بسم الله الرحمن الرحيم

∞∞∞∞

تم رفع هذه الرسالة بواسطة /صفاء محمود عبد الشافى

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

ملاحظات: لايوجد

Role of microRNA 204-5p (miR-204) as putative diagnostic marker in Non Small Cell Lung Cancer

Thesis

Submitted for partial fulfillment of M.D. degree in
Chest Diseases

By

Ramy Adel Younan
M.B.B.Ch, M.SC in chest diseases

Supervised by

Prof. / Emad ElDine Abd Elwahab Korraa
Professor of Chest Diseases
Faculty of Medicine-Ain Shams University

Prof. / Mohamed Ali El-Sayed Ali
Professor of Chest Diseases
Faculty of Medicine-Ain Shams University

Associate prof. / Maryam Ali Abd Elkader
Associate Professor of Chest Diseases
Faculty of Medicine-Ain Shams University

Associate prof. / Nashwa Nagy EL-Khazragy
Associate Professor of Clinical Pathology / Hematology
Faculty of Medicine-Ain Shams University

Faculty of Medicine – Ain Shams University
2022

Acknowledgment

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

I'd like to express my respectful thanks and profound gratitude to ***Prof. / Emad ElDine Abd Elwahab Korraa,*** *Professor of Chest Diseases - Faculty of Medicine- Ain Shams University for his keen guidance, kind supervision, valuable advice, instructions and continuous encouragement, which made possible the completion of this work.*

I am also delighted to express my deepest gratitude and thanks to ***Prof. / Mohamed Ali El-Sayed Ali,*** *Professor of Chest Diseases, Faculty of Medicine, Ain Shams University, for his guidance, active participation and constant help throughout this work.*

I am deeply thankful to ***Associate prof. / Maryam Ali Abd Elkader,*** *Associate Professor of Chest Diseases, Faculty of Medicine, Ain Shams University, for her great help, cooperation and guidance.*

I would also like to express my appreciation to ***Associate prof. / Nashwa Nagy El-Khazragy,*** *Associate Professor of Clinical Pathology / Hematology, Faculty of Medicine-Ain Shams University, for her continuous help, guidance and encouragement.*

I would like to express my hearty thanks to all my family for their support till this work was completed.

Last but not least my sincere thanks and appreciation to all patients participated in this study.

Ramy Adel Younan

List of Contents

Title Page No.

List of Tables

List of Figures

List of Abbreviations

Abb.	Full term

AFB: *.....Auto-fluorescence bronchoscopy*
AML: *.....Acute myeloid leukemia*
ANOVA: *.....Analysis of variance*
BC: *.....Bladder cancer*
CC: *.....Cervical cancer*
cDNA: *.....Complementary DNA*
COPD: *.....Chronic obstructive pulmonary disease*
$C_{T:}$ *.....Cycle threshold*
CT: *.....Computed tomography*
CTCs: *.....Circulating tumor cells*
cTDNA: *.....Circulating tumour DNA*
CT-TTNA: *.....CT guided transthoracic needle aspiration*
CXR: *.....Chest x ray*
EpCAM: *.....Epithelial Cell Adhesion Molecule*
GC: *.....Gastric cancer*
HCC: *.....Hepatocellular carcinoma*
LC: *.....Lung cancer*
LDCT: *.....Low dose CT*
lincRNAs: *.....Long intergenic noncoding RNAs*
lncRNA: *.....Long non coding RNA*
miRNA: *.....microRNA*
MPNSTs: *.....Malignant peripheral nerve sheath tumors*
MRE: *.....miRNA response element*
mRNA: *.....Messenger RNA*
nc: *.....Non coding*
NCBI: *.....National center for biotechnology information*
NLST: *.....National lung screening trial*
NSCLC: *.....Non small cell lung cancer*
nt: *.....Nucleotides*

List of Abbreviations (Cont...)

Abb.	Full term
PACT:	*Protein kinase, interferon inducible double stranded RNA dependent activator*
PBS:	*Phosphate buffer saline*
PCR:	*Polymerase chain reaction*
PET:	*Positron emission tomography*
Piwi:	*Precursor of non coding RNA*
PLL:	*Peripheral lung lesion*
qRT- PCR:	*Quantitative reverse transcription PCR*
RA:	*Rheumatoid arthritis*
RB:	*Retinoblastoma*
R-EBUS:	*Radial probe endobronchial ultrasound*
RISC:	*RNA induced silencing complex*
RNAi:	*RNA interference*
ROBO4:	*Roundabout Guidance Receptor 4*
RPM:	*Revolution per minute*
rRNA:	*Ribosomal RNA*
SCC:	*Squamous cell carcinoma*
SCLC:	*Small cell lung cancer*
SNPs:	*Single nucleotide polymorphisms*
SPSS:	*Statistical package for Social Science*
TCGA:	*Cancer genome atlas*
TP53:	*Tumor protein*
TRBP:	*Transactivation response element RNA binding protein*
tRNA:	*Transfer RNA*
UTR:	*Untranslated regions*
VOC:	*Volatile organic component*

1. INTRODUCTION

The leading cause of deaths related to cancer in men and women worldwide is the lung cancer ***(Fitzmaurice et al., 2015)***.

Incidence and mortality of lung cancer are strongly related to cigarette smoking patterns. As smoking rates peak first in men, then women, incidence and mortality of lung cancer increase in subsequent decades before decreasing following the beginning of tobacco control programs ***(Thun et al., 2013).***

Lung cancer is usually suspected in individuals who have an abnormal chest radiograph or have symptoms caused by either local or systemic effects of the tumor. The method of diagnosis of lung cancer depends on the type of lung cancer (small cell lung cancer or non-small cell lung cancer [NSCLC]), the size and location of the primary tumor, the presence of metastasis, and the overall clinical status of the patient ***(Rivera et al., 2013).***

Lung cancer screening:

A lot of strategies aimed to detect lung lesions at an earlier stages: public awareness of the 'alarming' symptoms of early stage lung cancer, screening with different imaging

modalities such as chest X-ray or chest CT, screening by blood biomarkers, bronchial lavage fluid or exhaled air, and bronchoscopy for those who are at high risk to develop lung cancer ***(Vansteenkiste et al., 2012).***

MicroRNA was evaluated in sputum as a tool of early detection of lung cancer. In a case control study of squamous cell lung cancer, miRNA-205, miRNA-210 and miRNA-708 were measured and showed a diagnostic sensitivity of 72% and specificity of 95% through determining patients with squamous cell lung cancer from controls ***(Xing et al., 2010).***

A further study in lung adenocarcinoma used a panel of four miRNAs (mir-21, mir-486, mir-375 and mir-200b) had a diagnostic sensitivity of 70% and a specificity of 80% of distinguishing NSCLC (including both squamous and adenocarcinoma), although these values were more accurate when only considering lung adenocarcinomas ***(Yu et al., 2010).***

The miRNA dysregulation is a screening tool that can be used even when there is no particular miRNAs regulatory function. Furthermore, miRNA dysfunction can make a significant contribution to tumorigenesis because miRNAs functions can accept tumor suppressors or oncogenes. MiRNAs expression that are increased or reduced in malignant tumours, even if there is no evidence of their role in tumorigenesis, are equally described as miRNAs that are oncogenic or cancer suppressant ***(Amuran et al., 2018).***

Aim of the Work

The aim of this study is to evaluate the efficacy of miR-204 as a novel early diagnostic marker in NSCLC via comparing its value in both serum and bronchial tissue samples.

Chapter 1

LUNG CANCER, DIAGNOSIS AND EARLY DETECTION

2.1. Overview:

The leading cause of deaths related to cancer in men and women worldwide is the lung cancer ***(Fitzmaurice et al., 2015)***.

A breakdown by level of economic development shows no differences in cancer deaths in men but a higher rate of lung cancer deaths in women in industrialized countries as compared with developing nations. Among females in developing countries, lung cancer deaths lag behind those due to breast cancer ***(Torre et al., 2012).***

Incidence and mortality of lung cancer are strongly related to cigarette smoking patterns. As smoking rates peak first in men, then women, incidence and mortality of lung cancer increase in subsequent decades before decreasing following the beginning of tobacco control programs ***(Thun et al., 2013).***

Non–small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers. Histologically, NSCLC is divided into adenocarcinoma, squamous cell carcinoma (SCC) and large cell carcinoma. NSCLC is often insidious, producing no symptoms until the disease is well advanced. Early recognition of symptoms may be beneficial to outcome.***(Rivera et al., 2013).***

At initial diagnosis, 20% of patients have localized disease, 25% of patients have regional metastasis, and 55% of patients have distant spread of disease. Symptoms depend on the location of cancer. ***(Rivera et al., 2013).***

2.2 Diagnosis of lung cancer:

Clinical Presentation of lung cancer:

Although approximately 10 percent of lung cancers in asymptomatic patients are detected on chest radiographs, most patients are symptomatic when diagnosed ***(Collins et al., 2007)***.

Patients may present with the nonspecific systemic symptoms of fatigue, anorexia, and weight loss, or with direct signs and symptoms caused by the primary tumor or intrathoracic or extrathoracic spread. The symptoms caused by primary tumor include chest discomfort, cough, dyspnea and hemoptysis ***(Beckles et al., 2003)***.

The intrathoracic spread include chest wall invasion, esophageal symptoms, horner syndrome, pancoast's tumor, phrenic nerve paralysis, pleural effusion, recurrent laryngeal nerve paralysis and superior vena cava obstruction ***(Beckles et al., 2003)***.

The symptoms caused by extrathoracic spread include bone pain and fractures, confusion, personality change, focal neurologic deficits, headache, nausea, vomiting, palpable lymphadenopathy, seizures, weakness and weight loss ***(Beckles et al., 2003)***.

A minority of patients present with paraneoplastic syndromes. Common endocrine syndromes include hypercalcemia, syndrome of inappropriate antidiuretic hormone, and Cushing's syndrome. Digital clubbing and hypertrophic pulmonary osteoarthropathy are common skeletal manifestations. Less well-defined neurologic syndromes include Lambert-Eaton myasthenic syndrome, peripheral neuropathy, and cortical cerebellar degeneration ***(Collins et al., 2007)***.

Diagnosis of lung cancer (tissue diagnosis):

Lung cancer is usually suspected in individuals who have an abnormal chest radiograph or have symptoms caused by either local or systemic effects of the tumor. The method of diagnosis of lung cancer depends on the type of lung cancer (small cell lung cancer or non-small cell lung cancer), the size and location of the primary tumor, the presence of metastasis, and the overall clinical status of the patient ***(Rivera et al., 2013).***

Selecting the most appropriate test usually requires consultation with a pulmonologist, interventional radiologist, or thoracic surgeon. In patients with apparent early non–small cell carcinomas, who are surgical candidates, thoracotomy is the recommended test for tissue diagnosis and staging. In patients with presumed small cell or metastatic non–small cell carcinomas, the diagnosis should be made using the most