CEBPA expression levels as a prognostic marker in Acute Myelogenous Leukemia

Thesis Submitted In Partial Fulfillment of
Master Degree
In Clinical and Chemical Pathology

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2009

بسم الله الرحمن الرحيم

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ACKNOWLEDGMENT

First and foremost I feel always indebted to **GOD** the Kindest and Most Merciful. I would like to express my deepest gratitude and deep thanks to **Prof. Nehal Salah Aldin**, I would like to express my sincere thanks to **Prof. Dr. Amina AbdAlwahed**, I am profoundly indebted to **Dr. Zeinab Ali**, Finally I would also like to express my deepest appereciation to all my colleagues in the laboratory unit of NEMROCK, and in the Department of Clinical Oncology, for their support and encouragement.

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBEREVIATIONS

AME	AML1-MDS1-EVI1 fusion protein.
AML	Acute myeloid leukaemia
AMLSG	German-Austrian AML Study Group
APL	Acute promyelocytic leukemia
Ara-C	Cytosine Arabinoside
ATP	Adenosine Triphosphates
ATPases	Enzymes involved in ATP hydrolysis
ATRA	All-trans retinoic acid
BAL	Biphenotypic acute leukemia
BAALC	Brain and acute leukemia gene cytoplasmic
BaP	Basophil progenitor
Bcl	B-cell leukemia/lymphoma
BCP-ALL	B-cell precursor-acute lymphoblastic leukemia
Bcr-Abl	c-Abl= codes for a tyrosine kinase protein Bcr= Breakpoint Cluster Region
BM	Bone marrow
β-ME	β-Mercaptoethanol
BMCP	Basophil/mast cell progenitor
BR	Basic region
Brm	Brahma
bZIP	Basic region and leucine zipper region
CBF	Core-binding-factor
Cdk	Cyclin-dependent kinase
C/EBPA	CCAAT/enhancer binding protein alpha
CLL	Chronic lymphocytic leukaemia
CLP	Common lymphoid progenitor
CML	Chronic myeloid leukemia
CMP	Common myeloid progenitor
CN	Cytogenetically normal
CR	Complete remission
CRD	CR duration
CSF	Cerebro-spinal fluid
DFS	Disease free survival
DIC	Disseminated intravascular coagulopathy
E2F	Transcription factor involved in cell-cycle regulation and synthesis of DNA in mammalian cells
EFS	Event-free survival
EGIL	European Group of Immunological Classification of Leukemias
EL	Erythrocyte Lysing

EoP	Eosinophil progenitor
ERG	V-ets erythroblastosis virus E26 oncogene like (avian)
ETO	Eight-twenty-one- according to the gene located on chromosome 8 which is involved in translocation with the AML1 gene, also named RUNX1 on chromosome 21
Ets transcription factors	Family named according to first member discovered as part of avian E26 (E-twenty-six) retrovirus genome
EVI1	Ecotropic viral integration site 1 transcription factor
FAB	French American-British
Flt3	Fms-like tyrosine kinase 3
FLT3-ITD	Internal tandem duplication of the fms related tyrosine kinase 3 (FLT3) gene
Fms	Oncogene of the McDonough strain of feline sarcoma virus
GPCR	Gharbiah Population-based Cancer Registry
G-CSF	Granulocyte-colony stimulating factor
GM-CSF	Granulocyte-Macrophage-Colony stimulating factor
GMP	Granulocyte-Monocyte progenitor
GSK-3	Glycogen synthase kinase-3
HLA-DR	Human Leucocytic Antigen-DR
HSC	Haematopoietic stem cell
LZ	Leucine zipper region
IACR	International Agency of Cancer Research
IGH	Immunoglobulin heavy chain locus
IL	Interleukin
ITD	Internal tandem duplication
IκB	Protein family of inhibitors of nuclear factor kappa B
JMD	Juxtamembrane domain
LDH	Lactate dehydrogenase
NPM1	Nucleophosmin 1
Mad	Transcription factor, an antagonist of the c-Myc transcription factor
Max	A member of the basic region-helix-loop-helix-leucine zipper protein family
MCP	Mast cell progenitor
MDS	Myelodysplasia syndrome
MEP	Megakaryocyte/Erythroid Progenitor
MLL	Myeloid/lymphoid or mixed-lineage leukemia () gene
MLL-PTD	Partial tandem duplication of the myeloid/lymphoid or mixed-lineage leukemia gene
MPDs	Myeloproliferative disorders
MPO	Myeloperoxidase
MRD	Matched related donor

NRAS	Neuroblastoma RAS viral oncogene homologue
NF-κB	Nuclear factor kappa B
NPM1	Nucleophosmin (nucleolar phosphoprotein B23, numatrin)
OS	Overall survival
Pax	Paired box family of transcription factors
PI3K	Phospatidylinositol 3-kinase
PP2A	Protein phosphatase 2A
PTD	Partial tandem duplication
PTMs	Post-translational modifications
PU.1	A member of the Ets transcription factors that is expressed specifically in myeloid and B cells
Rb	Retinoblastoma protein
RFS	Relapse-free survival
RUNX1	Runt-related transcription factor 1 gene
RT-PCR	Real-Time Polymerase Chain Reaction
SBB	Sudan Black B
SC	Synergy control
SUMO-1	Small ubiquitin related modifier-1
SWI/SNF	A nucleosome remodelling complex composed of several proteins-products of the SWI and SNF genes as well as several other polypeptides
TAD	Transactivation domains
Taq	Thermus aquaticus
TBP	TATA box-binding protein
TFIIB	Transcription factor which binds directly to TBP and recruits RNA polymerase II
TKD	Tyrosine kinase domain
UPD	Uniparental disomy
WHO	World Health Organization
WT1	Wilms tumor gene

Abstract

Background:

The transcription factor CCAAT/enhancer binding protein Alpha (C/EBPA) is a myeloid specific transcription factor that regulates the balance between cell proliferation and differentiation in haematopoietic and non-hematopoietic tissues. C/EBPA plays a major role during the commitment of hematopoietic stem cells towards granulocytic and monocytic differentiation. Impairments in C/EBPA signalling such as reduced mRNA and protein expression are often observed in human myeloid leukemias which may subsequently contribute to leukaemic transformation ***(Trivedi et al, 2007)***. In the last few years, various mechanisms have been suggested through which C/EBPA is negatively regulated in certain AML FAB subtypes.

Objectives:

Studying the heterogeneity of CEBPA m-RNA expression among different FAB subtypes of newly diagnosed AML patients, as a first step in unraveling the impact of newly discovered genetic abnormalities on the pathophysiology of AML.

Patients and Methods:

Fourty nine AML patients were enrolled in the study, 19(38.8%) males, 30(61.2%) females and mean age 32 years (range 2–77). Controls included 20 healthy subjects, 11(55%) males and 9(45%) females with mean age 33 years (range 16–59). The diagnosis of AML was established according to morphology and immunophenotyping (FAB classification). The quantitative assessment of C/EBPA gene expression in AML patients at diagnosis and healthy subjects was performed using RT-PCR.

Results:

CEBPA expression levels among the ***Healthy population*** and ***AML patients*** had an average expression of (1.41±1.14 & 0.52±1.13 respectively, p=0.001). The majority of our study population (40/49) had ***Low*** CEBPA expression levels (range 0.001-0.547). Few (6/49) cases showed ***Intermediate*** expression levels (range 0.812-1.866) while only 3 cases showed ***High*** expression level (range 2.828-5.278). Clinically, Low expression subgroup had poor survival rates while Intermediate and High subgroups had better outcome.

Conclusion:

Using real-time PCR we were capable of defining three prognostic subgroups, that is, high, intermediate and low CEBPA mRNA expressing patients. Accordingly, CEBPA expression analysis should be carried out on a larger cohort prior to treatment to predict AML patient's outcome.

Keywords:

AML: acute myeloid leukemia

CEBPA: CCAAT/enhancer binding protein Alpha

FAB:French-American-British

CONTENTS

Introduction

Introduction

The transcription factor CCAAT/enhancer binding protein A (C/EBPA) is a myeloid specific transcription factor that regulates the balance between cell proliferation and differentiation in haematopoietic and nonhematopoietic tissues ***(McKnight et al, 2001)***. C/EBPA is encoded by an intronless gene and maps to human chromosome 19q13.1; C/EBPA is a member of the basic region leucine zipper (bZIP) class of DNA-binding proteins ***(Fuchs et al, 2007)***. C/EBPA plays a major role during the commitment of hematopoietic stem cells towards granulocytic and monocytic differentiation ***(Dahl et al, 2003)***. A critical role for the function of C/EBPA in granulopoiesis was demonstrated in mice harboring a disruption of the C/EBPA gene ***(Wang et al, 1995)***. These mice show a selective early block in granulopoiesis, with the appearance of many myeloid blasts in foetal liver and peripheral blood ***(Zhang et al, 1997)***.

C/EBPA mutations have been observed in AML patients with the approximate frequency of 5-14% ***(Shih et al, 2005, 2006)***. The mutations can be largely divided into two common types. First, carboxy-terminal in-frame mutations disrupt the basic zipper region that is responsible for the formation of homodimers or heterodimers with other C/EBP proteins as well as with transcription factors of other families to precisely modulate the transcription of target genes ***(Lekstrom and Xanthopolus, 1998)***.

Second, amino-terminal frame shift mutations result in premature termination of the normal 42 KDa form of the C/EBPα protein while preserving the 30 KDa form that acts as inhibitor of the C/EBPA p42 mediated transactivation of transcription of target genes resulting in induction of proliferation ***(Zhang et al, 2004)***.

Decreased expression of C/EBPA but not the mutation has been shown in patients with granulocytic leukaemias that are associated with translocations t(8;21) ,inv(16) or t(15;17) .Derived fusion proteins repress C/EBPA expression. Differentiation therapy of some AML types is based on restoring C/EBPα function. C/EBPA expression may be a biomarker for early detection of AML and DNA modifying drugs such as demethylating agents and/or histone deacetylase inhibitors could be used in the treatment ***(Fuchs et al, 2007)***.

Review of Literature