بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

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MONA MAGHRABY

Ain Shams University
Faculty of Science
Biochemistry Department

Synergism of siRNA and Doxorubicin on Breast Cancer Cell Lines

A thesis

Submitted for the degree of Master of Science in Biochemistry as a partial fulfillment for requirements of the Master of Science

By

Salma Abdelmonem Mahmoud Aboelela

Pre-Master in Biochemistry Ain Shams University (2013)

Under Supervision of

Prof. Dr / Amina M. Medhat

Professor of Biochemistry
Biochemistry Department
Faculty of Science
Ain Shams University

Prof. Dr / Abeer M. Ashmawey

Professor of Medical Biochemistry
Cancer Biology Department
National Cancer Institute
Cairo University

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Declaration

I declare that this thesis has been composed and the work recorded in has been done by myself

It has not been submitted for any other degree at this or any other university.

Salma Aboelela

Dedication

I dedicate this thesis to my beloved family; my parents and my brothers, without whom this thesis was not to be accomplished, I am so grateful for their endless love and support.

I am sincerely thankful for all my friends and colleagues who helped me throughout the entire process; I will always appreciate their great effort and help.

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Contents

Abstract

Telomerase activity is up regulated in most breast cancer subtypes but not in the adjacent normal tissues. Thus, it is a promising target for anticancer therapy. The present work investigated the effects of telomerase inhibition by siRNA on breast cancer cell lines and studied the feasibility of whether the combined effect of doxorubicin with siRNA treatment on breast cancer cells potentiates a rapid cellular response to the cytotoxic effect of chemotherapy. This study was performed on luminal A (MCF-7), triple negative (MDA-MB-468), and HER-2/neu (SKBR-3) human breast cancer cell lines, wherein telomerase activity inhibition by hTERT siRNA and doxorubicin was detected by measuring telomerase activity using telomeric repeat amplification protocol (TRAP assay), assessing cell viability through MTT assay, and evaluating apoptosis through scanning electron microscopy (SEM) and through estimating caspase-3 and -8 activities using enzyme-linked immunosorbent assay (ELISA). In the present study, hTERT siRNA effectively reduced telomerase activity and cell viability to more than 90% and 60%, respectively, in most breast cancer cell lines within 72 hours after transfection. The combination of hTERT siRNA and doxorubicin showed a cumulative effect compared with either treatment alone. Meanwhile, SEM demonstrated apoptotic morphologic cell changes. Telomerase inhibition is a promising strategy for the effective treatment of breast cancer. When used in combination with doxorubicin, it could potentiate the cytotoxic effect of the drug on breast cancer cells.

Keywords: telomerase- siRNA- doxorubicin- breast cancer.

List of Abbreviations

Abbreviated name	Full name
Ago	Argonaute
AIF	Apoptosis-inducing factor
ANOVA	Analysis of variance
APAF1	Apoptotic protease activating factor 1
ATCC	American type culture collection
ATM	Ataxia telangiectasia mutated
ATP	Adenosine triphosphate
Bad	BCL2 associated agonist of cell death
Bax	BCL-2-associated X protein
BCL2	B-cell lymphoma-2
BH3	BCL-2 homology
BID	BH3 interacting-domain death agonist
BLAST	Basic local alignment search tool
BRCA1	Breast cancer 1
BRCA2	Breast cancer 2
CAD	Caspase-activated DNase
CDH1	E-cadherin
CICD	Caspase independent cell death
CIS	Carcinoma in situ
CK	Cytokeratin
CLDN	Claudin
CPP	Cell-penetrating peptide
DABSYL	4-(dimethylamine) azo benzene sulfonic acid
dATP	Deoxyadenosine triphosphate
DCIS	Ductal carcinoma in situ
DDR	DNA damage response
DISC	Death-inducing signaling complex
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulphoxide
DR	Death receptor
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor

EGFR	Epidermal growth factor receptor
EIO	European institute of oncology
ELISA	Enzyme-linked immunosorbent assay
ER	Estrogen receptor
ET	Energy transfer
FADD	Fas-associated protein with death domain
FAS	FS-7-associated surface antigen
FASL	Fas ligand
FBS	Fetal bovine serum
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
HER2	Human epidermal growth factor receptor 2
HRP	Horseradish peroxidase
HRT	Hormone replacement therapy
hTERC	Human telomerase RNA component
hTERT	Human telomerase reverse transcriptase
hTR	Human telomerase RNA
IBC	Inflammatory breast cancer
IC	Inhibitory concentration
IDC-NST	Invasive ductal carcinomas of no special type
IgG	Immunoglobulin G
ILC	Invasive lobular carcinoma
LSD	Least significant difference
MCF-7	Michigan cancer foundation-7
MBC	Metaplastic breast cancer
MDR1	Multidrug resistance
miRNA	Micro RNA
MLV	Murine leukemia virus
MOMP	Mitochondrial outer membrane permeabilization
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide
NADH	Nicotinamide adenine dinucleotide(NAD) + hydrogen (H)
NCBI	National center for biotechnology information
NCRP	National cancer registry program
NFS	Nanoparticle formation solution
NTC	No template control