

Biochemical Study On Stem Cells Isolated From Patients With Hepatocellular Carcinoma (HCC)

Thesis

**Submitted for Partial Fulfillment of Ph.D. degree in Cancer Biology
(Medical Biochemistry & Molecular Biology)**

By

Amany Yahia Abdel-Hamid

M.Sc. Biochemistry 2005-Faculty of Science

Ain Shams University

Under supervision of

Prof. Dr. Motawa E. El Hussein

Prof. of Medical Biochemistry

Cancer Biology Department

National Cancer Institute

Cairo University

Dr. Sherif B. El Din Zaid

Assistant prof. of Surgical Oncology,

Surgery Department

National Cancer Institute

Cairo University

Dr. Abeer M. El-Sayed

Assistant Prof. of Pathology

Pathology Department

National Cancer Institute

Cairo University

Cancer Biology Department

National Cancer Institute

Cairo University

2017

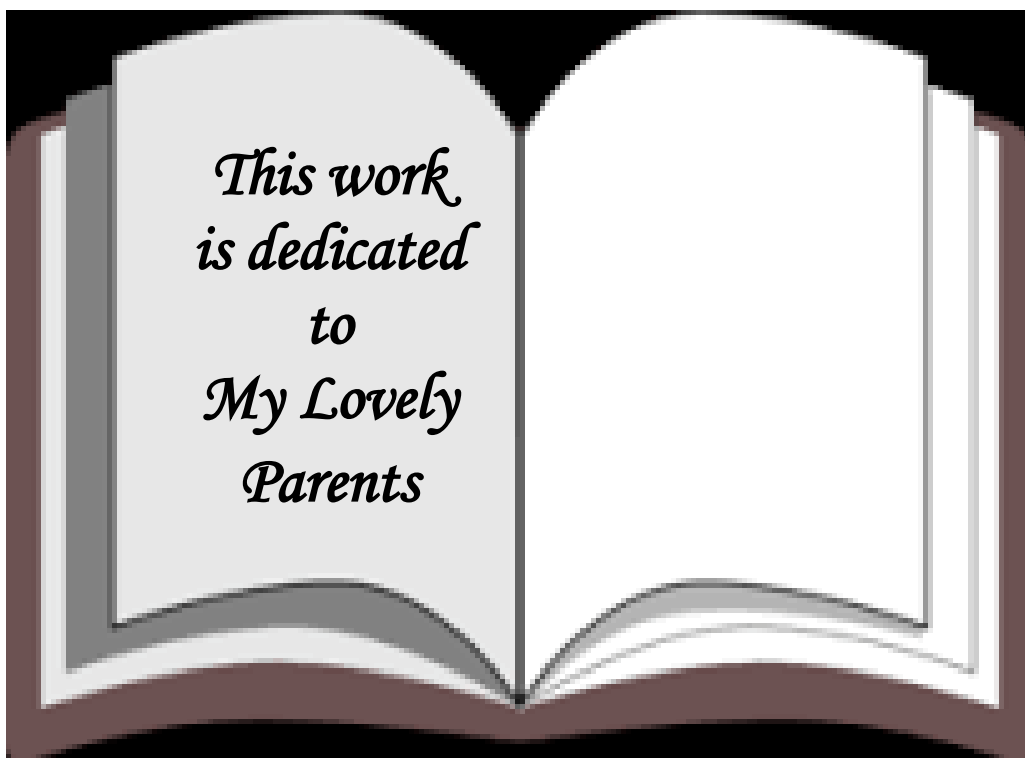
بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

"قَالُوا سِحْرَانِكَ لَا عِلْمَ لَنَا

إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ

الْعَلِيمُ الْحَكِيمُ"

"سورة البقرة – آية 32"



ACKNOWLEDGEMENT

*First and foremost, I feel always indebted to **ALLAH AZZA WA JAL**, the most Merciful.*

*Second, I am greatly honored to express my sincere gratitude, deepest appreciation to **Prof. Dr. Motawa E. El Houseini** (Prof. of Medical Biochemistry, Cancer Biology Department, National Cancer Institute) for suggesting the research proposal and designing the work plan.*

*I would like to express my deepest gratitude and appreciation to **Dr. Sherif B. El Din Zaid** (Assistant Prof. of Surgical Oncology, Surgery Department, National Cancer Institute) and **Dr. Abeer M. EI-Sayed** (Assistant Prof. of Pathology, Pathology Department, National Cancer Institute) for their help, time, guidance and kind support throughout the work.*

*I would like to express my deepest gratitude to **Prof. Dr. Amr A. Abdel Aal** (Professor of Surgery, Faculty of Medicine, Ain Shams University) for his generous help and faithful support.*

*I would like also to thank **Dr. Zainab Fathy** (Lecturer of Virology and Immunology, Cancer Biology Department, National Cancer Institute, **Dr. Mahmoud Kamal** (M.Sc in Biochemistry, Faculty of Science, Ain Shams University) and **Prof. Dr. Mahmoud El Rouby** (Prof. of Virology and Immunology, Cancer Biology Department, National Cancer Institute) for their great help they have done for this study.*

*May **ALLAH** accept the work of all those and reward them for it.*

CONTENTS

<i>Title</i>	<i>Page No.</i>
List of Tables	I - III
List of Figures	IV - VII
List of Abbreviations	VIII – X
1- Introduction.....	1-3
2- Aim of work.....	3
3-Literature Review.....	4-34
1. Hepatocellular Carcinoma (HCC)	4
2- Liver cells types and functions	19
3. Stem Cells	23
4- Material and methods.....	35-46
5- Results.....	47-73
6- Discussion.....	74-80
7- Summary.....	81-83
8- References.....	84-105
Arabic summary	

LIST OF TABLES

<i>Table No.</i>	<i>Title</i>	<i>Page No.</i>
Tables of Literature Review		
Table (1):	Causes of liver cirrhosis that could result in the development of hepatocellular carcinoma	6
Tables of Materials and Methods		
Table (1):	Primer sequences used for detection of c-Kit gene	42
Tables of Results		
Table (1):	The patients characteristics under the study	47
Table (2):	CD90 expression in tumor-derived stem cells before and after treatment with different anti HCV therapies.	51
Table (3):	CD44 expression in tumor derived-stem cells before and after treatment with different anti HCV therapies.	52
Table (4):	CD133 expression in tumor-derived stem cells before and after treatment with different anti HCV therapies.	53
Table (5):	CD90 + CD44 co-expression in tumor-derived stem cells before and after treatment with different anti HCV therapies.	55
Table (6):	CD133 +CD44 co-expression in tumor-derived stem cells before and after treatment with different anti HCV therapies.	56

<i>Table No.</i>	<i>Title</i>	<i>Page No.</i>
Table (7):	CD90 expression in non tumor-derived stem cells before and after treatment with different anti HCV therapies.	57
Table (8):	CD44 expression in non tumor-derived stem cells before and after treatment with different anti HCV therapies.	58
Table (9):	CD133 expression in non tumor-derived stem cells before and after treatment with different anti HCV therapies.	59
Table (10):	CD90+CD44 co-expression in non tumor-derived stem cells before and after treatment with different anti HCV therapies.	61
Table (11):	CD133 +CD44 co-expression in non tumor-derived stem cells before and after treatment with different anti HCV. therapies.	62
Table (12):	Comparison between CD90 expression in non tumor and tumor-derived stem cells before and after treatment with different anti HCV therapies.	63
Table (13):	Comparison between CD44 expression in non tumor and tumor-derived stem cells before and after treatment with different anti HCV therapies.	64
Table (14):	Comparison between CD133 expression in non tumor and tumor-derived stem cells before and after treatment with different anti HCV therapies.	65

<i>Table No.</i>	<i>Title</i>	<i>Page No.</i>
Table (15):	Comparison between CD90+CD44 co-expression in non tumor and tumor-derived stem cells before and after treatment with different anti HCV therapies.	66
Table (16):	Comparison between CD133 + CD44 co-expression in non tumor and tumor-derived stem cells before and after treatment with different anti HCV therapies,	67
Table (17):	Percent change in CD90 expression in non tumor and tumor-derived stem cells treated with different anti HCV therapies.	68
Table (18):	Percent change in CD44 expression in non tumor and tumor-derived stem cells treated with different anti HCV therapies.	69
Table (19):	Percent change in CD133 expression in non tumor and tumor-derived stem cells treated with different anti HCV therapies.	70
Table (20):	Percent change in CD90+CD44 co-expression in non tumor and tumor-derived stem cells treated with different anti HCV therapies.	71
Table (21):	Percent change in CD133+CD44 co-expression in non tumor and tumor-derived stem cells treated with different anti HCV therapies.	72

LIST OF FIGURES

<i>Fig.</i>	<i>Title</i>	<i>Page No.</i>
Figures of Literature Review		
Fig. (1):	Calculated age specific incidence rates among Egyptian population in the period 2008-2011 according to the National Population-Based Cancer Registry Program, Department of Biostatistics & Cancer Epidemiology, National Cancer Institute.	5
Fig. (2):	Strategies for primary and secondary liver cancer prevention in healthy subjects and in those with chronic hepatitis infection.	13
Fig. (3):	Differentiation potential of pluripotent stem cells.	26
Fig. (4):	Pathophysiological changes take place during long-term inflammation	29
Figures of Results		
Fig. (1):	H&E section of tumor tissue of case (2) showing poorly differentiated hepatocellular carcinoma, grade III (200x).	48
Fig. (2):	H & E section of tumor tissue of case (3) showing hepatocellular carcinoma, grade II, (100x).	48
Fig. (3):	A & B photomicrographs of cultured tumor-derived stem cells showing proliferated spindle cells after 12 days of culture (100x).	49
Fig. (4):	A & B photomicrographs of cultured non-tumor derived stem cells showing low proliferation rate after 12 days of culture A(100x),B(200x).	50

<i>Fig.</i>	<i>Title</i>	<i>Page No.</i>
Fig. (5):	Means of expression of CD90 in tumor-derived stem cells after treatment with different anti HCV therapies.	51
Fig. (6):	Means of expression of CD44 in tumor-derived stem cells after treatment with different anti HCV therapies.	52
Fig. (7):	Means of expression of CD133 in tumor-derived stem cells after treatment with different anti HCV therapies.	53
Fig. (8):	Means of expression of CD90, CD44 and CD133 in tumor-derived stem cells after treatment with different anti HCV therapies.	54
Fig. (9):	Means of co-expression of CD90+CD44 in tumor-derived stem cells after treatment with different anti HCV therapies.	55
Fig. (10):	Means of co-expression of CD133+CD44 in tumor-derived stem cells after treatment with different anti HCV therapies	56
Fig. (11):	Means of expression of CD90 in non tumor-derived stem cells after treatment with different anti HCV therapies.	57
Fig. (12):	Means of expression of CD44 in non tumor-derived stem cells after treatment with different anti HCV therapies.	58
Fig. (13):	Means of expression of CD133 in non tumor -derived stem cells after treatment with different anti HCV therapies.	59

<i>Fig.</i>	<i>Title</i>	<i>Page No.</i>
Fig. (14):	Means of expression of CD90,CD44,and CD133 in non tumor-derived stem cells after treatment with different anti HCV therapies.	60
Fig. (15):	Means of co-expression CD90+CD44 in non tumor derived stem cells after treatment with different anti HCV therapies.	61
Fig. (16):	Means of expression of CD133 +CD44 co-expression in non tumor-derived stem cells after treatment with different anti HCV therapies.	62
Fig. (17):	Means of expression of CD90 in non tumor and tumor-derived stem cells after treatment with different anti HCV therapies.	63
Fig. (18):	Means of expression of CD44 in non tumor and tumor-derived stem cells after treatment with different anti HCV therapies.	64
Fig. (19):	Means of expression of CD133 in non tumor and tumor-derived stem cells after treatment with different anti HCV. therapies.	65
Fig. (20):	Means of co-expression of CD90+CD44 in non tumor and tumor-derived stem cells after treatment with different anti HCV therapies.	66
Fig. (21):	Means of co-expression of CD133 +CD44 in non tumor and tumor-derived stem cells after treatment with different anti HCV therapies.	67

<i>Fig.</i>	<i>Title</i>	<i>Page No.</i>
Fig. (22):	Means of percent change of CD90 expression in non tumor and tumor-derived stem cells treated with different anti HCV therapies.	68
Fig. (23):	Means of percent change of CD44 expression in non tumor and tumor-derived stem cells treated with different anti HCV therapies.	69
Fig. (24):	Means of percent change of CD133 expression in non tumor and tumor-derived stem cells treated with different anti HCV therapies.	70
Fig. (25):	Means of percent change of CD90+CD44 co-expression in non tumor and tumor-derived stem cells treated with different anti HCV therapies.	71
Fig. (26):	Means of percent change of CD133+CD44 co-expression in non tumor and tumor-derived stem cells treated with different anti HCV therapies.	72
Fig. (27):	Gel electrophoresis for expression of β -actin gene (internal control) on (tumor and non-tumor tissue of HCC patients) lane M (100bp molecular weight ladder).	73
Fig. (28):	Gel electrophoresis for expression of c-kit gene on (tumor and non-tumor tissue of HCC patients). The c-kit expressed in HCC, and expression was negative in non-tumor liver tissue. Lane M (100bp molecular weight ladder).	73

LIST OF ABBREVIATIONS

5-FU	5-flouro uracil
AFP	Alpha fetoprotein
AFU	Alpha –L- fucosidase
AIH	Autoimmune hepatitis
b-FGF	Basic fibroblast growth factor
BMI	Body mass index
CD	Cluster differentiation
CDK	Cyclin- dependent kinase
cDNA	Complementary deoxyribonucleic acid
CKI	Cyclin- dependent kinase inhibitor
c-kit	Stem cell factor receptor
CLD	Chronic liver disease
CSCs	Cancer stem cells
CT	Computed tomography
DN	Dysplastic nodule
DENA	Diethyl Nitrosamine
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
EDTA	Ethylene diamin tetra- acetate
EGF	Epidermal growth factor
ER	Endoplasmic reticulum

ESCs	Embryonic Stem Cells
FCM	Flow cytometry
FGF	Fibroblast growth factor
FITC	Fluorescein isothiocyanate
H&E	Hematoxillin – Eosin
HBSS	Hanks balanced salt solution
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HSCs	Hepatic stellate cells
PSCs	Peri-sinusoidal cells
INF-α	Interferon alpha
iPSCs	Induced pluripotent stem cells
Mm	Micro – meter
MRI	Magnetic resonance imaging
MSCs	Mesenchymal stem cells
MW	Microwave
NAFLD	Non- alcoholic fatty liver disease
NASH	Non alcoholic steato-hepatitis
NPCs	Non parenchymal cells
NT	Non tumor
PTFs	Portal Tract Fibroblasts
O S M	Oncostatin

RBCs	Red blood cells
RF	Radiofrequency
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
SCF	Stem cell factor
SECs	Sinusoidal endothelial cells.
SVR	Sustained viral response
T	Tumor
TGF-β	Transforming growth factor beta
UCB	Umbilical cord blood
US	Ultrasonography
VEGF	Vascular endothelial growth factor