ALTERATIONS OF MICRORNA EXPRESSION PATTERNS IN HUMAN CERVICAL CARCINOMA CELLS (CA SKI) TOWARDS 1'S-1'-ACETOXYCHAVICOL ACETATE (ACA) AND CISPLATIN (CDDP)

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ABSTRACT

The main aims of this study were to investigate the combined effects of a natural compound 1'S-1'-acetoxychavicol acetate (ACA) with cisplatin (CDDP) on human cervical carcinoma cells Ca Ski, and to identify microRNAs (miRNAs) asscociated with response towards ACA and/or CDDP. Data obtained from MTT cell viability assays indicated that both ACA and CDDP induced dose- and time-dependent cytotoxicity on Ca Ski cells when used as a standalone agent. The *in vitro* studies also demonstrated that ACA potentiates the cytotoxic effects of CDDP when used in combination through synergistic interactions. The miRNA microarray was used to identify global miRNA expression profiles on Ca Ski cells following administration of ACA and/or CDDP, and it was found that 25 miRNAs were differentially expressed in response towards ACA and/or CDDP with markedly different pattern of miRNA expressions between different treatment regimens. Three promising miRNA candidates (hsa-miR-138, hsa-miR-210 and hsa-miR-744) which exhibited the highest fold-change in combination chemotherapy and whose expressions were among those validated by qRT-PCR, were selected for bioinformatic analyses. The hypothetical pathway model comprising interaction between candidate miRNAs with their putative target genes indicated that the cytotoxic effects induced by ACA in combination with CDDP may be regulated by miRNA expression. Therefore, our study provides a platform for potential therapeutic approaches in chemotherapy, whereby miRNA expression can be exploited to further improve efficacy in combination chemotherapy.

ABSTRAK

Tujuan utama penyelidikan ini adalah untuk menyiasat kesan gabungan kompaun semulajadi 1'S-1'-acetoxychavicol acetate (ACA) dengan cisplatin (CDDP) ke atas sel-sel karsinoma serviks manusia Ca Ski, serta mengenal pasti miRNA yang bertindak balas terhadap rawatan ACA dan/atau CDDP. Data yang diperolehi daripada eksperimen MTT menunjukkan bahawa ACA dan CDDP menyebabkan sitotosik yang bergantung pada dos dan tempoh rawatan apabila digunakan sebagai ejen 'standalone'. Selain itu, kajian *in vitro* juga menunjukkan bahawa ACA mempotensikan kesan sitotoksik CDDP melalui hubungan sinergistik apabila digabungkan. MiRNA microarray digunakan untuk mengenal pasti profil ekspresi global miRNA setelah dirawat dengan ACA dan/atau CDDP, dan didapati bahawa terdapat sebanyak 25 miRNA yang diekspreskan secara berlainan apabila dirawat dengan ACA dan/atau CDDP dengan corak ekspresi miRNA yang berbeza antara regimen rawatan yang berlainan. Tiga calon miRNA yang menjanjikan (hsa-miR-138, hsa-miR-210 and hsa-miR-744), yang merupakan antara beberapa miRNA yang disahkan ekspresinya dengan qRT-PCR, dipilih untuk analisis bioinformatik kerana mereka mempamerkan 'fold-change' yang paling tinggi dalam kemoterapi kombinasi. 'Hypothetical pathway model' yang melibatkan interaksi miRNA dengan target gen putatif miRNA menunjukkan bahawa kesan-kesan sitotoksik yang ditunjukkan oleh ACA dan/atau CDDP berkemungkinan besar disebabkan oleh ekspresi miRNA. Maka, kajian kami menyediakan satu platform bagi pendekatan terapeutik dalam kemoterapi, dimana ekspresi miRNA boleh dimanipulasikan untuk meningkatkan efikasi dalam kemoterapi kombinasi.

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3’ UTR	Three prime untranslated region
5’ UTR	Five prime untranslated region
%	Percentage
×	Times
°C	Degree Celsius
µ	Micro
µg	Microgram
µg/ml	Microgram per militre
µl	Microlitre
µM	Micromolar
®	Registered
(v/v)	Volume per volume
(w/v)	Weight per volume
A	Absorbance
ASR	Age-standardized incidence rate
ATP	Adenosine triphosphate
Bfl-1/A1	Bcl-2-related protein A1
ACA	1’S-1’-acetoxychavicol acetate
AIF	Apoptosis inducing factor
Apaf-1	Apoptotic protease-activating factor-1
ATM	Ataxia-telangiectasia-mutated
Bad	Bcl-2-agonist death promoter
Bax	Bcl-2 associated X protein
Bcl-2	B-cell lymphoma 2

Bcl-xL	B-cell lymphoma-extra large
bp	Base pairs
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
CARIF	Cancer Research Initiative Foundation
Caspase	Cysteine aspartate protease
CaV	Voltage-gated calcium channels
CBP	CREB-binding protein
CDC	Cell-division cycle
CDDP	Cisplatin
CDK	Cyclin-dependent kinase
cDNA	Complimentary deoxyribonucleic acid
CI	Combination index
CIN	Cervical intraepithelial neoplasia
CKI	Cyclin-dependent kinase inhibitor
cm	Centimeter
cm^2	Centimeter square
CO_2	Carbon dioxide
CREB	cAMP response element-binding
DAVID	Database for Annotation, Visualization and Integrated Discovery
DEPC	Diethylpyrocarbonate
dH_20	Distilled water
DISC	Death-inducing signaling complex
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimetyl sulfoxide
DNA	Deoxyribonucleic acid

DNase	Deoxyribonuclease
dNTP	Deoxy-nucleoside-triphosphate
DR	Death receptor
Dsh	Dishevelled
EDTA	Ethylenediaminetetraacetic acid
ELOSA	Enzyme Linked Oligosorbent Assay
ERK	Extracellular signal-regulated protein kinase
et al	And others
FADD	Fas-associated death domain
FAK	Focal adhesion kinase
FDA	Food and Drug Administration
FBS	Fetal bovine serum
FIGO	Fédération of Internationale de Gynécologic et d'Obstétrique
FLIP	FLICE-like inhibitory protein
Fzd	Frizzled
g	Gravity
g	Gram
GSH	Glutathione
h	Hour
hCG	Human chorionic gonadotropin
HCl	Hydrochloric acid
HIF	Hypoxia-inducible factor
HPV	Human papillomavirus
HSV-2	Herpes simplex virus-2
IACR	International Agency for Research on Cancer
IAP	Inhibitor of apoptotic protein

IC_{50}	50% inhibitory concentration
ICO	Institut Català d'Oncologia
IGF	Insulin growth factor
IKK	IκB kinase
IL-3	Interleukin-3
Inc.	Incorporated
JNK	c-Jun N-terminal kinases
kDA	Kilo Dalton
KEGG	Kyoto Encyclopedia of Genes and Genomes
kg	Kilogram
KSFM	Keratinocyte serum-free medium
LEF	Lymphoid enhancer factor
M	Molar
MAPK	Mitogen-activated protein kinase
MEK	MAPK kinase
mg	Milligram
mg/ml	Milligram per millitre
$MgCl_2$	Magnesium chloride
min	Minute
miRNA	Micro ribonucleic acid
ml	Milliliter
mm	Millimetre
mM	Millimolar
$MnCl_2$	Manganese chloride
mRNA	Messenger ribonucleic acid
MPT	Mitochondrial permeability transition

MTT	3-(4,5-dimethylthiazol-2-γl)-2,5-diphenyl-tetrazoliumbromide
NCI	National Cancer Institute
NCR	National Cancer Registry
NCX	Sodium-calcium exchanger
NF-κB	Nuclear factor kappa B
ng	Nanogram
nm	Nanometer
nM	Nanomolar
p53	Protein 53
Pap	Papanicolaou
PBS	Phosphate buffered saline
PDGF	Platelet-derived growth factor
PDGFR	Platelet-derived growth factor receptor
pH	Potential of hydrogen
PI3K	Phosphoinositide 3-kinase
PMCA	Plasma membrane Ca2+ ATPase
pRb	Retinoblastoma protein
psi	Pound force per square inch
r	Pearson correlation coefficient
RIN	RNA Integrity Number
RIP	Receptor interacting protein
RISC	RNA-induced silencing complex
RMA	Robust Multichip Average
RNA	Ribonucleic acid
RNase	Ribonuclease
ROC	Receptor-operated calcium channels

rpm	Revolutions per minute
rRNA	Ribosomal ribonucleic acid
S	Svedberg
s	Second
SA-HRP	Streptavidin-horseradish peroxidase
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SOS	Son of sevenless
SSC	Saline sodium citrate
TCF	T-cell factor
TGF-β	Transforming growth factor-β
™	Trademark
TMB	3,3′,5,5′-Tetramethylbenzidine
TNF	Tumor necrosis factor
TNFR	Tumor necrosis factor receptor
TRADD	Tumor necrosis factor receptor associated death domain
TRAF2	Tumor necrosis factor receptor-associated factor 2
U	Unit
USA	United States of America
UV	Ultraviolet
WHO	World Health Organization
WNT	Wingless-type MMTV integration site family