In Vitro Anti-skin Cancer Properties and Mechanisms of Action of Xanthones from the Mangosteen Pericarp

A thesis submitted in fulfillment of the requirement for the degree of

Doctor of Philosophy

By

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Bachelor of Clinical Medicine

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Candidate's Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

Jing Jing Wang

September 2012

Thesis Summary

The incidence of skin cancer has increased more than 600% worldwide since the 1940s, and Australians have the highest incidence in the world, with at least 2 in 3 Australians diagnosed with skin cancer before the age of 70. The current chemotherapy is not effective, with new drugs in high demand. Plants are important sources for anti-cancer drugs. Mangosteen (*Garcinia mangostana* Linn.) is a tropical tree from South East Asia and its fruit pericarp is a well-known traditional medicine.

This study investigated the potential anti-skin cancer activity of the crude extract and major xanthone compounds from the pericarp of mangosteen by investigating the cytotoxicity and underlying cellular and molecular mechanisms. Two types of human skin cancer cell lines were used as *in vitro* models: melanoma SK-MEL-28 and squamous cell carcinoma A-431.

There were five major research outcomes. (i) Development of a methodology for extraction of mangosteen based on chemical composition and antioxidant activity. (ii) Demonstration of anti-proliferative activity towards skin cancer cell lines. The crude extract and six xanthone compounds tested had significant anti-cancer activities, with IC_{50} values ranging from 2.39 to 7.61 µg/ml. The activity was selective against skin cancer cells with less effect on human normal skin fibroblast CCD-1064Sk and the keratinocyte HaCaT cell lines. IC_{50} values of the xanthones were similar to, or much lower than, those of two most commonly used commercial drugs (5-fluorouracil and dacarbazine). (iii) Identification of cellular and molecular pathways. The anti-cancer action of xanthone compounds was found to be via activation of caspases together with the loss of mitochondrial membrane potential

and inhibition of Akt and NFκB survival pathways. In melanoma SK-MEL-28 cells, downregulation of BRAF V600E mutation expression was observed after treatment with some xanthones, e.g. a maximum 6.8-fold decrease in the level of BRAF V600E relative to the untreated control. (iv) Identification of synergistic effects. Synergistic effects between α-mangostin and the other individual compounds were observed. However, no synergistic effect was found between xanthone compounds and commercial drugs under the tested conditions in the current study. (v) Evaluation of anti-metastatic activity of α-mangostin. Skin cancers, especially melanoma, have a high potential to metastasise. α-Mangostin exhibited significant inhibitive activity of invasion and migration at non-toxic doses on both skin cancer cell lines tested. The anti-metastatic activity of α-mangostin was associated with downregulation of mRNA expression of MMP-2 and MMP-9 through inhibiting NFκB and Akt pathways.

This study provides important scientific evidence of the potential antioxidant and antiproliferative activity of extracts and xanthone compounds from the pericarp of mangosteen, and increases understanding of their underlying mechanisms. These findings can contribute to the development of novel plant-derived antioxidant strategies in the treatment of skin cancers.

Keywords: skin cancer; mangosteen; xanthones; cytotoxicity; apoptosis; survival pathway; metastasis

Acknowledgements and Dedication

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In memory of my special close friend, Mrs. Alita Larsens.

List of Abbreviations

%	Percentage
AAPH	2, 2'-Azobis (2-amidinopropane) dihydrochloride
Akt	Protein kinase B
ANOVA	Analysis of Variance
ATCC	American type culture collection
AU	Arbitrary unit
BRAF	Serine/threonine-protein kinase *B-Raf*
BSA	Bovine serum albumin
ºC	Degree celcius
CDK	Cyclin-dependent kinases
CKI	Cyclin-dependent kinases inhibitors
COX-2	Cyclooxygenase-2
Ct	Threshold of cycle
DAPI	4′, 6-Diamidino-2-phenylindole dihydrochloride
DMBA	7,12-dimethyl[a]benzanthracene
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulfoxide
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
%DRSA	Percentage DPPH radical scavenging activity
DTIC	Dacarbazine
DW	Dry weight
EMEA	European agency for the evaluation of medicinal products
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
ERK	Extracellular signal-regulated kinase
EtOH	Ethanol
FAK	Focal adhesion kinase
FBS	Foetal bovine serum
FDA	Food and drug administration
FRAP	Ferric reducing antioxidant power
5-FU	5-Fluorouracil
µg	Microgram
GAE	Gallic acid equivalents
GOI	Gene of interest
h	hour
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
HO^-	Hydroxide ion
IC_{50}	50% inhibitory concentration
IκB	Inhibitor of kappaB
IKK	IκB kinase
IL-8	Interleukin-8
IMDM	Iscoves Modified Dulbecco's Medium
IU	International units
JNK	c-Jun NH_2-terminal kinase
l	litre
LDL	Low density lipoprotein
µl	microlitre

µM	micromolar
ml	millilitre
MAPK	Mitogen-activated protein kinase
MMP	Matrix metalloproteinase
MPEE	Mangosteen pericarp ethanol extract
MPWE	Mangosteen pericarp water extract
MQ	Milli Q
mRNA	Messenger ribonucleic acid
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
NFκB	Nuclear factor kappa B
NSAIDS	Nonsteroidal antiinflammatory drugs
OD	Optical density
ORAC	Oxygen radical absorbance capacity
PBS	Phosphate-buffered saline
$ONOO^-$	Peroxynitrite
PCR	Polymerase chain reaction
PI	Propidium iodide
PI3K	Phosphoinositide 3-kinase
qRT-PCR	Quantitative real-time reverse transcription PCR
ROS	Reactive oxygen species
RPMI	Roswell Park Memorial Institute
R^2	R square
RT	Room temperature
s	second
SD	Standard deviation
SDS	Sodium Dodecyl Sulfate
SEM	Standard error of the mean
SPSS	Statistical Package for the Social Sciences
TE	Trolox equivalents
TF	Total flavonoids
TNF	Tumor necrosis factor
TP	Total phenolics
TPA	12-O-tetradecanoylphorbol-13-acetate
TRAP	Total radical trapping antioxidant parameter
UCA	Urocanic acid
uPA	Urokinase-type plasminogen activator
UV	Ultraviolet
VEGF	Vascular endothelial growth factor

Publications, Presentations and Awards

Publications

- Jing J. Wang, Barbara J.S. Sanderson, Wei Zhang, 2011. Cytotoxic effect of xanthones from pericarp of the tropical fruit mangosteen (*Garcinia mangostana* Linn.) on human melanoma cells. Food and Chemical Toxicology. 49: 2385–2391

- Jing J. Wang, Barbara J.S. Sanderson, Wei Zhang, 2012. Significant anti-invasive activities of α-mangostin on metastasis of human skin cancer cells. Anticancer Research (Article in press)

- Jing J. Wang, Qing H. Shi, Wei Zhang, Barbara J.S. Sanderson, 2012. Anti-skin cancer properties of phenolic-rich extract from the pericarp of mangosteen (*Garcinia mangostana* Linn.). Food and Chemical Toxicology. 50: 3004-3013.

Publications in Submission

- Jing J. Wang, Wei Zhang, Barbara J.S. Sanderson, 2012. Altered mRNA expression related to the apoptotic effect of three xanthones on human melanoma SK-MEL-28 cell line. Food and Chemical Toxicology (Under review; manuscript No. FCT-6676).

Publications in preparation

- Jing J. Wang, Barbara J.S. Sanderson, Wei Zhang. Anti-proliferative and apoptotic effect of 6 pure xanthone compounds on human squamous cell carcinoma A-431 cells.

- Jing J. Wang, Wei Zhang, Barbara J.S. Sanderson. Xanthones isolated from mangosteen pericarp induce apoptosis in human melanoma SK-MEL-28 cells.

- Jing J. Wang, Barbara J.S. Sanderson, Wei Zhang. Potential synergistic skin cancer suppression by combination of xanthones.

Presentations

- ASMR SA Scientific Meeting 6th June 2012. Adelaide, Australia. Oral Presentation "Inhibitory effect of α-mangostin on proliferation and metastasis of human melanoma SK-MEL-28 cell line"

- Cancer Research Day. 25th November 2011. Adelaide, Australia. Oral Presentation. “Xanthones from mangosteen pericarp: anti-skin cancer properties”

- AusBiotech 2011 National Conference. 16th -19th October 2011. Adelaide, Australia. Oral and Poster Presentation “*In vitro* anti-skin cancer properties and mechanisms of action of α-mangostin from the mangosteen pericarp”

- Asian Congress on Biotechnology. 11th-15th May 2011. Shanghai, China.

Poster Presentation. “Anti-skin cancer activity of crude extract of mangosteen (*Garcinia mangostana* Linn.)”

- Three Minute Thesis Competition 2011. 6th May 2011. Adelaide, Australia. Oral Presentation “Mangosteen combats skin cancer”

- Chemeca 2010. 26th -29th Septemper 2010. Adelaide, Australia. Oral Presentation “Compounds from pericarp of mangosteen (*Garcinia Mangostana* Linn.) induce cell cycle arrest and apoptosis in human melanoma cells”

- 13th World Congress on Cancers of the Skin. 7th -10th April 2010. Madrid, Spain. Oral Presentation “Evaluation of antiproliferation properties of xanthones from pericarp of mangosteen (*Garcinia mangsotana* L.) on human melanoma cells”

Awards

- 2012 Ross Wishart Memorial Award finalist

- 2011 AusBiotech-GSK Student Excellence Award – State Winner

- 2011 Chinese Government Award for Outstanding Self-financed Students Abroad

- 2010 “Top Ten Cited Author in 2007 & 2008” from Mutation Research

- 2010 AusBiotech-GSK Student Excellence Award – State Finalist

- 2008 AusBiotech-GSK Student Excellence Award – State Finalist

- 2008 – 2012 EPRIS Scholarship, Flinders University, Australia

Professional Membership

- 2010- current AusBiotech (Australia’s Biotechnology Organisation)

- 2012 – 2013 Australian Society for Medical Research

- 2012-2013 Bioprocessing Network

Table of Content

List of Tables

List of Figures