

“The role of autophagy in patients with acute leukemia”

**A thesis submitted for partial fulfillment of Doctor of Philosophy Degree in
Pharmaceutical Sciences (Biochemistry)**

Submitted by

Sara Mostafa Abd El-Salam Radwan

Assistant Lecturer of Biochemistry, Faculty of Pharmacy, Ain Shams University

Master Degree in Pharmaceutical Sciences (Biochemistry), Ain Shams University, 2009

Under supervision of

Dr. Hala Osman El-Mesallamy

**Professor of Biochemistry
Head of Biochemistry Department
Faculty of Pharmacy
Ain Shams University**

Dr. /Nadia Hamdy El-Hefny

**Assistant Professor of Biochemistry
Biochemistry Department
Faculty of Pharmacy
Ain Shams University**

Dr./ Hany Mohamed Hegab

**Assistant Professor of clinical hematology
Department of internal medicine
Faculty of Pharmacy
Ain Shams University**

***Biochemistry Department
Faculty of Pharmacy
Ain Shams University***

(2016)

"دور الالتهام الذاتي في مرضي سرطان الدم الحاد"

مقدمة كمطلب جزئي للحصول علي درجة دكتوراة الفلسفة في العلوم الصيدلية رسالة
(كيمياء حيوية)
مقدمة من

سارة مصطفى محمد السلام رضوان

مدرس مساعد بقسم الكيمياء الحيوية - كلية الصيدلة - جامعة عين شمس
ماجستير الكيمياء الحيوية - كلية الصيدلة - جامعة عين شمس - ٢٠٠٩

تحت إشراف

أ.د/ هالة عثمان المسلمي

أستاذ الكيمياء الحيوية
رئيس قسم الكيمياء الحيوية
كلية الصيدلة - جامعة عين شمس

أ.م.د./ هاني محمد حجاب

أستاذ مساعد أمراض الدم الاكلينيكية
قسم الباطنه العامة
كلية الطب - جامعة عين شمس

أ.م.د. / نادية حمدي الحفني

أستاذ مساعد الكيمياء الحيوية
قسم الكيمياء الحيوية
كلية الصيدلة - جامعة عين شمس

قسم الكيمياء الحيوية

كلية الصيدلة

جامعة عين شمس

(٢٠١٦)

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

"قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا
إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ"

صدق الله العظيم

سورة البقرة آية (٣٢)

Acknowledgements

Acknowledgements

First of all I thank **ALLAH** for lightening the way for me to reach my aim, as without His help, this work would have never been accomplished and may we always gain His kind mercifulness and forgiveness.

I can't find words to express my thanks and deepest sense of gratitude to **Dr. Hala Osman El-Mesallamy**, Professor and Head of Biochemistry Department, Faculty of Pharmacy, Ain Shams University, for her precious time, great efforts, kind cooperation, faithful help and of course for suggesting the idea of this work. I hope that one day I can return back a part of her great favor.

I would also like to express my gratefulness and appreciation for **Dr. Nadia Hamdy El-Hefny**, Assistant Professor of Biochemistry, Biochemistry Department, Faculty of Pharmacy, Ain Shams University, for her keen supervision, continuous guidance and valuable opinions. I hope her every success in her life and work.

I would like to thank **Dr. Hany Mohamed Hegab**, Assistant Professor of Hematology, Faculty of Medicine, Ain Shams University, for his continuous help, cooperation and his effort in following up the patients, and for giving me chance to collect samples from the Clinical Hematology and Stem Cell Transplantation Unit, Ain Shams University Hospital.

I am greatly thankful to all members of the Clinical Hematology and Stem Cell Transplantation Unit, Ain Shams University Hospital, for their friendly cooperation.

Last but not least, neither appreciation nor gratitude could ever pay back to **my family** for their patience and understanding during the tiring period of this work.

LIST OF CONTENTS

Subjects	Page
PUBLICATIONS RELATED TO THE THESIS.....	i
LIST OF ABBREVIATIONS.....	ii
LIST OF TABLES.....	iv
LIST OF FIGURES.....	v
INTRODUCTION AND AIM OF THE WORK.....	1
REVIEW OF LITERATURE.....	4
Acute Leukemia.....	4
Acute Myeloid Leukemia (AML).....	9
Acute Lymphocytic Leukemia (ALL).....	12
Treatment of acute leukemia.....	15
Autophagy.....	17
Autophagy and Apoptosis.....	22
Autophagy and Cancer.....	25
Potential mechanisms linking autophagy and tumor suppression.....	28
Potential mechanisms linking autophagy and tumor progression.....	29
Beclin-1.....	31
Microtubule-associated protein 1 light chain 3B (MAP1LC3B).....	34
B-cell lymphoma-2 (BCL-2)	37
Hypoxia.....	40
Hypoxia-Inducible Factor-1 alpha (HIF-1 α).....	42
SUBJECTS AND METHODS.....	46
RESULTS.....	60
Discussion.....	72

List of Contents

Summary and Conclusions.....	85
Recommendations.....	89
References.....	90
Arabic Summary.....	١

PUBLICATIONS RELATED TO THE THESIS

Radwan, S. M., Hamdy, N. M., Hegab, H. M. and El-Mesallamy, H. O. (2016). Beclin-1 and hypoxia-inducible factor-1 α genes expression: Potential biomarkers in acute leukemia patients. *Cancer Biomarkers*, DOI: 10.3233/CBM-160603.

Cancer Biomarkers journal impact factor: 1.721

Cancer Biomark. 2016 Mar 18;16(4):619-26. doi: 10.3233/CBM-160603.

Beclin-1 and hypoxia-inducible factor-1 α genes expression: Potential biomarkers in acute leukemia patients.

Radwan SM¹, Hamdy NM¹, Hegab HM², El-Mesallamy HO¹.

Author information

¹Biochemistry Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

²Department of Internal Medicine-Clinical Hematology Unit, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Abstract

BACKGROUND: Beclin-1, an important autophagic gene, and hypoxia-inducible factor-1 α (HIF-1 α), the master regulator of the hypoxic response, are reported in several human cancers. However, their expressions in acute leukemia haven't yet been well investigated.

OBJECTIVE: This study was designed to investigate the gene expression of beclin-1, microtubule-associated protein-1 light chain-3B (MAB1LC3B), the anti-apoptotic marker Bcl-2, and HIF-1 α , as well as to evaluate the relationship between their expressions profile and prognosis in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) adult patients.

METHODS: The study involved 30 AML patients, 25 ALL patients, and 20 controls. Gene expression was analyzed using quantitative reverse transcriptase polymerase chain reaction (QRT-PCR).

RESULTS: In both AML and ALL groups, beclin-1 and MAB1LC3B expressions were significantly down-regulated ($p < 0.001$), while HIF-1 α ($p < 0.01$) and Bcl-2 ($p < 0.001$) expressions were significantly up-regulated compared to the control group. HIF-1 α fold expression was significantly negatively correlated with beclin-1 ($p < 0.01$). Moreover, decreased beclin-1 gene expression and increased HIF-1 α gene expression were both associated with poor survival, supporting their pivotal role in the development and progression of acute leukemia.

CONCLUSIONS: Both Beclin-1 and HIF-1 α could be considered as important biomarkers determinants of pathogenesis and survival in acute leukemia.

LIST OF ABBREVIATIONS

ALL	Acute lymphocytic leukemia
AML	Acute myeloid leukemia
ASUH	Ain shams university hospitals
ATG	Autophagy-related genes
Bcl-2	B-cell lymphoma-2
B-CLL	B-chronic lymphocytic leukemia
Bcl-xL	B-cell lymphoma-extra large
BH	B-cell lymphoma-2 homology
CCD	Central coiled-coiled domain
CD13	Cluster of differentiation 13
CMA	Chaperone-mediated autophagy
CNS	Central nervous system
CR	Complete remission
CSF	Cerebrospinal fluid
CT	Computed tomographic
ECD	Evolutionarily conserved domain
EFS	Event free survival
ER	Endoplasmic reticulum
FSC	Forward scatter
HIF-1α	Hypoxia-inducible factor-1 alpha
HLA-DR	Human leukocyte antigen - D related
Hsc70	The 70 kd heat shock cognate protein
IHC	Immunohistochemistry
IPT	Immunophenotyping
LDH	Lactate dehydrogenase

List of Abbreviations

MAb	Monoclonal antibodies
MAP1LC3B	Microtubule-associated protein-1 light chain-3B
ODD	Oxygen-dependent degradation
OS	Overall survival
PAS	Pre-autophagosomal structures
PCD	Programmed cell death
PHD	Prolyl hydroxylase domain protein
PI3Ks	phosphatidylinositol-3-kinases
ROS	Reactive oxygen species
RT-PCR	Reverse transcription polymerase chain reaction
SSC	Side scatter
USP	Ubiquitin specific peptidase
VEGF	Vascular endothelial growth factor
VHL	Von Hippel–Lindau
WHO	World health organization

LIST OF TABLES

Table No.	Table Title	page
1	The general characteristics of the studied groups.....	60
2	Cluster of differentiation 13 and HLA-DR expression in the studied groups	61
3	Hemoglobin concentration and platelet count in the studied groups	62
4	Correlation coefficients (r) of beclin-1, MAP1LC3B, Bcl-2 and HIF-1 α expression levels with respect to both platelets count and hemoglobin level in ALL patients...	69
5	Correlation coefficients (r) of beclin-1, MAP1LC3B, Bcl-2 and HIF-1 α expression levels investigated in acute leukemia patients (n=55)	70

LIST OF FIGURES

Figure No.	Figure Title	page
1	Diagnostic work-up for specimens from patients with a suspected diagnosis of acute leukemia	7
2	Different roles of autophagy in mammals.....	18
3	Different types of autophagy	20
4	Autophagosome and autolysosome formation.....	22
5	Cellular processes: apoptosis, autophagy, and cellular senescence are distinct cellular response to stress.....	24
6	Potential roles of autophagy in tumor suppression or progression	27
7	Tumor-suppressing and tumor-promoting roles of autophagy during tumorigenesis	31
8	Structure of the beclin-1 protein and its regulators.....	32
9	Mechanisms underlying the regulation of Bcl-2/Bcl-xL interactions with beclin-1	34
10	Autophagy-related gene 8 and MAP1LC3 N-termini mediate membrane fusion processes required for autophagosome biogenesis.....	35
11	The locations of Bcl-2 homology domains	38

List of Figures

12	Spatial relationship between a blood vessel, hypoxic conditions and a malignant solid tumor in the context of O ₂ and HIF-1 concentrations	43
13	Hypoxia-inducible factor-1 alpha regulation under normoxic conditions	44
14	Overview of HIF-1 α regulation both in hypoxic and normoxic conditions	45
15	The age of different studied groups	60
16	Beclin-1, MAP1LC3B, HIF-1 α , and Bcl-2 Expression levels in different studied groups	63
17	Beclin-1 fold expression with respect to CD13 expression...	64
18	Microtubule-associated protein-1 light chain-3B fold expression with respect to CD13 expression.....	65
19	B-cell lymphoma-2 fold expression with respect to CD13 expression.....	65
20	Hypoxia-inducible factor-1 alpha fold expression with respect to CD13 expression.....	66
21	Hypoxia-inducible factor-1 alpha fold expression with respect to HLA-DR expression.....	67
22	Beclin-1 fold expression with respect to HLA-DR expression.....	67
23	Microtubule-associated protein-1 light chain-3B fold	68

List of Figures

	expression with respect to HLA-DR expression.....	
24	B-cell lymphoma-2 fold expression with respect to HLA-DR expression.....	68
25	Correlation of Beclin-1 fold expression with (a) overall survival and (b) event free survival	71
26	Correlation of HIF-1 α fold expression with (a) overall survival and (b) event free survival	71

INTRODUCTION AND AIM OF THE WORK

Acute leukemia is a heterogenous group of neoplasms that affect hemopoietic stem cells. It is broadly classified based on the cell of origin into non lymphoid (commonly referred to as myeloid) and lymphoid (*Raj, 2013*).

The relationship between autophagy and cancer has been a hot topic for years, and currently numerous new studies are shedding light onto different aspects of this relationship (*Ekiz et al., 2012*). However, the exact role of autophagy in carcinogenesis is still unclear (*Brech et al., 2009*).

Both beclin-1 and microtubule-associated protein-1 light chain-3 (MAP1LC3) genes play a pivotal role in mammalian autophagy (*Eskelinen and Saftig, 2009*). Beclin-1 is a key factor for initiation and regulation of autophagy (*Niu et al., 2014*). Moreover, it is a key molecule in the interaction between both autophagy and apoptosis by binding with the anti-apoptotic molecules, B-cell lymphoma-2 (Bcl-2) and B-cell lymphoma-extra large (Bcl-xL) (*Lee and Lee, 2012*). Decreased expression of beclin-1 has been correlated with tumor progression and was found to be associated with low survival rate (*Jin and White, 2008*).

Microtubule-associated protein-1 light chain-3 is thought to be essential for autophagy, as it is associated with the dynamic process of autophagosome formation. Microtubule-associated protein-1 light chain-3 has three isoforms (including MAP1LC3A, MAP1LC3B and

Introduction and Aim of the Work

MAP1LC3C). It has been demonstrated that detecting MAP1LC3B expression is a simple and specific technique for monitoring autophagy (*Huang et al., 2010*). However, to the best of our knowledge, expressions of beclin-1 and MAP1LC3B, as well as any correlation between their expression and outcome in acute leukemia, have not been well characterized.

As the tumor grows, it develops extensive regions of poor oxygenation and high acidity due to the inconsistency between the rapid rate of tumor growth and the capacity of existing blood vessels to supply oxygen. At a cellular level, a hypoxic stress generates an adaptive response which is mediated by the transcription factor hypoxia-inducible factor-1 α (HIF-1 α) (*Manolescu et al., 2009*). Hypoxia-inducible factor-1 α is a transcriptional factor that plays significant role in tumorigenesis, including the processes of angiogenesis, metabolism, proliferation and differentiation (*Rankin and Giaccia, 2008*). Although HIF is not an oncogene, a large amount of evidence has accumulated linking HIF-1 α regulation with cancer pathogenesis (*Evens et al., 2010*).

Despite a well-recognized role of hypoxia and its major downstream mediator HIF-1 α in solid tumors, the effects of hypoxia in leukemia cell survival and chemoresistance have not yet been completely elucidated (*Frolova et al., 2012*).

Accordingly, autophagy, apoptosis and hypoxia may be potential candidates playing role in the pathogenesis and prognosis of acute leukemia. Therefore, the current study was designed to investigate the
