بسم الله الرحمن الرحيم

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Identification of Tim-3 Expression in Acute Myeloid Leukemia: Its Clinical and Laboratory Correlation

Thesis

Submitted for Partial Fulfilment of Master Degree in Clinical Pathalogy

By

Shaymaa Ahmed EL-Sayed

M.B., B.Ch.

Under Supervision of

Prof. / Mona Ahmed Hassan Wahba

Professor of Clinical Pathology

Faculty of Medicine, Ain Shams University

Dr. Nesma Ahmed Safwat

Assistant Professor of Clinical Pathology

Faculty of Medicine, Ain Shams University

Faculty of Medicine

Ain Shams University

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Shaymaa Ahmed EL-Sayed

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List of Abbreviations

Abb.	Full term
AML	Acute Myeloid Leukemia
AML-MRC	AML with myelodysplasia-related changes
ANAE	Alpha-naphthyl acetate esterase
ANC	Absolute neutrophil count
ASH	American Society of Hematology
BAALC	Brain and acute leukemia, cytoplasmic gene
CAP	College of American Pathologists
CAR	Chimeric antigen receptor
CEBPA	CCAAT/enhancerbinding protein alpha gene
CML	Chronic myeloid leukemia
CR	Clinical remission
CR	Complete remission
DAB	Diaminobenzidine
DAG	Diacylglycerol
DCs	Dendritic cells
DNMT3A	DNA methyltransferase 3A gene
EC	Endothelial cells
ERG	ETS-related gene
ET	Essential thrombocytopenia
FC	Flow cytometry
FISH	Fluorescence in situ hybridization
FITC	Fluorescein isothiocyanate
FLT3	FMS-like tyrosine kinase 3 gene
GAL-9	Galectin-9
HPC	Hematopoietic progenitor cell
HS	Highly significant
HSCs	Hematopoietic stem cells
IDH ½	Isocitrate dehydrogenase gene
IHC	Immunohistochemistry
IP_3	Inositol-triphosphate
IP3R	IP_3 receptor
IQR	Inter-quartile range
ITD	Internal tandem duplication
IV	Intravenous

List of Abbreviations cont...

Abb.	Full term
LPHN1	Ligand-dependent activation of ectopically expressed latrophilin 1
LPHN1	Neuronal receptor latrophilin 1
LPS	Pro-inflammatory stimulation
LSC	leukemic stem cells
MDS	Myelodysplastic syndrome
MLFS	Morphologic leukemia-free state
MLL-AML/PTD	Partial tandem duplication (PTD) of the mixed-lineage leukemia (MLL) gene
MN1	Meningioma 1 gene
MoAbs	Monoclonal antibodies
MPN	Myeloproliferative neoplasm
MPO	Myeloperoxidase
MRD	Minimal residual disease
Na F	Sodium flouride
NCR	Non Complete Remission
NGS	Next-generation sequencing
NK	Natural killer
NOS	Not otherwise specified
NPM1	Nucleophosmin 1gene
NRAS	Neuroblastoma RAS viral oncogene homolog gene
NS	Non-significant
PAS	Periodic acid shiff.
PBS	Phosphate buffered saline
PD-1	Programmed cell death 1
PE	Phycoerythrin
PI3K	Phosphatidylinositol 3 kinas
PIP2	Phosphatidyl-inositol-bisphosphate
PMF	Primary myelofibrosis
PV	Polycythemia vera
RUNX1	Runt-related transcription factor 1 gene
S	Significant
SCF	Growth factor

List of Abbreviations cont...

Abb.	Full term
SD	Standard deviation
SH2	Src homology 2
SPSS	Statistical package for Social Science
t-AML	Therapy-related AML
TET	Ten-Eleven translocation proteins
TIM-3	T-cell immunoglobulin mucin -3
TNF-α	Tumor necrosis factor-α
WBC	White blood cell.
WHO	World Health Organization
WT1	Wilms tumor gene
αKG-DD	α-Ketoglutarate-dependent dioxygenase

INTRODUCTION

Acute Myeloid Leukemia (AML) is a clonal malignant disorder derived from a small number of self-renewing leukemic stem cells (LSC). LSC are the main cause of relapse and refractoriness because of its uncontrollable proliferation, blocked apoptosis and differentiation obstacle caused by the malignant clonal disorder and tumor immune escape ***(Li et al., 2016).***

Therefore, it is necessary to identify surface and molecular markers that are specific to the LSC in order to eliminate them without any damage to the normal hematopoietic stem cells ***(Sands et al., 2013).***

T-cell immunoglobulin mucin -3(Tim-3) has recently been described as a unique AML stem cell antigen that is not present on normal hematopoietic stem cells, It has been shown to be expressed in the majority of AML subtypes ***(Roth et al., 2013)***. Tim-3 is a type 1cell surface glycoprotein that belongs to TIMs family. In AML, ligation of Tim-3 by its ligand galectin-9 (GAL-9) will induce simultaneous activiation of the Nuclear factor kappa light chain and B-catenin signaling in leukemic cells, which in turn will induce marked gene expression changes including up-regulation of Myeloid cell leukemia-1, the important survival factor for LSCs, enhancing the pro-survial signaling of the leukemic clone ***(Kikushige et al., 2013).***

Therefore, Tim3/Gal-9 axis constitutes an essential autocrine loop for LSC to outgrow normal HSCs, representing a

universal machinery for development and maintenance of human myeloid malignant stem cells. Moreover, TIM-3 upregulation and ligation were always associated not only with primary AML, but also with leukemic transformation from avariety of pre-leukemic diseases ***(Kikushige et al., 2015).***

AIM OF THE WORK

Explore the impact of Tim-3 expression on the clinic-laboratory characteristics and prognostic behavior of denovo AML patients.

ACUTE MYELOID LEUKEMIA

Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy characterized by the clonal expansion of myeloid blasts in peripheral blood, bone marrow ***(O'Donnell et al., 2017)***.

I. Incidence and Epidemiology:

AML represents 15-20% of acute leukemia cases in children and 80% in adults. AML is the predominant form of leukemia in neonatal and adult periods but represents a small fraction of cases during infancy and adolescence ***(Song et al., 2018)***. Males are 1.2–1.6 times more likely to develop AML ***(Howlader et al., 2019).***

II.Predisposing Factors:

The development of AML has been associated with several risk factors including the following:

A. Genetic Factors:

There are several genetic disorders that have predominantly systemic manifestations but are also associated with the development of acute leukemia such as Down syndrome ***(Creutzig et al., 2012).*** In addition, inherited bone marrow failure syndromes such as Fanconi anemia and Shwachman-Diamond syndrome ***(West et al., 2014).*** Rare forms of familial acute