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Expression of Livin and its prognostic significance in acute leukemia

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List of Abbreviations

AIDS	Acquired immune deficiency syndrome
AIF	Apoptosis inducing factor
ALL	Acute lymphoid leukemia
AML	Acute myeloid leukemia
APL	Acute promyelocytic leukemia
BCL2	(B-cell leukemia/ lymphoma)2
BIR	Baculoviral IAP repeat
CARD	Caspase-associated recruitment domain
CBFB-MYH11	Core binding factor protein beta-myosin 11 fusion genes
CD	Cluster of differentiation
CEBPA	CCAAT/enhancer-binding protein alpha encoding gene
cIAP	Cellular inhibitor of apoptosis protein
CNS	Central nervous system
CR	Complete remission
CT	Threshold cycle
DAPI	Diamidine phenyl indole
DIABLO	Direct IAP binding protein with low pI

DNA	Deoxyribonucleic acid
dNTP	Deoxy nucleotide triphosphate
DR	Death receptor
EFS	Event free survival
FAB	French – American – British
FCS	Fetal calf serum
FISH	Florescence in situ hybridization
FITC	Fluorescein isothiocyanate
FLT3	Fms-like tyrosine kinase receptor-3
FSC	Forward scatter
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
Hb	Hemoglobin
HLA-DR	Human leukocyte antigen
HTLV-1	Human T-cell Lymphotropic Virus Type 1
IAP	Inhibitor of apoptosis proteins
IPT	Immunophenotyping
ITD	Internal tandem duplications
JNK-1	Jun N terminal kinase
kDa	Kilo Dalton
KIAP	kidney inhibitor of apoptosis protein

LDH	Lactate dehydrogenase
LSI	Locus specific identifier
MDS	Myelodysplastic syndrome
ML-IAP	Melanoma inhibitor of apoptosis protein
MLL	Mixed lineage leukemia
MoAB	Monoclonal antibody
MPD	Myeloproliferative syndrome
MRD	Minimal residual disease
NAD	Nicotineamide adenine dinuclutide
NAIP	Neuronal apoptosis inhibitor protein
NGF-R	Nerve growth factor receptor
NK	Natural killer
NPM1	Nucleophosmin
NRAS	Neuroblastoma RAS viral oncogene homolog
OL	Overt leukemia
OS	Overall survival
PB	Peripheral blood
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PCD	Programmed cell death

PE	Phycoerythrin
RING	Really interesting new gene
RNA	Ribonucleic acid
RPMI	Roswell Park Memorial Institute medium
RT-PCR	Reverse Transcription Real Time Polymerase Chain Reaction
RUNX-RUNX1T1	Runt-related transcription factors fusion genes
SMAC	Second mitochondria-derived activator of caspases
SSC	Side scatter
t- AML	Therapy related AML
t-livin	Truncated livin
TNF	Tumor necrosis factor
TRAIL	TNF-related apoptosis-inducing ligand
UNG	Uracil-N-glycosylase
WBC	White blood cells
WHO	World Health Organization
WT1	Wilm's tumor protein
XIAP	X-chromosome linked IAP
β-ME	β-mercaptoethanol

1-Introduction and Aim of the Work

The acute leukemias are a heterogenous group of neoplasms that affect hemopoietic stem cells. Acute leukemias are broadly classified into non lymphoid (commonly referred to as myeloid) and lymphoid based on the cell of origin. Myeloid and lymphoid leukemias differ from one another with regard to clinical presentation, course and response to therapy ***(Greer et al., 2007).***

Molecular analysis of the common genetic alterations in leukemic cells has contributed greatly to our understanding of the pathogenesis and prognosis of acute leukemias ***(Gilliland and Tallman, 2002).***

Response to therapy in acute leukemia is ultimately linked to the expression of genes that control cellular drug sensitivity and propensity to apoptosis. The discovery of such genes is important because it could provide a means to enhance classification systems based on relapse hazard ***(Zaza et al., 2005)***.

Apoptosis is an active biologic mechanism leading to programmed cell death. A tight regulation is required in biologic systems to ensure delicate balance between life and death. The loss of apoptosis may result in a variety of diseases, including cancers ***(John, 2000).***

During the last decade, a complex network of proapoptotic and antiapoptotic proteins, which strictly regulate apoptotic pathways have been identified ***(Zhang et al., 2004).*** Studies investigating the expression of these molecules in acute leukemia have demonstrated that the expression of proapoptotic or antiapoptotic

regulatory molecules varies depending on the types of leukemia and individual patient's characteristics ***(Prokop et al., 2000; Wrzesień-Kuś et al., 2004).*** These differences can be potentially important for the prediction of the response to treatment ***(Prokop et al., 2000).***

The inhibitor of apoptosis proteins (IAP family proteins) are known to inhibit apoptosis induced by a variety of stimuli ***(Nachmias et al., 2004).*** They are cellular factors that act on initiator and effector caspases ***(Vucic et al., 2000).***

The IAP family inhibit caspases which are a group of cysteinyl proteases with substrate specificity for aspartic acid at the P1 C-terminal site through two distinct mechanisms. The first is through a direct interaction between IAP and caspases, while the second is through really interesting new gene (RING)-dependent ubiquitination and proteosomal degradation of caspases ***(LeBlanc, 2003).***

Livin is a relatively new member of the IAP family and had been initially associated with the development of malignant melanoma ***(Kasof and Gomes, 2001).*** More recently however, it has been found that livin is also expressed in additional cancers, including leukemias, bladder cancer, and different forms of lung cancer ***(Qiuping et al., 2004).***

Livin has been shown to antagonize both the death receptor and mitochondria - based apoptotic pathways through the inhibition of caspases 3,7 and 9 as well as the participation of c-Jun N terminal kinase 1 (JNK 1) ***(Kasof and Gomes, 2001).***

Therefore, livin expression has been regarded as poor prognostic marker in malignancies, however only a limited number of studies are available to date. Thus the clinical relevance of livin expression is still controversial in different types of malignancies.

The ultimate goals of the current study was to evaluate the gene expression of livin in acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL) and correlate it to other prognostic factors in both types of leukemia as well as to determine the potential role of livin expression as a prognostic marker in order to achieve a more refine risk-oriented treatment stratification with intensification of therapy in those patients prone to resistance and/or relapse.

2. Review of literature

2.1 The acute leukemias

Acute leukemias are a heterogeneous group of malignant diseases of hematopoietic progenitor cells with different molecular genetic abnormalities, clinical characteristics, and variable outcomes with currently available treatments ***(Gilliland and Tallman, 2002).***

2.1.1 Etiology

The etiology of leukemia remains rather unclear. Ionizing radiation is a known cause of leukemia in humans. Other suspected risk factors include pesticides; medical conditions such as infectious mononucleosis, autoimmune diseases, immunodeficiency and tonsillectomy. Except for Human T-cell Lymphotropic Virus Type 1 (HTLV-1), a rare type of leukemia, no viruses or infections have been implicated in the etiology of leukemia. Adult leukemia has been associated with working in the chemical industry ***(Olufemi et al., 2003).***

2.1.2 Epidemiology

Age-specific incidence of AML rises linearly after age 40 with a median age of approximately 65 years. Most cases are sporadic, but congenital disorders such as Fanconi's, Bloom's, Down's, Kostmann's, and Diamond–Blackfan syndromes can increase the relative risk of developing AML ***(Scheinberg et al., 2001)***. Risk is also increased in individuals with acquired hematologic disorders including the