Transient Receptor Potential Vanilloid 1 (TRPV1) in Haematological Malignancies

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Dedication

To my parents, husband Asal, son Awsam and my newborn Karam.

With all my love....

Declaration of Originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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Statement of Ethical Conduct

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University. This study was approved by the Human Research Ethics Committee Network, Tasmania (Approval No. H0011050).

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List of Abbreviations

A-425619	1-isoquinolin-5-yl-3-(4-trifluoromethyl-benzyl)urea
A-778317	1-((R)-5-tert-butyl-indan-1-yl)-3-isoquinolin-5-yl-urea
AA	Arachidonic acid
ACA	N-(p-amylcinnamoyl)anthranilic acid
ADP	Adenosine diphosphate
AEA	N-arachidonoylethanolamine (anandamide)
ALL	Acute lymphocytic leukaemia
AMG628	(R)-N-(4-(6-(4-(1-(4-fluorophenyl)ethyl)piperazin-1-yl)pyrimidin-4-yloxy)benzo[d]thiazol-2-yl)acetamide
AML	Acute Monocytic Leukaemia
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
AP-1	Activator protein-1
ATL	Adult T-cell leukaemia
ATP	Adenosine triphosphate
Bax	Bcl-2–associated X protein
Bcl-2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma-extra large
BCTC	N-(4-tertiarybutylphenyl)-4-(3-chloropyridin-2-yl) tetrahydropyrazine-1(2H)-carbox-amide.
BDNF	Brain-derived neurotrophic factor
bFGF	Basic fibroblast growth factor
BTP2	4-methy-4′-[3,5-bis(trifluoromethyl)-1H-pyrazol-1-yl]-1,2,3-thiadiazole-5-carboxanilide
C- terminus	Carboxy terminus
CaM	Calmodulin
CaMKII	Ca^{2+}-calmodulin-dependent kinase II
cAMP	Cyclic adenosine monophosphate
CAP	Capsaicin
CAZ	Capsazepine
CD	Cluster of Differentiation
CDK	Cyclin-dependent kinase
CGRP	Calcitonin gene-related peptide
CLL/SLL	Chronic lymphocytic leukaemia/ small lymphocytic lymphoma
CML	Chronic myelogenous leukaemia
CMML	Chronic Myelomonocytic Leukaemia
CNS	Central Nervous System
COPD	Chronic Obstructive pulmonary disease
CRP	C- reactive protein
DAG	Diacylglycerol
DC	Dendritic cell
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DPTHF	Diphenyltetrahydrofuran
DPBA	Diphenylboronic anhydride
DRG	Dorsal root ganglion

ECL	Enhanced Chemiluminescence
eIF2α	Eukaryotic translation initiation factor 2, subunit 1 (α, 35kDa)
eIF2αK3	Eukaryotic translation initiation factor-2α kinase-3
EIPA	Ethylisopropyl amiloride
ER	Endoplasmic reticulum
ET	Essential thrombocythaemia
ETC	Electron transport chain
FBS	Foetal Bovine Serum
FITC	Fluorescein isothiocyanate
FSC	Forward Scatter
GADD153	Growth arrest- and DNA damage-inducible transcript 3
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GARD	Gastroesophegal reflux disease
GM-CSF	Granulocyte-macrophage colony stimulating factor
GTPγS	Guanosine gamma thiophosphate
HCL	Hairy-Cell Leukaemia
HEK293	Human Embryonic Kidney cells
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HL-60	Human myelocytic leukaemia
HRP	Horseradish Peroxidase
ICDA	Inhibitor of caspase activated DNase
IDN	Identification number
IFN-γ	Interferon-gamma
IL-1/ 2/ 6	Interleukin-1/ 2/ 6
ILD	Interstitial lung disease
Ins(1,4,5)P3	Inositol 1,4,5-trisphosphate
IP_3	Inositol triphosphate
JNJ17203212	4-(3-trifluoromethyl-pyridin-2-yl)-piperazine-1-carboxylic acid (5-trifluoromethyl-pyridin-2-yl)-amide
JYL1421	N-(4-tert-butylbenzyl)-N′-[3-fluoro-4-(methylsulfonylamino)benzyl]thiourea
KB-R7943	2-[2-[4-(4-nitrobenzyloxy)phenyl]ethyl]isothiourea methanesulfonate
LGH	Launceston General Hospital
LNCaP	Androgen-dependent prostate cancer cells
MAPK	Mitogen-activated protein (MAP) kinases
MFI	Median Fluorescence intensity
ML-9	1-(5-chloronaphtalene-1-sulphonyl) homopiperazine
MM	Multiple myeloma
MPD	Myeloproliferative Disorder
MTT	3-(4,5-dimethylthia-zol-2-yl)-2,5-diphenyltetrazolium bromide
M.W	Molecular weight
N- terminus	Amino- terminus
NADA	N-arachidonoyldopamine
NADH	Nicotinamide adenine dinucleotide
NFAT	Nuclear factor of activated T-cells

NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NFM	Non-Fat Milk
NGF	Nerve growth factor
NHBE	Normal human bronchial epithelial
NHL	Non-Hodgkin's Lymphoma
OAG	1-oleoyl-2-acetyl-sn-glycerol
p21 $^{WAF1/CIP1}$	Cyclin-dependent kinase inhibitor
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffer saline
PBSA	Phosphate buffer saline-sodium azide
PBST	Phosphate buffer saline tween- 20
PC3	Androgen-independent prostate cancer cells
PHB2	Prohibitin
PI3K	Phosphatidylinositol 3-kinase
PIP_2	Phosphatydyl-inositol-4,5-bisphosphate
PKA	Protein kinases A
PKC	Protein kinases C
PLC	Phospholipase C
PMA	Phorbol 12-myristate 13-acetate
PMT	Photo multiplier tube
PgE2	Prostaglandin E_2
PP	Peyer's patch
PTCL-NOS	Peripheral T-cell lymphoma/ not otherwise specified
PVDF	Polyvinylidene difluoride
Q-PCR	Quantitative real-time PCR
RCF	Relative Centrifugal Force
RHC80267	1,6-di[O-(carbamoyl)cyclohexanone oxime]hexane
ROS	Reactive oxygen species
RT-4	Human well-differentiated low-grade papillary
RT-PCR	Reverse transcription polymerase chain reaction
RTX	Resiniferatoxin
SB366791	N-(3-methoxyphenyl)-4-chlorocinnamide
SDS	Sodium Dodecyl Sulphate
SSC	Side Scatter
STAT	Signal transducer and activator of transcription
TBMC	6-tert-butyl-m-cresol
THC	Δ^9-tetrahydrocannabinol
TNF-α	tumour necrosis factor-alpha
TRAIL	Tumour necrosis factor-related apoptosis-inducing ligand
TRIM	1-(2-(trifluoromethyl)phenyl) imidazole
trkA	Tyrosine kinase A
TRP	Transient receptor potential
TRPV1	Transient receptor potential vanilloid type 1
URB597	3′-carbamoylbiphenyl-3-yl cyclohexylcarbamate.
UTAS	University of Tasmania
^{Va}	Varitint-waddler phenotype

WS-12	2-isopropyl-5-methyl-cyclohexanecarboxylic acid (4-methoxy-phenyl)-amide
2-APB	2-aminoethoxydiphenyl borate
4α-PDD	4α-phorbol 12,13-didecanoate
5-HT	Serotonin
5(6)-EET	5',6'-epoxyeicosatrienoic acid
5-(S)-HETE	5-(S)-hydroxyeicosatetraenoic acid
12-(S)-HPETE and 15-(S)-HPETE	12- and 15-(S)-hydroperoxyeicosatetraenoic acids
20-HETE	20-hydroxyeicosatetraenoic acid
$\Delta\Psi_m$	Mitochondrial membrane potential

Presentations at Conferences during PhD Candidature

Conference Presentations

- **Omari, S** and Adams, MJ and Khalafallah AA and Mohamed, M and Geraghty, DP, TRPV1 expression in haematological malignancies, Annual Combined ASM of APSA and ASCEPT, 1 - 4 December, Melbourne, Australia (2013) [Conference Extract].

- **Omari, S** and Geraghty, DP and Kunde, DA and Adams, MJ, Inhibition of Human Haematological Malignant Cell Lines by Capsaicin is not TRPV1-Mediated, Annual Combined ASM of the HSANZ/ANZBT/ASTH and the APSTH, 28 – 31 October, Melbourne, Australia (2012) [Conference Extract].

- **Omari, S** and Kunde, DA and Adams MJ and Geraghty DP, Inhibition of human haematological malignant cell line growth by capsaicin is not TRPV1-mediated, Annual Combined ASM of APSA and ASCEPT, 2-5 December, Sydney, Australia (2012) [Conference Extract].

Presentations related to but not directly arising from this thesis

Conference Presentation

- Shegog, YM and **Omari, S** and Adams, MJ and Ragg, S and Eastley, B and Geraghty, DP, Flow cytometric analysis of transient receptor potential vanilloid 1 (TRPV1) in human leukocyte populations, Proceedings of the ASCEPT, December 4- 9, Perth, Australia, pp. p77 (2011) [Conference Extract].

Abstract

Transient receptor potential vanilloid-1 (TRPV1) is a member of the TRP family of channels that are responsible for nociceptive, thermal and mechanical sensations. It is primarily associated with neuronal cells, but has been detected in different non-neuronal cells, including leukocytes. Capsaicin (CAP), the active ingredient of hot chilli peppers, is one of a number of related endogenous and plant-derived compounds (broadly termed 'vanilloid-like agents') that have been shown to induce apoptosis and inhibit cell proliferation in some cancer cells, through both TRPV1-dependent and -independent mechanisms. The expression and function of TRPV1 in haematological malignancies however, has not been extensively investigated. Specific targeting by vanilloid-like agents toward TRPV1 on cancerous cells in patients with haematological malignancies may represent a novel therapeutic approach to treating these diseases.

This thesis investigated the expression and function of TRPV1 in haematological malignancies, using both blood cancer cell lines and blood samples obtained from patients with different blood cancers. The specific aims were to; 1) study the effect of TRPV1 agonists and antagonists on the viability of THP-1, U266B1 and U937 haematological malignant cell lines, 2) validate and optimise Western blotting and flow cytometry protocols to detect TRPV1 expression in leukocytes, 3) investigate TRPV1 expression in THP-1, U266B1 and U937 cells, and 4) compare TRPV1 expression in leukocytes obtained from patients with blood cancers to normal subjects.

The thesis begins with a comprehensive review and discussion on TRPV1 structure and function, as well as its expression and role in health and disease. In particular, there is a focus on the role of TRPV1 in cancer, including haematological malignancies (Chapter 1).

In Chapter 2, the effect of CAP on the metabolic activity of three malignant haematological cell lines, THP-1, U266B1 and U937, was investigated. Metabolic activity assays were performed using the alamarBlue® method. CAP induced cytotoxicity in all three cell lines in a concentration-dependent manner. A biphasic effect on metabolic activity was observed on THP-1 cells [EC_{50}, IC_{50} (95% CI) = 32.9 (19.9-54.3), 219 (144-246) μM]. U266B1 cells were more resistant to CAP-induced death than THP-1 and U937 cells. TRPV1 and CB1 antagonists (SB452533 and AM251, respectively) suppressed the CAP-induced increase in THP-1 cell metabolic activity ($P<0.001$). These experiments suggest that CAP inhibits the metabolic activity of malignant haematological cells through a non-TRPV1-dependent mechanism.

Chapters 3 and 4 represent the experimental work and trouble-shooting conducted to develop, validate and optimise methods for the detection of TRPV1 expression in human cells. Western blotting (Chapter 3) and flow cytometric (Chapter 4) methods have been previously published, however few have documented the use of appropriate controls for the detection of TRPV1, suggesting that data in the literature may not necessarily be valid. A problem identified in the current study was the correct application of negative controls, particularly to assess the specificity and therefore suitability of the primary antibody used in these methods. These optimised

protocols were then used to investigate the expression of TRPV1 in human malignant haematological cell lines (Chapter 5) and leukocytes obtained from patients with blood cancers (Chapter 6).

Increased expression of TRPV1 protein was observed in THP-1, U266B1 and U937 cells compared to normal leukocytes. Furthermore, a TRPV1 dimer was detected in U266B1 cells. Interestingly, TRPV1 was detected in non-haematological cell lines that have previously been used as TRPV1-negative cells for Western blotting, including untransfected- and TRPV1-transfected (without tetracycline to switch TRPV1 transcription off) HEK293 and RAW264.7 cells. This latter finding highlights the need for appropriate negative (and positive) controls in both flow cytometric and Western blotting studies of TRPV1.

Expression of TRPV1 in leukocytes obtained from patients with a range of haematological malignancies, including multiple myeloma (MM) and B-cell non-Hodgkin's Lymphoma (B-NHL), was then investigated (Chapter 6). TRPV1 expression was detected in all patients and controls using flow cytometry, but not Western blotting. Using flow cytometry, a sub-group of patients (4/49=8.2%, MM=2, B-NHL=2) demonstrated increased expression of TRPV1 relative to the remainder of the cohort. TRPV1 was found to be similar to the control group for 91.8% of all patients. There were no significant differences in TRPV1 expression (assessed using flow cytometry) between patients with MM and B-NHL, or between *de novo* patients and those undergoing treatment. Using Western blotting, TRPV1 (~95kDa) was detected in one MM and four B-NHL patients, although interestingly, a 240kDa band was also detected in both a B-NHL and a MM patient. In addition,

although C-reactive protein was elevated (≥5 mg/L) in 25% of all patients, it was not associated with higher TRPV1 expression. These results indicate that TRPV1 expression in leukocytes is relatively increased in a small subset of patients with blood cancers, and is not associated with inflammation. Furthermore, some patients may have a unique isoform of TRPV1 that warrants further investigation.

In summary, this study has generated new data and knowledge on the role of TRPV1 in haematological cells, including those from patients with blood cancers. A number of novel findings have been reported. Firstly, the inhibition of cell metabolic activity by the TRPV1 agonist, CAP, was found to be independent of TRPV1 activation in malignant haematological cell lines. Secondly, optimised Western blotting and flow cytometric methods for the detection of TRPV1 expression were developed and successfully validated. Thirdly, increased TRPV1 expression was demonstrated in the THP-1, U266B1 and U937 malignant haematological cell lines. Finally, increased TRPV1 expression was observed in some patients with MM and B-NHL, but was not associated with inflammation. The results presented in this thesis can be used as a basis for future studies of TRPV1 function in other human cells and cancers.