

Effect of Human Adipose Tissue Derived Mesenchymal Stem Cells on Squamous Cell Carcinoma Cell Line

Thesis Submitted to the Faculty of Dentistry, Ain Shams University, In the Partial Fulfillment of the Requirements for the
Degree of Doctor of Philosophy
In Oral Pathology

By

Iman Mostafa Abdel Meguid

B. D. S (Ain Shams), M. D. Sc. (Ain Shams)
Assistant Lecturer of Oral Pathology, Faculty of Dentistry
Future University

Faculty of Dentistry
Ain Shams University

2017

Supervisors

Dr. Mohamed Salah El-Din Ayoub

Professor of Oral Pathology

Faculty of Dentistry

Ain Shams University

Dr. HouryMoustafa Baghdadi

Professor and Head of Oral Pathology

Faculty of Dentistry

Ain Shams University

Dr. Dina Sabry Abdel Fattah

Professor of Medical Biochemistry and Molecular Biology

Faculty of Medicine

Cairo University

Table of Contents

List of Abbreviations	i
List of Figures	vii
List of Tables	ix
Introduction	1
Review of Literature	4
Cell Cycle.....	4
Cell Cycle and Carcinogenesis.....	6
P16 tumor suppressor gene.....	6
Apoptosis.....	8
Apoptosis and Carcinogenesis.....	11
Livin (an inhibitor of apoptosis protein).....	13
Livin as a prognostic marker.....	17
Livin as a potential therapeutic target.....	18
Squamous Cell Carcinoma.....	18
Stem Cells.....	20
Types of Stem Cells.....	21
Embryonic Stem Cells.....	22
Adult Stem Cells.....	24
MSCs are promising therapeutic tools	27
Wnt signaling and MSCs.....	32
Adipose Tissue Derived Mesenchymal Stem cells (AD-MSCs).....	32
Cell Surface Characterization of AD-MSCs.....	33
Multilineage Differentiation Capacity.....	33
Other Applications of AD-MSCs.....	35
MSC Mediated Effects on Tumor Support or Suppression.....	36
Aim of the Study	39
Material and Methods	40
I. Isolation and propagation of human adipose tissue derived MSCs	

Table of Contents

AD-MSCs	40
II.Propagation of HEp2 cell line.....	42
III.Addition of stem cells conditioned medium to HEp2 cells.....	45
IV.Coculture of AD-MSCs and HEp2 cells.....	45
V.QRT-PCR gene expression of P16 and Livin genes.....	45
VI. Cell Proliferation Assay.....	52
V. Assessment of the Results.....	53
Results	54
A. Microscopic examination results	54
B.Real Time PCR results.....	59
1. P16 gene expression.....	59
2. Livin gene expression.....	62
C. MTT Assay Results.....	66
Discussion	70
Conclusion	82
Recommendation	83
English Summary	84
References	86
Arabic Summary	118

Table of Contents

List of Abbreviations

AD-MSCs: Adipose Derived Mesenchymal Stem Cells

ALCAM: Activated Leukocyte Cell Adhesion Molecule

ASCs: Adult Stem Cells

Bcl-2: B-cell lymphoma 2

bFGF: basic Fibroblast Growth Factor

BIR: Baculovirus IAP Repeat

BIRC1/NAIP: Baculoviral IAP Repeat Containing 1/NLR family Apoptosis Inhibitory Protein

BIRC2/cIAP1: Baculoviral IAP Repeat Containing 2/ cellular Inhibitor of Apoptosis Protein 1

BIRC3/cIAP2: Baculoviral IAP Repeat Containing 3/ cellular Inhibitor of Apoptosis Protein 2

BIRC4/XIAP: Baculoviral IAP Repeat Containing 4/X-linked Inhibitor of Apoptosis

BIRC5: Baculovirus IAP RepeatContaining 5

BIRC6:Baculovirus IAP RepeatContaining 6

BIRC7:Baculovirus IAP RepeatContaining 7

BIRC8: Baculovirus IAP RepeatContaining 8

BM: Bone Marrow

BM-MSCs: Bone Marrow Mesenchymal Stem Cells

List of Abbreviations

BMP2: Bone Morphogenic Protein

CAFs: Carcinoma Associated Fibroblasts

CD 95: Cluster of Differentiation 95

CDKs: Cyclin-Dependent Kinases

cDNA: complementary Deoxyribonucleic Acid

CFU-Fs: Colony Forming Unit Fibroblasts

Ct: Cycle threshold

CTL: Cytotoxic T-Lymphocyte

dATP: deoxyadenosine Triphosphate

DCs: Dendritic Cells

dCTP: deoxycytidine Triphosphate

DDR:DNA Damage Response

DEPC: Diethyl pyrocarbonate

dGTP:deoxyguanosine Triphosphate

DMEM: Dulbecco's Modified Eagle's Medium

DNA: Deoxyribonucleic Acid

DNAase: Deoxyribonuclease

dNTPs: Deoxynucleotide Triphosphate

DPSCs: Dental Pulp Stem Cells

DR: Death Receptor

List of Abbreviations

dTTP: deoxythymidine Triphosphate

E1: ubiquitin-activating enzyme

E2: ubiquitin-conjugating enzyme or UBC

E3: ubiquitin protein ligase

E4: ubiquitin chain-assembly factor

ECM: Extra-Cellular Matrix

EDTA: Ethylene Diamine Tetra Acetate

ELISA: Enzyme-Linked Immunosorbent Assay

ER: Endoplasmic Reticulum

ESCs: Embryonic Stem Cells

FasL: Fas Ligand

FBS: Fetal Bovine Serum

GAPDH: Glyceraldehyde 3-Phosphate Dehydrogenase

HeLa Cells: Cell line derived from cervical cancer cells taken “Henrietta Lacks”

HEp2: Human Epithelial type 2 cells

HGF: Hepatocyte Growth Factor

HKGs: Housekeeping Genes

HLA: Human Leucocyte Antigen

HLA-DR: Human Leukocyte Antigen - antigen D Related

List of Abbreviations

HNSCC: Head and Neck Squamous Cell Carcinoma

HPRI: Human Placental Ribonuclease Inhibitor

HSCs: Hematopoietic Stem Cells

IAPs: Inhibitor of Apoptosis of Proteins

ICAM-1: Intra-Cellular Adhesion Molecule-1

IFN- γ /IFN- α /IFN- β : Interferon gamm/alpha/beta

IGF-1: Insulin-like Growth Factor-1

IL-6: Interleukin 6

Ink4: INhibitors of CDK4

iPS cells: induced Pluripotential Stem cells

I κ B α : Inhibitor of kappa B alpha

JNK: c-Jun N-terminal Kinase

lacZ: bacterial beta-galactosidase

LIF: Leukemia Inhibitory Factor

MAPK: Mitogen Activated Protein Kinase

MCF-7: Michigan Cancer Foundation-7 (breast cancer cell line)

M-CSF: Macrophage Colony-Stimulating Factor

MHC:Major Histocompatibility Complex

ML-IAP: Melanoma Inhibitor of Apoptosis

MMLV: Moloney Murine Leukemia Virus

List of Abbreviations

mRNA: Messenger Ribonucleic Acid

MSCs: Mesenchymal Stem Cells

MTT: Mosmann's Tetrazole Test/ Methyl Thiazol Tetrazlium

NeuN: neuron specific protein

NF- κ B: Nuclear Factor kappa B

NK: Natural Killer

NSE: Neuron Specific Enolase

OSCC: Oral Squamous Cell Carcinoma

PARP: Poly ADP-Ribose

PBS: Phosphate Buffer Saline

PGE2: Prostaglandin E2

PGF: Placental Growth Factor

pH: potential of Hydrogen

PI3K/AKT: Phosphoinositide 3-Kinase pathway/ AK murine Thymoma

PLA: Processed Lipoaspirate

PS: Phosphatidylserine

Rb protein: Retinoblastoma protein

RING: Really Interesting New Gene

Rpm: Rounds per minute

RQ: Relative Quantification

List of Abbreviations

RT-PCR: Reverse Transcriptase Polymerase Chain Reaction

SCC: Squamous cell Carcinoma

SCF: Stem Cell Factor

SDF-1: Stromal Cell Derived Factor-1

SHEDS: Stem Cells from Human Exfoliated Deciduous

siRNA: small interfering Ribonucleic Acid

SMAC/Diablo: Second Mitochondria-Derived Activator of Caspase/Direct IAP binding protein with low pH

SPSS: Statistical Package for the Social Science

SVF: Stromal Vascular Fraction

TAFs: Tumor Associated Fibroblasts

TFG- β : Transforming Growth Factor beta

TNFR1: TNF Receptor type

TNF- α : Tumor Necrosis Factor- alpha

TRAF2: TNF Receptor Associated Factor 2

UBC: Ubiquitin-Conjugating enzyme

VEGF: Vascular Endothelial Growth Factor

VSEL: Very Small Embryonic- Like

List of Figures

Fig. 1	Cell cycle checkpoints	4
Fig. 2	The intrinsic and extrinsic pathways of apoptosis	10
Fig.3	Mechanisms contributing to evasion of apoptosis and carcinogenesis	12
Fig. 4	The biological functions of Livin	14
Fig. 5	Classes of IAPs members	14
Fig. 6	Ubiquitin pathway	15
Fig. 7	Paracrine effects of cultured MSCs	31
Fig. 8	Photomicrographs of AD-MSCs culture	41
Fig. 9	Incubator	41
Fig. 10	Flask containing HEp-2 cell line	43
Fig. 11	Cooling Centrifuge (Sigma)	43
Fig. 12	Pipetting cell pellet to disaggregate clumps	43
Fig. 13	Spectrophotometer	47
Fig. 14	Real time PCR	51
Fig. 15	Photomicrograph of HEp2 Cells after 24 hours	56
Fig. 16	Photomicrograph of HEp2 Cells after 48 hours	56
Fig. 17	Photomicrograph of HEp2 Cells after 72 hours	56
Fig. 18	Photomicrograph of HEp2 cells cultured with AD-MSCs medium after 24 hours	57
Fig. 19	Photomicrograph of HEp2 cells cultured with AD-MSCs medium after 48 hours	57
Fig. 20	Photomicrograph of HEp2 cells cultured with AD-MSCs medium after 72 hours	57
Fig. 21	Photomicrograph of HEp2 cells cocultured with HEp2 and AD-MSCs media after 24 hours	58
Fig. 22	Photomicrograph of HEp2 cells cocultured with HEp2	58

List of Figures

	and AD-MSCs media after 48 hours	
Fig. 23	Photomicrograph of HEp2 cells cocultured with HEp2 and AD-MSCs media after 72 hours	58
Fig. 24	Bar Chart representing p16 expression in the three groups at different time durations	60
Fig. 25	Line graph showing p16 expression in the three groups at different time durations	60
Fig. 26	Bar Chart representing Livin gene expression in the three groups at different time durations	63
Fig. 27	Line graph showing Livin expression in the three groups at different time durations	63
Fig. 28	Bar Chart representing cell viability within the three groups at different time durations	67
Fig. 29	Line graph representing cell viability within the three groups at different time durations	67

List of Tables

Table 1	Groups used in this study	44
Table 2	Primer sequence for Livin, P16 and GAPDH genes	47
Table 3	List of volumes of each component used.	49
Table 4	Volumes of reagents used	50
Table 5	Thermal cycling conditions	50
Table 6	P16 expression within the three groups at different time durations using Kruskal-Wallis test	59
Table 7	Comparison of p16 expression in group HEp2 + AD-MSCs medium at different time durations using Wilcoxon Signed Rank test	61
Table 8	Comparison of p16 expression in HEp2+cocultured media at different time durations using Wilcoxon Signed Rank test	61
Table 9	Comparison of Livin expression the three groups at different time durations using Kruskal-Wallis test	62
Table 10	Comparison of Livin expression in HEp2 group at different time durations using Wilcoxon Signed Rank test	64
Table 11	Comparison of Livin in HEp2 + AD-MSCs at different time durations using Wilcoxon Signed Rank test	64
Table 12	Comparison of Livin expression in HEp2+cocultured media at different time durations using Wilcoxon Signed Rank test	65
Table 13	Comparison between the 3 main cultures at different time durations using Kruskal-Wallis test	66

List of Tables

Table 14	Comparison of cell viability in HEp2 group at different time durations using Wilcoxon Signed Rank test	68
Table 15	Comparison of cell viability in HEp2+AD-MSCs at different time durations using Wilcoxon Signed Rank test	68
Table 16	Comparison cell viability in HEp2+cocultured media at different time durations using Wilcoxon Signed Rank test	69