis, have a prognostic significance and provide new targets for therapeutic interventions ***(Lee et al., 2007; Lin et al., 2007; Sarina et al., 2009).***

AIM OF THE WORK

The present work aims to detect del 9p21 in pediatric ALL patients, by FISH technique; in order to evaluate the impact of such deletion on patients response to therapy and to correlate it to standard prognostic factors.

ACUTE LYMPHOBLASTIC LEUKEMIA

DEFINITION:

Acute lymphoblastic leukemia (ALL) is a malignant (clonal) disease of the bone marrow in which early lymphoid precursors proliferate and replace the normal hematopoietic cells of the marrow. ALL may be distinguished from other malignant lymphoid disorders by the immuno-phenotype of the cells, which is similar to B- or T-precursor cells. Immunochemistry, cytochemistry, and cytogenetic markers may also aid in categorizing the malignant lymphoid clone ***(Seiter, 2013).***

ALL is considered the most common cancer in children and is among the most curable of the pediatric malignancies ***(Alison et al., 2006).***

INCIDENCE AND EPIDEMIOLOGY:

I. Age

ALL is the most common cancer diagnosed in children and represents 23% of cancer diagnoses among children younger than 15 years. There has been a gradual increase in the incidence of ALL in the past 25 years. A sharp peak in ALL incidence is observed among children aged 2 to 3 years (>80 per million per year), with rates decreasing to 20 per million for ages 8 to 10 years. The incidence of ALL among children aged 2 to 3 years is approximately fourfold greater than that for infants and is nearly tenfold greater than that for adolescents aged 16 to 21 years ***(Shah and Coleman, 2007).***