GENE THERAPY FOR METHYLMALONIC ACIDURIA

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B.Biotechnology (Hons)

Thesis submitted for the degree of

Doctor of Philosophy

in

Discipline of Paediatrics

School of Paediatrics and Reproductive Health

Faculty of Health Sciences

University of Adelaide

Genetics and Molecular Pathology

SA Pathology

Women's and Children's Hospital

South Australia

November 2012

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ABBREVIATIONS

AAV	Adenoviral-Associated Virus
AdoCbl	Adenosylcobalamin
Adv	Adenovirus
APC	Antigen Presenting Cells
Apo	Apolipoprotein
BIV	Bovine Immunodeficiency Virus
C3-Carnitine	Propionyl-carnitine
C4DC	Methylmalonylcarnitine
CAE	Caprine Arthritis-Encephalitis Virus
CAI	Codon Adaptation Index
CAM	Chloroamphenicol
CF	Cystic Fibrosis
CFD	Codon Frequency Distribution
CIP	Alkaline Phosphatase Calf Intestine
CNS	Central Nervous System
CTL	Cytotoxic T-lymphocytes
DCA	Dicarboxylic Acid
DCC	Dicarboxylic Acid Carrier
DMEM	Dulbecco's Modified Eagles Medium
DMG	3'3-dimethylglutaric Acid
DTT	Dithiothreitol
EDTA	Ethylenediamine Tetraacetic Acid
EF1-α	Elongation Factor 1- alpha
F. IX	Factor IX
FCS	Foetal Calf's Serum

FH	Familial Hypercholesterolemia
FIV	Feline Immunodeficiency Virus
GagPol	*p*HCMVGagpollstmlwhvpre
GC/MS	Gas Chromatography/Mass Spectrometry
GFP	Green Fluorescent Proteins
GFR	Glomerular Filtration Rate
GH	Growth Hormone
GP64-FIV	GP64 pseudotyped FIV vector
GPI	Glycosylphosphatidylinositol
GPT	Glutamic Pyruvic Transaminase
GSH	Glutathione
HCCL	Hydroxycobalamin-[L-lactin]
HeBS	Hepes Buffered Saline
HEK293T	Human Embyonic Kidney
HEPES	N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]
HIV-1	Human Immunodeficiency Virus Type 1
HIV-2	Human Immunodeficiency Virus Type 2
hNAC	Na^+-Coupled Carboxylate Transporters
HPLC	High-Performance Liquid Chromatography
HRP	Horse Radish Peroxide
HSV	Herpes Simplex Virus
IFN	Type 1 Interferons
IG	Immunoglobulin
IH	Intrahepatic
IL-I	Interleukin-1
IM	Inner Membrane

IMS	Inner Membrane Space
IP	Intraperitoneal
IV	Intravenous
IU	Infectious Unit
LDH	Lactate Dehydrogenase
LPC	Lysophosphatidylcholine
LV	Lentivirus
MCA	2-methylcitric Acid
MCM	Methylmalonyl Coenzyme A mutase
MeOH	Methanol
MHC	Major Histocompatibility Complex
MMA	Methylmalonic Acid
MMAuria	Methylmalonic Aciduria
MMLV	Moloney Murine Leukaemia Virus
MPP	Mitochondrial Process Peptidase
MPS IIIA	Mucopolysaccharidosis Type IIIA
Mut^0	Complete MCM Deficiency
Mut^-	Partial MCM Deficiency
Mw	Molecular Weigh
m/z	Mass/Charge
NEMPs	Nuclear Encoded Mitochondria Proteins
Neo	Neomycin Phosphotransferase
NIH3T3	Mouse Embryo Fibroblasts
OH-Cbl	Hydroxycobalamin
OM	Outer Membrane
OTC	Ornithines Transcarbamylase
OXPHOS	Oxidative Phosphorylation

PA	Propionic Acid
PAuria	Propionic Aciduria
PBS	Dulbecco's Phosphate Buffered Saline
PEP	Processing Enhancing Proteins
pHe	Extracellular pH
PKU	Phenylketonuria
PS	Penicilin-Streptomycin
Rev	*p*HCMVRevmlwhvpre
ROS	Reactive Oxygen Species
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
SIN	Self-Inactivating
SIV	Simian Immunodeficiency Virus
Tat	*p*cDNA3.1Tat101ml
TBG	Thyroid Hormone-Binding Globulin
TBS	Tris-buffered Saline
TCA	Citric Acid Cycle
TEMED	N,N,N'N'-tetramethylenediamine
TIM	Translocase of Inner Membrane
TOM	Translocase of Outer Membrane
TMB	3'3'5'5'-tetramethylbenzidine
TNF-α	Tumour Necrosis Factor-α
Tris base	Tris[hydroxymethyl]amino methane
TVS	Tubulovesicular Structures
VSV	Vesicular Stomatitis Virus

VSV-G	Vesicular Stomatitis Virus-G Envelope Glycoprotein
X1-SCID	X-linked Severe Combined Immunodeficiency

THESIS ABSTRACT

Methylmalonic aciduria (MMAuria) most commonly results from a deficiency of methylmalonyl coenzyme A mutase (MCM). Current treatments for MMAuria remain unsatisfactory and research on novel therapies remains a high priority. A lentiviral (LV) vector was developed to treat *in vitro* and *in vivo* models of MMAuria.

The overall aim of this project was to examine the therapeutic effect of a LV vector that expresses human MCM transgene in MCM knockout fibroblasts and a MMA affected, mut -/- muth2, murine model.

In the first study, a self-inactivating LV vector that expressed human MCM, HIV-1SDmEF1αhMCM, was constructed and transduced into MCM knockout fibroblasts. Normal cells and untransduced MCM knockout fibroblasts served as controls. Real-time PCR showed a high level of vector copy number, 8 ± 2 copies/cell in LV-treated MCM-knockout fibroblasts, resulting in correction of both the MCM enzyme activity and propionate metabolism in MCM-knockout fibroblasts.

The HIV-1SDmEF1αhMCM was then delivered intravenously into mut -/- muth2 mice (n=2). Untreated mut -/- muth2 mice (n=2) and normal mice (n=5) were used as controls. Vector was detected at a copy number of 0.19 ± 0.04 copies/cell in liver. Nevertheless, the MCM enzyme analysis showed only a modest restoration of enzyme activity in the treated mice, resulting in a mild reduction of plasma and urine MMA levels in the treated animals. These data suggest success in targeting the liver with the intravenous gene delivery approach. Nevertheless, it was required to improve the human MCM transgene expression in order to enhance the level of restoration of MCM enzyme activity to further reduce the MMA levels.

In the second study, a LV vector that expresses a codon-optimised human MCM transgene, HIV-1SDmEF1αmurSigHutMCM, was produced and transduced into MCM-knockout fibroblasts. High levels of vector, 20 ± 0.8 copies/cell, were detected in LV-treated MCM-knockout fibroblasts. Western blot analysis and MCM enzyme activity analysis by HPLC demonstrated a high level of MCM expression in the treated fibroblasts, resulting in the correction of MCM enzyme activity, with the formation of a significant level of succinyl coenzyme A (179 ± 19 nM/min/µg of total cell protein).

The HIV-1SDmEF1αmurSigHutMCM was then injected intravenously into mut -/- muth2 mice (n=5). Untreated mut -/- muth2 (n=6) and normal mice (n=6) were used as controls. The HIV-1SDmEF1αmurSigHutMCM-treated mice achieved near-normal weight for sex. The western blot analysis demonstrated significant MCM enzyme expression in the liver of treated mice, with the measurement of high level of enzyme activity (66 ± 21 nM/min/µg of total cell protein). Biochemical analyses demonstrated that the normalization of MCM enzyme activity in the treated group was associated with a reduction in plasma and urine MMA levels. Furthermore, that a significantly lower MMA concentration, 133± 20 µM/g tissue, was measured in the liver compared to the untreated mice, 1003 ± 124 µM/g tissue.

These results confirm that HIV-1SDmEF1αmurSigHutMCM provides significant, if incomplete, biochemical correction for the treatment of this disease, suggesting that gene therapy is a potential treatment for MMAuria.

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.

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ACKNOWLEDGEMENTS

I especially want to thank my supervisors, Dr. Janice Fletcher and Associate Professor Donald S. Anson for their guidance, support and patience throughout my PhD.

I would also like to thank the following people for their contribution to the work described in this thesis:

1) Dr. Heidi L. Peters and Nicole Buck, from Murdoch Children's Research Institue, who have supported my work through providing the mouse model for my research;

2) Dr. David Johnson, Rosemarie Gerace and Minh-Uyen Trinh, from Women's and Children's Hospital, who have provided technical assistance for biochemical analyses in my research;

3) Dr. Peter Clements and Enzo Ranieri, from Women's and Children's Hospital, for their technical assistance with the High-Performance Liquid Chromatography experiments;

4) Lynn Scarman for her assistance and expertise with the mouse and animal care attendants at the Women's and Children's Hospital animal facility.

Thank you to the University of Adelaide for providing me with the opportunity to undertake my PhD and supporting me with scholarship. I would also like to thank the members of the Department of Genetic Medicine, Women's and Children's Hospital for providing me with a supportive place to conduct my research.

To all the other members of the gene therapy group: Dr. David Parsons, Dr. Julie Bielicki, Dr. Rachel Koldej, Dr. Karlea Kremer, Dr. Alice Stocker, Dr. Martin Donnelly, Dr. ChuanHe Liu, Chantelle McIntyre, Richard Bright, Patricia Cmielewski, Stanley Tan, SuePing Lim, SinLay Kang, and Nigel Farrow. Thank you for your support and critical analyses on my research.

My deepest gratitude goes to my family for their love and support throughout my PhD. I would not get this far without your encouragement.