Metallothionein involvement in mitochondrial function and disease:

a metabolomics investigation

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ABSTRACT

One of the many recorded adaptive responses in respiratory chain complex I deficient cells is the over-expression of the small metal binding proteins, metallothioneins (MTs). The antioxidant properties of MTs putatively protect the deficient cells against oxidative damage, thus limiting further damage and impairment of enzymes involved in energy production. Moreover, the role of metallothioneins in supplying metal cofactors to enzymes and transcription factors in order to promote energy metabolism was previously proposed, which could accompany their role as antioxidants. This view is supported by the observations that MT knockout mice tend to become moderately obese, implying a lower energy metabolic rate. Hence, the involvement of metallothioneins in mitochondrial function and disease cannot be ignored. However, this association is still very vague due to the diversity of their functions and the complexity of the mitochondrion. The use of systems biology technology and more specifically metabolomics technology was thus employed to clarify this association by investigating the metabolic differences between wild type and MT knockout mice in unchallenged conditions as well as when mitochondrial function (energy metabolism) was challenged with exercise and/or a high-fat diet. The metabolic differences between these mice were also studied when complex I of the respiratory chain was inhibited with rotenone. The metabolome content of different tissues and bio-fluids were examined in an untargeted fashion using three standardized analytical platforms and the data mined using modern metabolomics and related statistical methods. Clear metabolic differences were found between the wild type and MT knockout mice during unchallenged conditions. These metabolic differences were persisted and were often amplified when mitochondrial metabolism was specifically challenged through exercise, high-fat intake or complex I inhibition. The data pointed to an overall reduced metabolic rate in the MT knockout mice and possible insulin resistance after the interventions which imply (and confirm) the involvement of MTs in promoting energy metabolism in the wild type mice.

Keywords: Metallothioneins, mitochondria, metabolomics, metallothionein-knockout, chemometrics

“When a thing was new, people said ‘*it is not true’*. Later when the truth became obvious, people said ‘*it is not important*’. And when its importance could not be denied, people said ‘*anyway, it is not new*’.” – William James (1842 – 1920)

“Whether we model the reality or only a shadow of it, we form a better understanding of the intricate biochemical processes and their scattering in living systems.” (Weckwerth, 2003)

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LIST OF ABBREVIATIONS AND SYMBOLS

SYMBOLS

α	alpha
β	beta
m	milli (10^{-3})
µ	micro (10^{-6})
n	nano (10^{-9})
℃	degrees Celsius
%	percentage
>	greater than
<	less than
< v.c	below visual cut-off
~	approximately
#	number
x *g*	g-force (9.80665 m/s^2)

ABBREVIATIONS

ACN	acetonitrile
ADP	adenosine diphosphate
Ag	silver
ATP	adenosine triphosphate
bp	base pairs
BSA	bovine serum albumin
BSTFA	O-bis(trimethylsilyl)trifluoroacetamide
CCMN	Cross-Contribution robust Multiple internal standard Normalization
Cd	cadmium
CI	complex I (NADH:ubiquinone oxidoreductase)
CII	complex II (succinate:ubiquinone oxidoreductase)
CIII	complex III (coenzyme Q - cytochrome *c* reductase)

CIV	complex IV (cytochrome *c* oxidase)
CoQ	coenzyme Q (ubiquinone)
Cu	copper
CV	coefficient of variance
cyt c	cytochrome c
Da	Dalton
DMPA	N,N-dimethyl-L-phenylalanine
DNA	deoxyribonucleic acid
EC	environmental control
EDTA	ethylene diamine tetra-acetate
ESI	electrospray ionisation
ETC	electron transport chain
EtOH	ethanol
FAD	flavin adenine dinucleotide
FbF	find by formula
FbI	find by ion
g	gram
GC	gas chromatography
GPx	glutathione peroxidase
GSH	reduced glutathione
GSSG	oxidized glutathione (dimer)
h	hour
HCl	hydrochloric acid
HFD	high-fat diet
HFD-E	high-fat diet & exercise
Hz	Hertz
IMs	important metabolites

kDa	kilo Dalton
KOH	potassium hydroxide
l	liter
LC	liquid chromatography
M	molar
MeOH	methanol
MFE	molecular feature extraction
min	minutes
ml	milliliter
mM	milli-molar
MPP	Mass Profiler Professional
MS	mass spectrometry
MSTFA	N-Methyl-N-trifluoroacetamide
MSTUS	mass spectrometry total useful signal
MT	metallothionein
MT-1	metallothionein isoform 1
MT-2	metallothionein isoform 2
MT-3	metallothionein isoform 3
MT-4	metallothionein isoform 4
MTKO	metallothionein knockout (unspecified, referring to both MT1+2KO and MT3KO)
MT1+2KO	metallothioneins isoforms 1 and 2 knockout
MT3KO	metallothioneins isoform 3 knockout
mtDNA	mitochondrial DNA
MTs	metallothioneins
m/z	mass to charge
NAD(H)	nicotinamide adenine dinucleotide (reduced)
nM	nano-molar
nm	nanometer
$OH^{\cdot}$	hydroxyl radical
OXPHOS	oxidative phosphorylation

PBS	phosphate buffered saline
PC	principal component
PCA	principal component analysis
PCR	polymerase chain reaction
PLS	Partial least squares (projection to latent structures)
PLS-DA	partial least squares discriminant analysis
PLSR	partial least squares regression
ppm	parts per million
QC	quality control
Q-TOF	quadrupole time of flight
RNS	reactive nitrogen species
ROS	reactive oxygen species
RP	reverse phase
RT	retention time; rotenone treatment
SD	standard deviation
SOD	superoxide dismutase
TIC	total ion chromatogram
TOF	time of flight
VAST	variable stability
VC	vehicle control
WT	wild type
Zn	zinc

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