"The role of autophagy in patients with acute leukemia"

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

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بســـــم الله الرحـــــمن الرحـــــيم

"قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا
إِنَّكَ أَنتَ الْعَلِيــمُ الْحَكِيمُ"

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PUBLICATIONS RELATED TO THE THESIS

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

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Abstract

BACKGROUND: Beclin-1, an important autophagic gene, and hypoxia-inducible factor-1α (HIF-1α), 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-1α, 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-1α ($p < 0.01$) and Bcl-2 ($p < 0.001$) expressions were significantly up-regulated compared to the control group. HIF-1α fold expression was significantly negatively correlated with beclin-1 ($p < 0.01$). Moreover, decreased beclin-1 gene expression and increased HIF-1α 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-1α 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

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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

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-1α (HIF-1α) ***(Manolescu et al., 2009)***. Hypoxia-inducible factor-1α 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-1α regulation with cancer pathogenesis ***(Evens et al., 2010)***.

Despite a well-recognized role of hypoxia and its major downstream mediator HIF-1α 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