Investigation of novel therapeutic strategies for epithelial ovarian cancer

Nobuhisa Yoshikawa

Faculty of health science

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Abstract

Objective: PRIMA-1MET is a small molecule compound that restores wild-type p53 to mutant p53, and is recently confirmed to be safe at therapeutic plasma levels. The aims of this study were to identify the anti-tumour activity of PRIMA-1MET on epithelial ovarian cancer (EOC) cells and elucidate the underlying mechanism in vitro.

Methods: We used nine EOC cell lines and their chronic cisplatin/paclitaxel-resistant cells and performed cell viability assay and cell apoptosis assay to evaluate the efficacy of PRIMA-1MET. Moreover, we assessed the functional role of reactive oxygen species (ROS) and their scavenger in the EOC cells.

Results: We examined the viability of the total 13 EOC cells after 48 h treatment with PRIMA-1MET. Measuring the half maximal inhibitory concentration (IC_{50}) of EOC cells revealed that the sensitivity was heterogeneous, and did not correlate with *TP53* status. PRIMA-1MET induced apoptosis, PARP cleavage, and intracellular ROS accumulation in a p53-independent manner. The anti-tumour effects of PRIMA-1MET were completely rescued by a ROS scavenger, N-acetyl cysteine. Furthermore, PRIMA-1MET reduced the expression of antioxidant enzymes, PRX3 and GPX1, in a dose-dependent manner.

Conclusion: We demonstrated that PRIMA-1MET had an anti-tumour effect on EOC cells regardless of *TP53* status and chemo-resistance. PRIMA-1MET is a promising therapeutic agent for chemo-resistant EOC patients and may contribute to a better prognosis in the future.

Declaration

I certify that this thesis contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution except Nagoya University. To the best of my knowledge and belief, it contains no material that has previously been published by any other person except where due reference is made. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any other university of other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Abbreviations

µg	microgram
µL	microLitre
µM	microMolar
bp	base pairs
cDNA	complimentaly DNA
DMEM	Dubecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Dinucleotide triphosphate
DSB	double strand brake
ECM	extracellular matrix
EMT	Epithelial-to-mesenchymal transition
EOC	epithelial ovarian cancer
FACS	Fluorescence activated cell sorting
FBS	fetal bovine serum
GFP	Green Fluorescent Protein
gRNA	guide RNA
HDR	homology directed repair
HGSOC	high-grade serous ovarian cancer
HPMCs	Human peritoneal mesothelial cells
IGF	Insuline like Growth Factor
M	Molar

mg	milligram
mL	millilitre
MQ	methylene quinuclidinone
mRNA	messenger ribonucleic acid
NaCl	sodium chloride
NHEJ	non-homologous end joining
PARP	Poly ADP-ribose Polymerase
RPMI	Roswell Park Memorial Institute medium
RT-PCR	Reverse transcription real time polymerase chain reaction
shRNA	short hairpin RNA
TGF	Transforming Growth Factor
VEGF	Vascular Endothelial Growth Factor

Conference Presentations

Nobuhisa Yoshikawa, Kathleen Pishas, Daniel Worthley, and David Callen.

Gene correction by CRISPR-Cas system in ovarian cancer. Faculty of Health Sciences 2014 Postgraduate Research Conference, National Wine Centre of Australia, University of Adelaide, Poster presentat