Expression of Melanoma Antigen Encoding Gene-A3 in Acute Myeloid Leukemia

Thesis

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By

Noha Said Semary

M.B.B.Ch

Under Supervision of

Prof. Dr. Hala Mohammed Farawela

Professor of Clinical and Chemical Pathology

Faculty of Medicine - Cairo University

Assist. Prof. Dr. Rania Ahmed Zayed

Assistant Professor of Clinical and Chemical Pathology

Faculty of Medicine - Cairo University

Assist. Prof. Dr. Manal Mohamed Mahmoud Makhlouf

Assistant Professor of Clinical and Chemical Pathology

Faculty of Medicine - Cairo University

Faculty of Medicine

Cairo University

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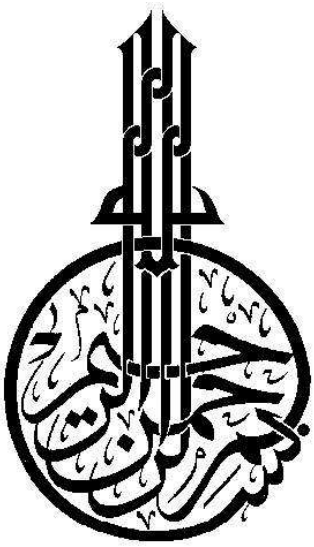

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عَلَّمَ الْإِنْسَانَ مَا لَمْ
يَعْلَمْ (٥)

سورة العلق (١-٥)

صدق الله العظيم

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Abstract

Melanoma antigen encoding gene-A3 (MAGE-A3), also called cancer/testis (CT) antigen, is a member of MAGE-multigene family which is located on the long arm of the X chromosome and its expression can be caused by promoter region demethylation. The MAGE-A3 proteins' functions are unknown but it was found to play a role in cell cycle progression, transcriptional regulation and drug resistance.

The present work aims to study the frequency of expression of MAGE-A3 on the transcriptional level in fresh leukemic blasts cells isolated from patients with acute myeloblastic leukemia to define its role in the development of AML as well as to evaluate future probable application in tumor - immunotherapy.

This study included 40 de novo AML patients as well as 30 age and sex matched normal healthy subjects as a control group. They were all subjected to reverse transcriptase-polymerase chain reaction (RT-PCR) assay for detection of MAGE-A3 gene expression.

Our study revealed that 23 AML patients (57.5%) expressed the MAGE-A3 gene, while none of the control group subjects (0%) expressed this gene. It was found that MAGE-A3 gene expression was associated with increased risk of AML with OR 2.763 and 95% CI 1.890-8.041.

Conclusion: MAGE-A3 gene expression may have a clinical relevance and important role as a risk factor in the development of AML.

Key words : AML : Acute myeloid leukemia - LAAs: leukemia associated antigens - MAGE: Melanoma antigen encoding gene - CTA: Cancer testis antigens **.**

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List of Abbreviations

ACS	American Cancer Society
alloSCT	Allogeneic hematopoietic stem-cell transplantation
ANAE	Alph naphthyl acetate esterase non specific esterase
AP	Acid phosphatase
ASCT	Autologous hematopoietic stem-cell transplantation
ATRA	All- trance retinoic acid
BAALC	Brain and acute leukemia cytoplasmic
BDCA-2/CD303	Blood dentritic cell antigen 2 protein/ c-type lectin-like domain
BM	Bone marrow
CAE	Chloracetate esterase
CBC	Complete blood count
CD2AP	CD2- associated protein
CEBPA	CCAAT/ enhancer binding protein alpha
CGH	Comparative genomic hybridization
CI	Confidence interval
CLA	Tran-smembrane tight junction protein claudin
CN-AML	Cytogenetically normal- AML
CNS	Central nervous system
CRP	C- reactive protein
CR	Complete remission
CSF	Cerebro- spinal fluid
CTA	Cancer testis antigens
DFS	Disease free survival
DIC	Disseminated intravascular coagulopathy
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix

EDTA	Ethylene diamine tetra- acetic acid
EM	Electron microscopy
FISH	Fluorescence in situ hybridization
FLK2	Fetal liver kinase 2
FLT3	Fms like tyrosine kinase 3
GATA	Glutamyl-t RNA amidotransferase subunit A
GVHD	Gravt versus host disease
HGFs	Hematopoietic growth factor
HSCs	Hematopoietic stem cells
HSCT	Hematopoietic Stem cell transplantation
LAAs	Leukemia associated antigens
LAT	Linker for activation of T cells
LDL	Low density lipoprotein
LM	Light microscopy
MAGE	Melanoma antigen encoding gene
MDS	Myelodysplastic Syndrome
MHC	Major histocompatibility complex
MIC	Morphologic immunologic cytogenetic
MKL	Megakaryocytic leukemia
MLL	Mixed lineage leukemia
MN1	Menenigioma 1
MoAB	Monoclonal antibody
MPL	Myeloproliferative leukemia
MPN	Myeloproliferative neoplasm
NAP	Neutrophil alkaline phosphatase
NASDE	Naphthol ASD chloroacetate esterase
NEC	Non erythroid cells
NK/T-cell	Natural killer/ T cell
NOS	Not otherwise specified

NPM1	Nucleophosmin
NSE	Non specific estrase
OR	Odds ratio
OS	Overall survival
PAS	Periodic acid Schiff
PB	Peripheral blood
PDGF-R	Platelet derived growth factor receptor
PMN	Polymorphonuclear leucocyte
PTD	Partial tandem duplication
RAR α	Retinoic acid receptor alpha
RB	Retinoblastoma
RNA	Ribonuclic acid
RTKIII	Receptor tyrosine kinase
RT-PCR	Reverse transcription polymerase chain reaction
SBB	Sudan black B
SKY	Specral karyotyping
STK1	Stem cell tyrosine kinase
TCL1	T cell lymphoma protein/A
TDT	Terminal deoxynucletoidyl transferase
TLC	Total leukocytic count
TSG	Tumor suppressor gene
UAL	Undeffrentiated acute leukemia
WBC	White blood cell
WT1	Wilm's tumor 1

Introduction

Leukemias, myelodysplastic syndromes (MDS), lymphomas, and multiple myeloma (MM) are complex diseases with a wide range of clinical, morphological, biologic, cytogenetic, molecular, and immunophenotypic features ***(Estey, 2001)***. 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. 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 ***(Lowenberg, 2004)***.

Leukemia-associated antigens such as proteins encoded by melanoma antigen-encoding genes (MAGE) might provide tools for immunotherapy of leukemia ***(Martínez et al., 2007)***.

Tumor-specific antigens recognized by autologous T lymphocytes are encoded by genes, including those of the MAGE gene family which includes MAGE-A, -B and –C families that encodes peptides recognized by autologous cytotoxic T lymphocytes in a MHC class-I restricted fashion ***(Lucas et al., 2000)***. MAGE-A genes are located in the chromosome Xq whereas MAGE-B, also called DAM-6, are located on chromosome Xp ***(Simpson et al., 2005)***.

MAGE-A3 gene is a member of a multigene family which is composed of three exons and the large open reading frame is entirely located in the third exon ***(Andrade et al., 2008)***.

MAGE-A3 gene is silent in normal healthy adult tissues with the exception of placenta and testis where it appears to be expressed in male germ-line cells that do not bear HLA class-I molecules ***(Eifuku et al., 2001)***.

MAGE-A3 is widely expressed in cancers of several histological types such as melanomas, lung carcinomas, head and neck tumors, bladder carcinomas, ovarian tumors, seminomas, neuroblastomas, hepatocellular carcinomas, colorectal carcinomas and osteosarcomas ***(Martínez et al., 2007)***. Also MAGE-A3 is expressed in hematological malignancies but in a lesser frequency than solid tumors ***(Adams et al., 2002)***.

Aim of the Work

The present work aims to study the frequency of expression of MAGE-A3 on the transcriptional level in fresh leukemic blasts cells isolated from patients with acute myeloblastic leukemia to define its role in the development of AML as well as to evaluate future probable application in tumor immunotherapy.

Acute Myeloid Leukemia

Definition:

Acute myeloid leukemia (AML) is a hematopoietic stem cell disorder characterized by a block in differentiation of hematopoiesis, resulting in growth of a clonal population of neoplastic cells or blasts ***(Shipley and Butera, 2009)***.

The cell of origin in AML is a blast that most often show myeloid or monocytic differentiation. In approximately 5 to 10% of patients blasts have erythroid or megakaryocytic differentiation. For this reason, acute non- lymphoblastic leukemia (ANLL) has been considered a more precise term but AML is more common and is the recommended term ***(Cheson et al., 2003)***.

Epidemiology:

Acute myeloid leukemia (AML) has an incidence of 2-3 per 100,000 in children rising to 15 per 100,000 in older adults. It can occur at all ages but has its peak incidence in seventh decade ***(Burnett, 2005)***.

AML accounts for 15 to 20 percent of the acute leukemias in children and 80 percent of acute leukemias in adults. It's slightly more common in males. Little difference in incidence is seen between individuals of African or European descent at any age, while a lower incidence is seen in persons of Asians descent. An increase in frequency of AML is seen in Jews, especially those of Eastern European descent ***(Liesveld and Lichtman, 2006)***.

Pathogenesis:

Acute myeloid leukemia is believed to begin in a single somatic hematopoietic progenitor that transforms to a cell incapable of normal differentiation. Many of these cells no longer possess the normal property of apoptosis resulting in a cell with prolonged life-span and unrestricted clonal proliferation. A Major cause of morbidity and mortality is the deficiency of normal functioning mature hemopoietic cells, rather than the presence of numerous malignant cells ***(Estey and Döhner, 2006)***.

Development of AML results in a block of differentiation, increased proliferation and inhibition of apoptosis. This has been hypothesized to be due to multiple genetic events, activation or inappropriate expression of surface membrane hemopoietic growth factors ***(Zhou et al., 2000)***.

1. Genetic Factors:

The somatic mutation results from a chromosomal translocation in nearly 80% of patients. The translocation results in rearrangement of a critical region of a proto-oncogene. The fusion of portions of two genes usually doesn't prevent the process of transcription and thus the fusion gene encodes a fusion protein that, because of its abnormal structure, disrupts a normal cell pathway and leads to malignant transformation of the cell. This protein product is often a transcription factor that disrupts the regulatory sequences that control differentiation, growth rate or survival of blood cell progenitors ***(Kelly and Gilliland, 2002)***.