Micro Rna-181a And Its Target Smad 7 As Potential Biomarkers For Tracking Child-Acute Lymphoblastic Leukemia

Thesis

Submitted for partial fulfillment of MD Degree in Medical Biochemistry and Molecular Biology

Presented by

Marwa Nabhan Mohamed
Assist. Lecturer of Medical Biochemistry & Molecular Biology
(M.B.B.Ch, M.Sc) Faculty of Medicine, Ain Shams University

Supervised by

Prof. Dr. Fathy Tash
Professor of Medical Biochemistry & Molecular Biology
Faculty of Medicine, Ain Shams University

Prof. Dr. Safinaz El-Habashy
Professor of Pediatrics
Faculty of Medicine, Ain Shams University

Prof. Dr. Randa Ali-Labib
Professor of Medical Biochemistry & Molecular Biology
Faculty of Medicine, Ain Shams University

Dr. Manal Louis Louka
Assist. Prof. of Medical Biochemistry & Molecular Biology
Faculty of Medicine -Ain Shams University

Dr. Eman KhairyFarahat
Lecturer of Medical Biochemistry & Molecular Biology
Faculty of Medicine-Ain Shams University

Faculty of Medicine
Ain Shams University
2017
☐

الحمض النووي الريبوزي الدقيق ١٨١ و سماد٧ كدلالات حيويه محتمله في سرطان الدم الليمفاوي الحاد في الأطفال

رسالة

توطئة للحصول علي درجة الدكتوراة في الكيمياء الحيوية
والبيولوجيا الجزيئيه

مقدمة من

☐ مروة نبهان محمد/الطبيبة
مدرس مساعد الكيمياء الحيويه والبيولوجيا الجزيئيه
كلية الطب- جامعة عين شمس

تحت إشراف

أ.د/ فتحي طاش
أستاذ الكيمياء الحيوية الطبيه و البيولوجيا الجزيئيه
كلية الطب- جامعة عين شمس
أ.د / سافيناز الحبشي
أستاذ طب الأطفال
كلية الطب – جامعة عين شمس
أ.د / راندا علي لبيب
أستاذ الكيمياء الحيوية الطبيه و البيولوجيا الجزيئيه
كلية الطب – جامعة عين شمس
د/ منال لويس لوقا
أستاذ مساعد الكيمياء الحيوية الطبيه و البيولوجيا الجزيئيه
كلية الطب – جامعة عين شمس
د/ ايمان خيري فرحات
مدرس الكيمياء الحيوية الطبيه و البيولوجيا الجزيئيه
كلية الطب - جامعة عين شمس

كلية الطب
جامعة عين شمس
٢٠١٧

بسم الله الرحمن الرحيم

قالوا

سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم الحكيم

صدق الله العظيم

سورة البقرة الآية: ٣٢

Acknowledgement

First of all, I would like to thank **Allah**, the most merciful, for giving me the ability to complete this work.

This work was supported by Ain Shams University Faculty of Medicine Grants Office, Grant No. 2016-42.

My deepest gratitude and appreciation to **Prof. Dr. FathyTash** Professor of Medical Biochemistry and Molecular Biology Faculty of Medicine, Ain Shams University, for his excellent supervision, useful advice and valuable cooperation all over the course of this work.

I would like sincerely to thank **Prof.Dr. Safinaz El-Habashy** Professor of Pediatrics Faculty of Medicine, Ain Shams University, for her meticulous supervision, and helpful instructions.

My deepest thanks and gratitude are specially expressed to **Prof.Dr. Randa Ali-Labib** Professor of Medical Biochemistry and Molecular Biology Faculty of Medicine, Ain Shams University, for her unlimited and talented efforts to accomplish this work.

I am especially indebted to **Dr. Manal Louis Louka** Assistant Professor of Medical Biochemistry & Molecular Biology Faculty of Medicine, Ain Shams University, for her unlimited help, and close supervision.

I must acknowledge the unlimited support and advises offered by **Dr. Eman Khairy Farahat** Lecturer of Medical Biochemistry & Molecular Biology Faculty of Medicine, Ain Shams University, for her infinite givens and advises would never be forgiven.

A special thanks to my family. Words can't express how grateful I am to the soul of my mother, and my father for all of the sacrifices that they've made on my behalf.

I would like also to thank my husband, and my children for their great support, and continuous encouragement to complete this work.

Marwa Nabhan

Contents

List of Abbreviations

3`-UTR	: 3`-UnTranslated Region
ALL	: Acute Lymphoblastic Leukemia
AML	: Acute Myeloid Leukemia
AUC	: Area Under the Curve
BCR/ABL	: Breakpoint Cluster Region/ Abelson murine leukemia viral oncogene homolog 1
BMI1	: B Lymphoma Murine Leukemia Viral Insertion Region 1 Homolog
C. elegans	: Caenorhabditiselegans
CASP8AP2	: Caspase-8-Associated Protein 2 gene
CDKs	: Cyclin-Dependent kinases
CLL	: Chronic Lymphocytic Leukemia
CML	: Chronic Myeloid Leukemia
Co-Smad	: Common-mediator Smad
CT	: Cycle Threshold
DEPC-water	: Di-Ethyl Pyro-Carbonate water
DGCR8	: DiGeorge syndrome Critical Region gene 8
Early pre B	: Early precursor B
EBV	: Epstein-Barr virus
EFS	: Event-Free Survival
EGIL	: European Group for the Immunological classification of Leukemia
EGR1	: Early Growth Response gene 1
EIA	: Enzyme Immunoassay
EMF	: Electro Magnetic Fields
EOC	: Epithelial Ovarian Cancer
FAB	: French-American-British
FN	: False Negatives
FP	: False Positives
GSK3β	: Glycogen Synthase kinase 3β
GVHD	: Graft Versus Host Disease
HRP	: Horse-Radish Peroxidase
HSCT	: Hematopoietic Stem Cell Transplantation
JAK2	: Janus Kinase 2
MAPKs	: Mitogen Activated Protein kinase
MH1	: Mad-Homology 1
MH2	: Mad-Homology 2
miRNAs	: MicroRNAs
MLL	: Mixed Lineage Leukemia
NCI	: National Cancer Institute

NPV	: Negative Predictive Value
OD	: Optical Density
PBX1/E2A	: Pre-B cell leukemia transcription factor 1 / Transcription factor E2 Alpha
PCAF	: P300/CBP Associated Factor
PPV	: Positive predictive value
Pre-B	: Precursor-B ALL
Pri-miRNA	: Primary micro RNA
RISC	: RNA-induced silencing complex
RNU6-2	: Small nuclear U6 2 RNA
ROC	: Receiver Operating Characteristics curve
RQ	: Relative Quantification
R-Smads	: Receptor-regulated Smads
Smad	: *Mothers against decapentaplegic homolog*
Smurf	: Smad-ubiquitination-regulatory factor
SP Conjugate	: Streptavidin-Peroxidase Conjugate
TEL/AML1 Or (ETV6/RUNX1)	: Translocation-Ets-Leukemia virus/ Run-related transcription factor 1
TGF-β1	: Transforming Growth Factor-β1
TMB	: 3,3',5,5'-Tetra Methyl Benzidine
TN	: True Negatives
TP	: True Positives
ZEB1	: *Zinc Finger E-Box Binding Homeobox 1*

List of Table

List of Figures

Abstract

Acute lymphoblastic leukemia (ALL) is the most common pediatric hematologic tumor. MiR-181a was expected to have a role in the development of hematological malignancies; it might act as tumor suppressor or oncogene. Smad7 was selected as miR-181a target pair. It is a negative regulator for the TGF-β1 signaling pathway. In this study, relative expression levels of miR-181a by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), both Smad 7 and TGF-β1 proteins levels by enzyme linked immunosorbent assay (ELISA) were all measured in serum of 60 child, 30 with ALL and 30 age and sex matched healthy child as control group. MiR-181a expression showed highly significant decrease; plus a significant increase and decrease of Smad7 and TGF-β1 protein levels respectively, in serum samples of ALL as compared to control group. MiR-181a expression achieved a highly significant positive and a significant negative correlation with TGF-β1 and Smad7 respectively. Furthermore, the levels of Smad7 and TGF-β1 were negatively correlated with each other ($p < 0.05$). Although, positivity rate of both Smad7 and TGF-β1 in ALL group increased with presence of hepatosplenomegaly, still there was no statistical significance. In conclusion, miR-181a could act as a tumor suppressor in pediatric ALL with over expression of its target pair, Smad7. Smad7 regulates TGF-β1 signaling via a negative feedback loop and mediates the interaction between TGF-β1 and other signaling pathways; suggesting that Smad7 over expression may have therapeutic potential in ALL.

Keywords: Lymphoblastic, Leukemia, miR181a, Smad7, TGF-β1

Introduction and aim of work

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children. The annual incidence is approximately four cases per 100.000 children in national cancer institute (NCI), Cairo University, Egypt. ALL constitutes 30% of all pediatric malignancies and 70% of pediatric leukemia. Cases show a male to female ratio of 2.3:1, 2-10 years age group constitutes 68.5% ***(Samuel and Kathleen, 2005).***

MicroRNAs (miRNAs) are a new class of endogenous non coding small RNA molecules that have been shown to be important regulators of gene expression in cells. miRNAs post-transcriptionally regulate gene expression by either cleavage or repression of mRNA through binding to the 3`-untranslated region (3`-UTR) of the target mRNAs ***(Bartel, 2004).*** They are known to be involved in a variety of functions in development, cell proliferation, apoptosis, differentiation, and tumorigenesis ***(Samuel and Kathleen, 2005).*** Thus, miRNA functional identification has become one of the most attractive research fields in biomedicine. Unfortunately, most of these miRNAs have unknown functions ***(Ambros, 2004).***

Mir-181 mature products miR-181a, miR-181b, miR-181c and miR-181d are thought to have regulatory roles at post transcriptional level, through complementarities to target mRNAs ***(Ambros, 2004).*** MiR-181 is highly expressed in B-lymphoid cells of the bone marrow and ectopic expression of miR-181 in progenitor cells increases the fraction of B-lineage cells in vitro and in adult mice, although the underlying mechanism is poorly understood ***(Chen et al., 2004).***

Dahlhaus et al. (2013) reported that Expression of miR-181a differed between various leukemia cell lines and mature blood cells.

The importance of individual miRNAs has been established in specific cancers. However, a comprehensive analysis of the contribution of miRNAs to the pathogenesis of any specific cancer is lacking ***(Mavrakis et al., 2011)***.

The Transforming Growth Factors-β (TGF-β) is a multifunctional cytokine belonging to the transforming growth factor superfamily that control growth, differentiation, and apoptosis of cells. TGF-β inhibits the proliferation of cells within the epithelial, endothelial, and hematopoietic lineages, and regulates the differentiation of immune, neuronal, mesenchymal, and epithelial cell types ***(Massagué, 1990; Piek et al., 1999).***

TGF-β binds to serine/threonine kinase receptors ***(Massagué, 2000),*** following the ligand binding, phosphorylation of downstream targets take place. These targets include Smad proteins, which are ~500 amino acids in length ***(Shi and Massagué, 2003).***

Eight Smad proteins are encoded in the human genome ***(Massagué 1998).*** Only five of the mammalian Smads Smad1, Smad2, Smad3, Smad5, Smad8 act as substrates for the TGF-β receptors; these are commonly referred to as Receptor-regulated Smads (R-Smads). Smad4, Common-mediator Smad (Co-Smad), serves as a common partner for all R-Smads. Smad6 and Smad7 are inhibitory Smads (I-Smads), and serve as decoys interfering with Smad–receptor or Smad–Smad interactions ***(Raftery and Sutherland, 1999).***

Parikh et al., 2014 suggested that the miR-181a may be a useful prognostic marker for predicting response to therapy of epithelial ovarian cancer (EOC), and reported that the target for miR-181a was the Smad7 gene. Inhibition of Smad7 by miR-181a would therefore remove the brake on TGF-β and encourage metastases. However still, the story of miR-181a in cancer is not straight forward, and it appears to have more than one target protein. Given the number of targets for miR-181a that have been identified in various cancer cells and the differing effects of the micro-RNA in breast cancer as opposed to ovarian, cervical and colorectal cancer, a lot of work lies ahead in any potential therapeutic use of miR-181a.

The present work was conducted to:

- Evaluate the expression level of miR-181a, Smad7 and TGF-β1 proteins in both child-ALL malignant group and normal healthy children control group, aiming to highlight a functional role of any of them in diagnosis of child-ALL.

Relate the previous data in child-ALL to each other and to the different clinicopathological factors, hoping that this may uncover a potential biomarker for prognosis and/or a molecular target for the treatment of child-ALL if possible.

Child Acute Lymphoblastic Leukemia

Acute leukemia is a rapidly progressing disease that produces immature cells. These cells can't carry out their normal functions. However chronic leukemia usually progresses slowly and patients have greater number of mature cells that can carry out some of their normal functions ***(Jemal et al., 2006)***.

Epidemiology:

Acute Lymphoblastic Leukemia (ALL) is the most common cancer diagnosed in children representing over a quarter of all cancers among children younger than 15 years ***(Howlader et al., 2013).*** ALL overall survival is about 80% ***(Gaynon et al., 2010).*** The peak age of incidence occurs between the ages of 2-4 years, then decreases to become a rare disease of adulthood. Additionally, another smaller peak appears in people aged over 50 years ***(Redaelli et al., 2005)***. Worldwide, the annual incidence is about 3 per 100,000 ***(Grigoropoulos et al., 2013)***. In Egypt, the annual incidence is approximately 4 cases per 100.000 children as reported by National Cancer Institute (NCI), Cairo University, Egypt ***(Samuel and Kathleen, 2005).*** The cancer incidence in Egypt will rise from 801 in males and 471 in females in 2013 to 1535 in males and 896 in females by the year 2050 ***(Ibrahim et al., 2014).***