



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم

بسم الله الرحمن الرحيم



HANAA ALY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلم



HANAA ALY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



HANAA ALY



HOTAIR Expression and Prognostic Impact in Acute Myeloid Leukemia Patients

Thesis

*Submitted for Partial Fulfillment of M.D degree
in Clinical and Chemical Pathology*

By

Rawda Ahmed Alaa El-Din

*M.B.,B.Ch and M. Sc Clinical and Chemical Pathology
Faculty of Medicine- Ain Shams University*

Supervised by

Prof. Amany Ahmed Osman

*Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University*

Prof. Amal Mostafa Mohammed El-Afify

*Professor of Internal Medicine and Hematology
Faculty of Medicine - Ain Shams University*

Dr. Mona Fathey Abdel Fattah Hassan

*Assistant Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University*

Dr. Yasmin Nabil El-Sakhawy

*Assistant Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University*

Dr. Shereen Abdel Monem Ibrahim

*Lecturer of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University*

*Faculty of Medicine
Ain Shams University*

2020 – 2021

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالَ

سُبْحَانَكَ لَا يُلْمُ لَنَا
إِلَّا مَا عَلِمْنَا إِنَّكَ أَنْتَ
الْعَلِيمُ الْعَظِيمُ

صدق الله العظيم

سورة البقرة الآية: ٢٢

Acknowledgment

*First and foremost, I feel always indebted to **ALLAH**, the Most Kind and Most Merciful.*

All praise to Allah and all thanks, he guided and enabled me by his mercy to fulfill this thesis, which I hope to be beneficial for people.

*I would like to express my deep appreciation and gratitude to **Prof. Amany Ahmed Osman**, for her enormous effort, excellent guidance, supervision, advice and help during the entire course of this research.*

*I am deeply grateful to **Prof. Amal Mostafa Mohammed El-Afify**, for her valuable help and supervision.*

*I wish to express my sincere thanks and gratitude to **Assistant Prof. Mona Fathey Abdel Fattah Hassan, Assistant Prof. Yasmin Nabil El-Sakhawy and Dr. Shereen Abdel Monem Ibrahim**, for their continuous help, valuable remarks, advice and supervision with continuous guidance through out this research.*

*I am also grateful to **Prof. Iman Omar**, for her excellent guidance.*

Dedicated to my family; my Parents, my husband and my colleagues. Thank you all for your continuous help, cooperation, powerful support, encouragement and understanding.

Raouda Ahmed Alaa El-Din

Abstract

Background: Acute myeloid leukemia (AML) is a disorder characterized by a rapid onset of symptoms attributable to bone marrow failure due to clonal proliferation of primitive hematopoietic stem cells or progenitor cells. Epigenetic abnormalities play an important role in the development and progression of acute leukemia. Long non-coding ribonucleic acid (lncRNA) plays an important role in epigenetic regulation. Homeobox (Hox) transcript antisense intergenic RNA (HOTAIR) is a lncRNA which has been determined to be a negative prognostic indicator in various solid-tumor patients. However; its role in hematopoietic tumors as AML is to be assessed. This study aimed at measuring lncRNA HOTAIR in newly diagnosed AML patients and correlating its expression with different clinicopathological prognostic variables. This provides a new prospective for novel marker involved in development and progression of AML which can be used as diagnostic marker and target of therapy. The current study included 65 subjects divided into 35 newly diagnosed AML adult patients (before initiation of therapy) and 30 non-leukemic adult patients as controls. HOTAIR expression was measured by quantitative reverse transcription polymerase chain reaction (qRT-PCR).

Results: HOTAIR expression was found to be significantly upregulated in AML patients ($p = 0.000$) and showed to have a diagnostic ability of AML as confirmed by significant difference between cases and controls using receiver operating characteristic curve (ROC) analysis. However; it was not significantly correlated with event free survival (EFS) or clinicopathological prognostic variables.

Conclusion: This study showed that the expression of HOTAIR is upregulated in de novo AML patients and can be used as a diagnostic marker. However; highly expressed HOTAIR is not associated with poor prognosis.

List of Contents

Title	Page No.
List of Tables	ii
List of Figures.....	iii
List of Abbreviations	iv
Introduction.....	1
Aim of the Work	3
Review of Literature	
Acute Myeloid leukemia.....	4
Epigenetics.....	19
HOTAIR	31
Subjects and Methods	36
Results.....	51
Discussion.....	64
Summary.....	73
Recommendations.....	75
References.....	76
Arabic Summary	---

List of Tables

Table No.	Title	Page No.
Table (1):	WHO, 2016 classification of myeloid neoplasms	11
Table (2):	Expression of cell-surface and cytoplasmic markers for the diagnosis of AML	13
Table (3):	Risk categories according to genetic abnormalities	16
Table (4):	Demographic, clinical and some laboratory data of AML patients and control groups.....	56
Table (5):	Cytogenetic data of AML patients	57
Table (6):	Response, relapse and EFS in AML patients	57
Table (7):	HOTAIR expression in both AML patients and control groups.....	57
Table (8):	Relation of HOTAIR expression and FAB classification.....	58
Table (9):	Relation of HOTAIR with the clinical parameters.....	58
Table (10):	Correlation of HOTAIR with age, laboratory data and EFS (months) of the studied patients.....	58
Table (11):	Diagnostic characteristics of HOTAIR expression in AML patients.....	59
Table (12):	Relationship of HOTAIR expression and EFS.....	59
Table (13):	Analysis of different prognostic variables of AML for patient group	59
Table (14):	Relation of HOTAIR expression to standard prognostic factors in AML	60

List of Figures

Fig. No.	Title	Page No.
Figure (1):	Methylation as an epigenetic mechanism	20
Figure (2):	HOTAIR gene is located on chromosome 12 inside the HoxC locus, specifically between HoxC11 and HoxC12.	32
Figure (3):	The RNAs recruiting PRC2 complex inhibit PRC2 function.	33
Figure (4):	RNA extraction using QIAamp RNA Blood Mini Kit (Qiagen)	42
Figure (5):	Demonstration of difference of HOTAIR expression level between AML patients and control groups	61
Figure (6):	Significant correlation between HOTAIR expression and increased TLC in AML patients	61
Figure (7):	Receiver operating characteristic curve (ROC) for HOTAIR to differentiate between AML patients and controls.....	62
Figure (8):	Percent of responders and non-responders among AML patients	62
Figure (9):	Percent of relapsed and nonrelapsed patients among AML patients	63
Figure (10):	Cummulative survival of AML patient group during study period	63

List of Abbreviations

Abb.	Full term
<i>AL</i>	<i>Acute leukemia</i>
<i>AML</i>	<i>Acute myeloid leukemia</i>
<i>ASXL1</i>	<i>additional sex combs like 1</i>
<i>AUC</i>	<i>Area under the curve</i>
<i>BM</i>	<i>Bone marrow</i>
<i>CASC15</i>	<i>Cancer susceptibility 15</i>
<i>CBC</i>	<i>Complete blood picture</i>
<i>CBF</i>	<i>Core binding factor</i>
<i>CCAAT</i>	<i>cytidine-cytidine-adenosine-adenosine-thymidine</i>
<i>CD</i>	<i>Cluster of differentiation</i>
<i>cDNA</i>	<i>Complementary deoxyribonucleic acid</i>
<i>CEBPA</i>	<i>CCAAT/enhancer-binding protein alpha</i>
<i>CML</i>	<i>Chronic myelogenous leukemia</i>
<i>CpG</i>	<i>Cytosine next to guanine</i>
<i>CT</i>	<i>Computed tomography</i>
<i>Ct</i>	<i>Cycle threshold</i>
<i>DIC</i>	<i>Disseminated intravascular coagulation</i>
<i>DLBCL</i>	<i>Diffuse large B cell lymphoma</i>
<i>DNA</i>	<i>Deoxyribonucleic acid</i>
<i>DNMT3A</i>	<i>DNA methyltransferase 3A</i>
<i>EFS</i>	<i>Event free survival</i>
<i>ELN</i>	<i>European Leukemia Net</i>
<i>ETV6</i>	<i>Translocation-Ets- leukemia virus variant gene 6 protein</i>
<i>EZH2</i>	<i>enhancer of zeste homolog 2</i>
<i>FAB</i>	<i>French-American-British</i>
<i>FISH</i>	<i>fluorescence in situ hybridization</i>
<i>FLT3-ITD</i>	<i>Fetal liver tyrosine kinase- internal tandem duplication</i>

List of Abbreviations (cont...)

Abb.	Full term
<i>g</i>	<i>Gram</i>
<i>g / dL</i>	<i>Gram / dL</i>
<i>GATA</i>	<i>Trans-acting T cell specific transcription factor</i>
<i>H1</i>	<i>Histone 1</i>
<i>H2A</i>	<i>Histone 2A</i>
<i>H2B</i>	<i>Histone 2B</i>
<i>H3B</i>	<i>histone 3</i>
<i>H4</i>	<i>Histone 4</i>
<i>HB</i>	<i>Hemoglobin</i>
<i>HCT</i>	<i>Hematopoietic cell transplantation</i>
<i>HLA</i>	<i>Human leucocyte antigen</i>
<i>HOTAIR</i>	<i>Homeobox transcript antisense intergenic ribonucleic acid</i>
<i>HOX</i>	<i>Homeobox</i>
<i>HS</i>	<i>Highly significant</i>
<i>HSM</i>	<i>Hepatosplenomegaly</i>
<i>IDH2R172</i>	<i>isocitrate dehydrogenase 2 R172</i>
<i>Inv</i>	<i>Inversion</i>
<i>IPI</i>	<i>international prognostic index</i>
<i>IPT</i>	<i>Immunophenotyping</i>
<i>IQR</i>	<i>Interquartile range</i>
<i>K2-EDTA</i>	<i>K2-ethylene diamine tetraacetic acid</i>
<i>KIT</i>	<i>Tyrosine protein kinase</i>
<i>KMT2A</i>	<i>lysine specific methyltransferase 2A</i>
<i>K-RAS</i>	<i>Kirsten rat sarcoma viral oncogene</i>
<i>LncRNA</i>	<i>Long non-coding ribonucleic acid</i>
<i>LP</i>	<i>Lumber puncture</i>
<i>LSD1</i>	<i>Lysine specific histone demethylase 1A</i>
<i>MBD</i>	<i>Methyl-CpG binding domain</i>

List of Abbreviations (Cont...)

Abb.	Full term
<i>MBP</i>	<i>Methyl-CpG binding proteins</i>
<i>MDS</i>	<i>Myelodysplastic syndrome</i>
<i>mL</i>	<i>Mililiter</i>
<i>MRI</i>	<i>Magnetic resonance imaging</i>
<i>MS</i>	<i>Myelosarcoma</i>
<i>ncRNA</i>	<i>Non-coding ribonucleic acid</i>
<i>NPM1</i>	<i>Nucleophosmin 1</i>
<i>NS</i>	<i>Non-significant</i>
<i>PB</i>	<i>Peripheral blood</i>
<i>PET</i>	<i>Positron emission tomography</i>
<i>pg</i>	<i>Picogram</i>
<i>PLT</i>	<i>Platelets</i>
<i>PRC2</i>	<i>Polycomb Repressive Complex 2</i>
<i>PV</i>	<i>Predictive value</i>
<i>p-value</i>	<i>Probability value</i>
<i>qRT-PCR</i>	<i>Quantitative reverse transcription polymerase chain reaction</i>
<i>r</i>	<i>Correlation</i>
<i>RBCs</i>	<i>Red blood cells</i>
<i>Rn -</i>	<i>Emission Intensity of Reporter PCR without template</i>
<i>Rn +</i>	<i>Emission Intensity of Reporter PCR with template Emission Intensity of Passive Reference</i>
<i>ROC</i>	<i>Receiver operating characteristic</i>
<i>RUNX1</i>	<i>Runt-related transcription factor 1</i>
<i>S</i>	<i>Significant</i>
<i>SFRSF2</i>	<i>splicing factor arginine / serine-rich 2</i>
<i>SOX4</i>	<i>SRY-box transcription factor 4</i>

List of Abbreviations (Cont...)

Abb.	Full term
<i>SPSS</i>	<i>statistical package for social science</i>
<i>t</i>	<i>Independent t-test</i>
<i>t</i>	<i>Translocation</i>
<i>t-AML</i>	<i>Therapy related AML</i>
<i>TERT</i>	<i>Telomerase reverse transcriptase</i>
<i>TET2</i>	<i>ten-eleven translocation-2</i>
<i>Th2</i>	<i>T-helper 2</i>
<i>TLC</i>	<i>Total leucocytic count</i>
<i>TP53</i>	<i>Tumor protein p53</i>
<i>TRDMT1</i>	<i>Transfer RNA cytosine 5 methyltransferase 1</i>
<i>uL</i>	<i>Microliter</i>
<i>WBCs</i>	<i>White blood cells</i>
<i>WHO</i>	<i>World Health Organization</i>
<i>X²</i>	<i>Chi-square test</i>

INTRODUCTION

Acute myeloid leukemia (AML) is a disorder characterized by a clonal proliferation derived from primitive hematopoietic stem cells or progenitor cells. It occurs at all ages, but predominantly in older people (>60 years of age). AML typically presents with a rapid onset of symptoms attributable to bone marrow failure (**O'Donnell et al., 2012**).

Progress in therapeutic approaches such as chemotherapy, radiotherapy, biological regulations and hematopoietic stem cell transplantation resulted in significant advancements; however, leukemia continues to be a significant health burden. An effective molecular marker for early diagnosis, prognosis and treatment guidance, is therefore, required (**Lin et al., 2018**).

Numerous studies have shown that epigenetic abnormalities play an important role in the development and progression of acute leukemia. Non-coding ribonucleic acid (ncRNA) showed to play an important role in epigenetic regulation (**Muto et al., 2015**).

The sequencing technologies and genome-wide analysis have indicated that the majority of the genome is the so-called dark matter that is transcribed into noncoding ncRNA (**Nagano and Fraser, 2011**).