The clinical Relevance of Non-Coding RNA in Colorectal Cancer

Thesis

Submitted for partial fulfillment of M.D in Medical Biochemistry and Molecular Biology

Presented by

Nehal Samir Abd Elhalim

Assistant lecturer of Medical Biochemistry and Molecular Biology
(M.B, B.Ch., MSc. Medical Biochemistry and Molecular Biology)
Faculty of Medicine - Ain Shams University

Supervised by

Prof. Dr. Mohamed Farid ElAsmer

Professor of Medical Biochemistry & Molecular Biology
Faculty of Medicine, Ain Shams University

Prof. Dr. Hanaa El Tayeb Nasser

Professor of Medical Biochemistry & Molecular Biology
Faculty of Medicine, Ain Shams University

Prof. Dr. Ahmed Hassan Fawzi EL-Tawdi

Professor of General and Plastic Surgery
President of Military Medical Academy

Prof. Dr. Hesham Abd Elraouf EL-Akkad

Professor of General Surgery
Faculty of Medicine, Ain Shams University

Dr. Marwa Matboly Sayed

Lecturer of Medical Biochemistry and Molecular Biology
Faculty of Medicine, Ain Shams University

Faculty of Medicine
Ain Shams University
2018

الارتباط الاكلينيكي للاحماض النوويه الريبوزيه الغير ناسخة في مرضى سرطان القولون والمستقيم

رسالة

توطئة للحصول علي درجة الدكتوراه فى الكيمياء الحيوية الطبية والبيولوجيا الجزيئية

مقدمة من

الطبيبة/ نهال سمير عبد الحليم علي

مدرس مساعد الكيمياء الحيوية الطبية والبيولوجيا الجزيئية

(ماجستير الكيمياء الحيوية الطبية و البيولوجيا الجزيئية)

تحت إشراف

أ.د/ محمد فريد الاسمر

استاذمفرغ الكيمياء الحيوية الطبية والبيولوجيا الجزيئية

كلية الطب- جامعة عين شمس

أ.د/ هناء الطيب ناصر

استاذ الكيمياء الحيوية الطبية والبيولوجيا الجزيئية

كلية الطب- جامعة عين شمس

أ. د/ أحمد حسن فوزى التاودى

استاذ الجراحه العامه والتجميل

رئيس الاكاديميه الطبيه العسكريه

أ.د / هشام عبد الرؤف العقاد

استاذ الجراحه العامه

كلية الطب -جامعة عين شمس

د/ مروة متبولى سيد

مدرس الكيمياء الحيوية الطبية و البيولوجيا الجزيئية

كلية الطب -جامعة عين شمس

كلية الطب

جامعة عين شمس

٢٠١٨

سورة البقرة الآية: ٣٢

Acknowledgement

First and faremost thanks to ALLAH, the Most Merciful.

I wish to express my deep appreciation and sincere gratitude to ***Prof. Dr. Mohamed Farid ElAsmer,*** *Professor of Medical Biochemistry & Molecular Biology , Ain Shams University, for his kind supervision, valuable instructions, continuous help, patience, advices and guidance. He has generously devoted much of his time and effort for planning and supervision of this study. It was a great honor to me to work under his direct supervision.*

I wish to express my great thanks and gratitude to ***Prof. Dr. Hanaa El Tayeb Nasser,*** *Professor of Medical Biochemistry & Molecular Biology, Ain Shams University, for her kind supervision, indispensable advice and great help in this work.*

I wish to express my great thanks and gratitude to ***Prof. Dr. Ahmed Hassan Fawzi EL-Tawdi,*** *Professor of General and Plastic Surgery, President of Military Medical Academy, for his kind supervision, indispensable advice and great help in this work.*

I wish to express my great thanks and gratitude to ***Prof. Dr. Hesham Abd Elraouf EL-Akkad,*** *Professor of General Surgery, Ain Shams University, for his kind supervision, indispensable advice and great help in this work.*

I wish to express my great thanks and gratitude to ***Dr. Marwa Matboly Sayed,*** *Lecturer of Medical Biochemistry and Molecular Biology, Ain Shams University, for her kind supervision, indispensable advice and great help in this work.*

I wish to express my great thanks and gratitude to, ***Prof. Dr. Mohamed Gameel Ramadan,*** *Professor of surgical oncology, National Institute of cancer and I wish to express my great thanks and gratitude to* ***Dr. Tarek Nabeel Elbekini,*** *Assistant professor of pathology, National Institute of cancer, for their kind help & support in this work.*

Last but not least, I want to thank all my family, my colleagues, for their valuable help and support.

Finally I would present all my appreciations to all my patients, without them, this work would not have been completed.

This work was supported by Ain Shams University, Faculty of medicine Grants office (Grant No: 2017/4)

This work was supported by Egyptian Science & Technology Development Fund RSTDG; ID: 12595& ID: 22935

Contents

List of Abbreviations

AJCC	: American joint committee of cancer
ANRIL	: Antisense noncoding RNA in the INK4 locus
ASXL1	: Additional sex combs like 1
ATP	: Adenine tri phosphate
BCAR4	: breast cancer anti estrogen resistance protein 4
BMI	: Body mass index
CA19.9	: Cancer Antigen19.9
CBX	: Chromo Box
CEA	: Carcino Embryonic Antigen
ceRNA	: competing endogenous RNA
CIN	: Chromosomal instability
circRNA	: Circular RNAs
CNOT6L	: CCR4-NOT transcription complex, subunit 6-like
CRC	: Colorectal cancer
DEPC	: Di Ethyl Pyro Carbonate
EDTA	: Ethylene Diamine Tetra Acetic acid
EED	: Embryonic ectoderm development
EGFR	: Epidermal growth factor receptor
elnRNA	: Enhancer long non coding RNA
EZH	: Enhancer Of Zeste
FAP	: Familial adenomatous polyposis
FN	: False negative
FOFX1	: Fork head Box F1
FP	: False positive
HDAC	: Histone deacetylase
HDMT	: Histone dimethyl transferase
HOTAIR	: HOX transcript anti sense RNA
HOX gene	: Homebox
IGF	: insulin-like growth factor
IL	: Interleukin
kb	: Kilo bites
K-RAS	: Kirsten rat sarcoma ma
L3MBTL1	: Lethal 3 malignant brain tumor like1
lncRNA	: long noncoding RNAs
LSD1	: lysine-specific demethylase 1

MALAT1	: metastasis associated lung adenocarcinoma transcript 1)
MAP	: Mut Y homolog -associated polyposis
miRNA	: micro RNA
MMR	: Mismatch Repair
MREs	: microRNA response elements
mRNA	: Messenger RNA
MSI	: Microsatellite instability
NCRP	: National Cancer Registry Program
NPV	: Negative predictive value
nt	: Nucleotides
O.D	: Optical density
P value	: Probability value
PATEN	: Phosphatase &Tensin
PcG	: Poly comb gene
PDGF	: Platelet Derived Growth Factor
PHC	: Polyhomeotic Homolog
PI3K/AKT	: Phosphoinositol 3 kinase/Protein kinase B
PITX2	: Paired Like Homedomain 2
PPV	: Positive predictive value
PRC	: Polycomb Repressive Complexes
qPCR	: Quantitative reverse transcriptase polymerase chain reaction assay
RING1	: Realy Interesting New Gene 1
RNA	: Ribonucleic acid
ROC	: Receiver Operating Characteristics curve
ROX	: 6 carboxyl X Rhodamine dye
RQ	: Relative quantification
RT-PCR	: Reverse transcription Polymerase Chain Reaction
SCML	: Sex Comb On Midleg Like
SD	: Standard Deviation
SFMBT	: Scm Like With Four Mbt Domains
SNP1	: Smad nuclear interacting protein 1
SPSS	: Statistical Package for the Social Sciences
SUZ12	: Suppressor of Zeste 12
TN	: True negative

TNF	: Tumor necrosis factor
TNM	: Tumor Nodes Metastases
TP	: True positive
TP53	: tumor protein 53
UICC	: Union International Cancer Control
UTR	: Untranslated region
VAPA	: vesicle-associated membrane protein-associated protein A
VEGF	: Vascular endothelial growth factor
vlincRNA	: Very long intergenic non coding RNA
xg	: Times gravity

List of Table

List of Figures

Abstract

The competing endogenous RNA networks play a pivotal role in cancer diagnosis and progression. Novel properstrategies for early detection of colorectal cancer (CRC) are strongly needed. We investigated a novel CRC-specific RNA-based integrated competing endogenous network composed of lethal3 malignant brain tumor like1 (L3MBTL1) gene, long non-coding intergenic RNA- (lncRNA RP11-909B2.1) and homo sapiens microRNA-595 (hsa-miRNA-595) using in silico data analysis. RTqPCR-based validation of the network was achieved in serum of 70 patients with CRC, 40 patients with benign colorectal neoplasm, and 20 healthy controls. Moreover, in cancer tissues of 20 of the 70 CRC cases were involved in the study. The expression of RNA-based biomarker network in both CRC and adjacent non-tumor tissues and their correlation with the serum levels of this network members was investigated. Lastly, the expression levels of the chosen ceRNA was verified in CRC cell line. Our results revealed that the three RNAs-based biomarker network (long non-coding intergenic RNA-[lncRNA RP11-909B2.1], Homo sapiens microRNA595 [hsa-miRNA-595], and L3MBTL1 mRNA), had high sensitivity and specificity for discriminating CRC from healthy controls and also from benign colorectal neoplasm. The data suggest that among these three RNAs, serum lncRNA RP11-909B2.1 could be a promising independent prognostic factors in CRC. The circulatory RNA based biomarker panel can act as potential biomarker for CRC diagnosis and prognosis.

Key words:

Bioinformatics, Colorectal Cancer, Competing Endogenous RNA, Diagnosis

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide after lung and breast cancers with two-thirds of all CRCs occurring in the more developed regions of the world. ***(American Cancer Society, 2016).***

CRC is a major economic public health problem due to lacking of screening program. ***(Ibrahim et al., 2014).*** The number of CRC cases in Egypt is increasing according to NCRP ***(Ibrahim et al., 2014).***

The incidence and mortality rates for CRC are virtually identical, reflecting the poor overall survival rates for patients with this kind of tumor ***(Weizman & Nguyen 2010).*** Most therapies are only effective if CRC is diagnosed at early stages. In 20-25% of patients with colonic cancer and in 18% of patients with rectal cancer, metastases are present at the time of the first diagnosis. A complete colonoscopy up to the cecum, coupled with biopsy for histopathological examination, is considered the gold standard to diagnose colorectal lesions. However, compliance with these screening tests has been far from adequate ***(Hewitson et al., 2008)***. These limitations underscore the need for novel biomarkers, particularly noninvasive biomarkers in serum or plasma, for diagnosis, prognosis, and prediction of response to chemotherapy ***(Filipowicz et al., 2008).***