





# *Investigation of Genetic Drivers of Early Carcinogenesis in Hepatocellular Carcinoma*

Submitted By

**Eman Abd El-Razek Metawea Abbas**

M.Sc. in Microbiology (2017)

Faculty of Science, Ain Shams University

**For the Fulfillment of PhD Degree of Science in Microbiology**

*Under supervision*

**Prof. Dr. Ahmed Barakat**  
**Barakat**

Professor of Virology  
Faculty of Science  
Ain Shams University

**Prof. Dr. Samar Samir**  
**Youssef**

Professor of Medical Biotechnology  
Microbial Biotechnology Department  
National Research Centre

**Dr. Mohamed Hassany Barbary**

Hepatology and Gastroenterology Fellow

Tropical Medicine Department

National Hepatology and Tropical Medicine Research Institute

Department of Microbiology

Faculty of Science

Ain Shams University

2022



Ain Shams University  
Faculty of Science  
Microbiology Department

## Approval sheet

# *Investigation of Genetic Drivers of Early Carcinogenesis in Hepatocellular Carcinoma*

**Submitted By**

**Eman Abd El-Razek Mettawa Abaas**

M.Sc. in Microbiology (2017)

Faculty of Science, Ain Shams University

**For the Fulfillment of PhD Degree of Science in Microbiology**

### Under supervision of

**Prof. Dr. Ahmed Barakat Barakat**

Professor of Virology- Faculty of Science- Ain Shams university

**Prof. Dr. Samar Samir Youssef**

Professor of Medical Biotechnology- National Research Centre

**Dr. Mohamed Hassany Barbary**

Hepatology and Gastroenterology Fellow- National Hepatology and Tropical Medicine Research Institute

### Examination committee

**Prof. Dr. Samah Ali Loutfy**

Professor of Virology and Immunology -National Cancer Institute -Cairo University

**Prof. Dr. Mohammad Mabrouk Mohammad Aboulwafa**

Professor of Microbiology and Immunology - Faculty of Pharmacy - Ain Shams university

**Prof. Dr. Ahmed Barakat Barakat**

Professor of Virology- Faculty of Science- Ain Shams university

**Prof. Dr. Samar Samir Youssef**

Professor of Medical Biotechnology- National Research Centre

**Date / /**

**Approval date / /**

**University council approved**

**/ /**



Ain Shams University  
Faculty of Science  
Microbiology Department

**Name** : Eman Abd El-Razek Metawea Abbas

**Scientific Degree** : PhD Degree of Science in Microbiology

**Department** : Microbiology

**Faculty** : Science

**University** : Ain Shams

**Graduation Year** : 2007

**Year of grants** : 2022

*I declare that the thesis titled "Investigation of Genetic drivers of early carcinogenesis in Hepatocellular Carcinoma" is my own work and has not previously submitted to any other university. The references were being checked when possible; show the extent to which I have availed myself of the work of others.*

*Eman Abd El-Razek Metawea*

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿ قالوا سبحانك لا علم لنا الا ما علمتنا

﴿ إنك انت العليم الحكيم

صدق الله العظيم  
الآيه (32) سورة البقره

# *Dedication*

*I dedicate this work*

*To My Family:*

*My Great Dad*

*My Kind Mum*

*My Beloved Brother*

*and His Family*

*My Dear Husband*

*My LoVely Daughters*

*I thank Allah for choosing you to be my family*

*Thank you for supporting me with kindness, patience,  
..... and love.*

*I Love you all*

*Yours,*

*Eman Abd El- Razeq Metawaa*

## *Acknowledgment*

First and foremost thanks for **ALLAH**, the most beneficent and merciful

I would like to express my gratitude and thanks to my dear supervisor **Prof. Dr. Ahmed Barakat Barakat** Prof. of Microbiology, Microbiology Department, Faculty of Science, Ain Shams University for his kind supervision, revision, guidance, encouragement and his continuous support and help during this work. I cannot express my gratitude to him. I am very lucky to have this great opportunity to be one of his students.

My deepest gratefulness, gratitude and thanks to **Prof. Dr. Samar Samir Youssef** Prof. of Medical Biotechnology, Microbial Biotechnology Department, Biotechnology Research Institute, National Research Center for suggesting the point of this thesis, her confidence in allowing me to follow my curiosity instincts towards columniation of my research goals. Also, I thank her for her kind supervision, support, guidance and encouragement in all of the theoretical and practical aspects during all stages of this thesis. It is a great gift from ALLAH to be one of her students.

Thanks are also expressed to **Prof. Dr. Mohamed Hassany Barbary**, Hepatology and Gastroenterology Fellow, Tropical Medicine Department, National Hepatology and Tropical Medicine Research Institute, Cairo, for his generous help in collection all the clinical cases and his guidance in the clinical aspects throughout this work.

Sincere thanks and respect to **Prof. Dr. Moataza Omran** head of Microbial Biotechnology Department, Biotechnology Research Institute, National Research Center.

No words would be sufficient to express my gratitude towards all the professors and staff members of Microbial

---



## Acknowledgment

---

Biotechnology Department, Biotechnology Research Institute, National Research Center and my friends and colleagues, for their sincere encouragement and support throughout this work.

Special thanks to all the patients without whom this work would not have been completed.

Last but not least, I Would like to thank my family: my father, my mother, my husband and my lovely daughters, my brother and his family and the rest of my family for their love, support and patience. Their love and encouragements help me to withstand stress fulltime.

*Eman Abd El- Razeq Metawaa*





---

# *Contents*

---



# Contents

	page
List of Figures.....	I
List of Tables.....	II
List of Abbreviations.....	III
Abstract.....	VII
1. Chapter I : Introduction.....	1
Aim of work.....	6
2. Chapter II : Review of Literature.....	7
I. Hepatocellular carcinoma (HCC).....	7
I.1. Background .....	7
I.2. Incidence of HCC .....	8
I.3. Risk factors of HCC .....	10
I.4. Pathology of HCC .....	16
I.5. Staging of HCC .....	17
I.6. Diagnosis and prevention of HCC .....	21
I.7. HCC Treatment .....	27
I.8. Genetic and epigenetic changes in the molecular carcinogenesis of HCC.....	35
II. Copy number variations .....	39
II.1. The classification of CNV.....	40
II.2. Genomic factors of CNV .....	41
II.3. Molecular mechanisms of CNV formation .....	43
II.4. Environmental factors contributing to the formation of CNVs .....	46
II.5. Pathological aspects of CNVs .....	47
II.6. Molecular mechanism by which CNV convey phenotype .....	48
II.7. The Biological roles of CNV .....	48
II.8. The role of CNV in Cancer .....	49
II.9. Common genomic alterations and CNV in HCC .....	51
II.10. Amplification of chromosome 1q genes .....	57
III. B-cell CLL/lymphoma 9 (BCL9) gene .....	58
III.1. Genomic Locations for BCL9 Gene .....	58
III.2. BCL9 binding partners .....	59
III.3. Function of BCL9 .....	59
III.4. BCL9 CNV in disease .....	60
III.5. BCL9 as an oncogene .....	60
3. Chapter III: Subjects and Methods.....	64
3.1. Subjects .....	64
3.1.1. Patients classification .....	66
3.1.2. Sample collection .....	67

3.2.	Sample Processing.....	67
3.2.1.	Plasma separation .....	69
3.2.2.	The processing and purification of cfDNA .....	69
3.2.3.	cfDNA identification, quantification and dilution .....	72
3.2.4.	Quantitative analysis of CN variant of BCL9 gene by quantitative polymerase chain reaction reaction .....	75
3.2.5.	Analysis and Interpretation of data .....	83
3.3.	Statistical analysis.....	85
4.	Chapter IV: Results.....	87
4.1.	Demographic and clinical features of HCV-related HCC patients and healthy individuals .....	87
4.2.	Checking the quality and quantity of the extracted cfDNA .....	90
4.3.	Circulating free DNA isolation from whole blood .....	91
4.4.	Quantification analysis of copy number of BCL9 gene in 1q21.2 genomic part.....	92
4.5.	Calculation of the relative quantity and BCL9 Copy Number.....	93
4.6.	Association of HCC characteristics and baseline parameters with HCC development .....	94
4.7.	Relation of relative quantity and Copy Number of BCL9 gene in control group versus HCC group.....	98
4.8.	Frequency of CN gain in HCC patients .....	100
4.9.	Correlation of CNV in BCL9 gene in HCC patients with HCC development .....	101
4.10.	Variation of copy number of BCL9 in male versus female HCC patients .....	102
4.11.	Correlation between variation in BCL9 gene CN in male patients and the clinical characteristics of HCC patients...	104
5.	Chapter V: Discussion.....	106
6.	Chapter VI:.....	114
	. Summary .....	114
	. Conclusion.....	119
	. Recommendation .....	121
7.	Chapter VII: References.....	122
	Arabic summary	
	Arabic abstract	

## *List of Figures*

No.	Title	Page
<b>Fig. 1</b>	The incidence of HCC according to geographical area and aetiology .....	10
<b>Fig. 2</b>	Schematic diagram of mechanism of hepatocarcinogenesis .....	16
<b>Fig. 3</b>	BCLC staging system and treatment strategy .....	18
<b>Fig. 4</b>	Flow diagram of HCC prevention .....	26
<b>Fig. 5</b>	Therapeutic modalities used for HCC treatment .....	27
<b>Fig. 6</b>	Current and future treatment options by line of therapy and patient subgroup .....	34
<b>Fig. 7</b>	Recurrent versus non-recurrent rearrangements .....	42
<b>Fig. 8</b>	The molecular mechanisms of CNV formation .....	45
<b>Fig. 9</b>	Distribution of common cancer CNVs in the human genome .....	51
<b>Fig. 10</b>	Focal amplification and deletion peaks identified by GISTIC2 in primary HCCs .....	53
<b>Fig. 11</b>	Workflow of Copy Number detection .....	68
<b>Fig. 12</b>	CNV Analysis Using TaqMan Copy Number Assays.	80
<b>Fig. 13</b>	Spectral display for the quantity and quality of cfDNA samples .....	90
<b>Fig. 14</b>	Agarose gel electrophoresis of cfDNA .....	91
<b>Fig. 15</b>	Quantitative real time polymerase chain reaction products .....	92
<b>Fig. 16</b>	The BCL9 relative quantity and CNVs degree in HCC and control groups .....	99
<b>Fig. 17</b>	The BCL9 CNV distribution .....	100
<b>Fig. 18</b>	Distribution of CNV in early and late HCC patients...	101
<b>Fig. 19</b>	The BCL9 CNV distribution according to gender of HCC patients .....	103

## **List of tables**

No.	Title	Page
<b>Table. 1</b>	Risk factors for HCC development .....	11
<b>Table. 2</b>	Top Focal Regions of Copy Number Gains and Losses in HCC .....	54-55
<b>Table. 3</b>	Description of the Copy Number assay and the Copy Number Reference assay .....	78
<b>Table. 4</b>	The thermal cycle of the qPCR reaction .....	83
<b>Table. 5</b>	Demographic and clinical features of patients .....	89
<b>Table. 6</b>	Calculations for determining BCL9 copy number .....	93
<b>Table. 7</b>	Univariate analysis for the clinical parameters of the early versus late HCC patients.....	95-96
<b>Table. 8</b>	Multivariate logistic regression to detect independent predictors of late HCC stage .....	97
<b>Table. 9</b>	Relative quantity and copy number levels of BCL9 gene in the studied groups .....	98
<b>Table. 10</b>	Distribution of CNV in HCC patients .....	102
<b>Table. 11</b>	BCL9 CN gain according to male gender, and clinical and HCC characteristics parameters .....	105

## **List of Abbreviations**

<b>AASLD</b>	American Association for the Study of Liver Disease
<b>A1ATD</b>	Alpha1-Antitrypsin deficiency
<b>Ab</b>	Antibody
<b>AFB1</b>	Aflatoxin B1
<b>AFP</b>	alpha-fetoprotein
<b>AFP-L3</b>	Lens culinaris-reactive AFP
<b>AIH</b>	Autoimmune hepatitis
<b>AJCC</b>	American Joint Committee on Cancer
<b>ALT</b>	Alanine transferase
<b>ASR</b>	Age-standardized incidence rate
<b>AST</b>	Aspartate aminotransferase
<b>AXIN1</b>	Axin 1
<b>β</b>	Beta
<b>BCLC</b>	Barcelona Clinic Liver Cancer
<b>BCL9</b>	B-cell CLL/ lymphoma 9
<b>BCL9L</b>	B-cell lymphoma 9-like
<b>Bil.T.</b>	Bilirubin Total
<b>BMI</b>	Body Mass Index
<b>bp</b>	Base Pair
<b>CCND1</b>	Cyclin D1
<b>CDKN2A</b>	Cyclin-dependent Kinase inhibitor 2A
<b>CDKN2B</b>	Cyclin-dependent kinase inhibitor 2B
<b>cDNA</b>	Complementary DNA
<b>cfDNA</b>	Circulating cell-free DNA
<b>CI</b>	Confidence interval
<b>CLIP</b>	Cancer of the Liver Italian Program
<b>CMV</b>	Cytomegalo virus
<b>CN</b>	Copy number
<b>CND1</b>	cyclin D1
<b>CNV</b>	Copy number variation
<b>Conc.</b>	Concentration