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Functional consequences of the inhibition of Malaria S-adenosylmethionine decarboxylase as a key regulator of polyamine and methionine metabolism

By

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Summary

Malaria presents a global health risk that is becoming increasingly difficult to treat due to increased resistance of both the parasite and mosquito to all known drugs. Identification of novel drug targets are therefore essential in the fight against malaria. Polyamines are small flexible polycations that are represented by three basic polyamines. The interaction of polyamines with various macromolecules may lead to stabilisation of DNA, regulation of transcription, replication, and also have an important role in cellular differentiation, proliferation, growth and division. Therefore, disruption of polyamine biosynthesis presents a unique drug target worth exploiting. Polyamine biosynthesis in *P. falciparum* is regulated by a unique bifunctional S-adenosylmethionine decarboxylase/ornithine decarboxylase (AdoMetDC/ODC) complex, which is unique to *P. falciparum* and differs completely from human polyamine biosyntehsis. The inhibition of AdoMetDC induces spermidine and subsequent spermine depletion within the parasite that ultimately results in cell cycle arrest. A functional genomics approach was used within this study to identify a global response of the parasite due to the inhibition of AdoMetDC with the irreversible inhibitor, MDL73811.

The proteomics approach was optimised for conditions specific to our laboratory with regard to protein extraction, Plasmodial protein quantification, spot detection and finally protein identification by mass spectrometry (MS). This methodology resulted in reliable spot detection and achieved a 95% success rate in MS/MS identification of protein spots. Application of this methodology to the analyses of the Plasmodial ring and trophozoite proteomes ultimately resulted in the identification of 125 protein spots from the Plasmodial ring and trophozoite stages, which also confirmed stage specific protein production. Various protein isoforms were present which may be of significant biological importance within the Plasmodial parasite during development in the intraerythrocytic developmental cycle.

Subsequent application of the 2-DE methodology to the proteome of AdoMetDC inhibited parasites resulted in the identification of 61 unique Plasmodial protein groups that were differentially affected by the inhibition of AdoMetDC in 2 time points. The transcriptome of AdoMetDC inhibited parasites were also investigated at 3 time points. Investigation into the transcriptome revealed the differential regulation of 549 transcripts, which included the differential regulation of polyamine specific transcripts. Inhibition of AdoMetDC provided a unique polyamine specific transcriptomic signature profile that demonstrated unique interactions between AdoMetDC inhibition and folate biosynthesis, redox metabolism and cytoskeleton biogenesis. The results presented provide evidence that the parasite responds to AdoMetDC inhibition by the regulation of

the transcriptome and proteome in an attempt to alleviate the effects of AdoMetDC inhibition. Further analyses of the metabolome also provided evidence for the tight regulation of the AdoMet cycle. Overall, this study demonstrated important functional consequences as a result of AdoMetDC inhibition.

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List of Abbreviations

µg	Microgram
µl	Microliter
1-DE	One-dimensional gel electrophoresis
2-DE	Two-dimensional gel electrophoresis
4mC	N4-methylcytosine
5mC	5-methylcytosine
6mA	N6-methyladenine
A	Adenosine
ACT	Artemisinin-based combination therapy
AdoHcy	S-adenosyl-L-homocysteine
AdoMet synthase	S-adenosylmethionine synthase
AdoMetDC	S-adenosylmethionine decarboxylase
AdoMetDC/ODC	S-adenosylmethionine decarboxylase/Ornithine decarboxylase
AHC	S-adenosyl-L-homocysteine hydrolase
AM	Artemether
AMA	Apical membrane antigen
Arg	Arginine
ART	Artemisinin
AS	Artesunate
ATP	Adenosine triphosphate
Ave	Average
AVQ	Atovaquone
BCA	Bicinchoninic acid
BSA	Bovine Serum Albumin
C	Cytosine
CAPS	3-(cyclohexylamino)-1-propane sulfonic acid
CCB	Colloidal Coomassie Blue
CG	Cycloguanil
CHAPS	3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate
CPG	Chlorproguanil
CpG	Cytosine Guanine dinucleotide with connecting phosphodiester bond
CQ	Chloroquine
CSP	Circumsporozoite protein
Ct	Cycle threshold of the real-time amplification cycle
CV	Coefficient of variation
Cyclo	Cyclophillin
Cys	Cysteine
Da	Daltons
DALY	Disability adjusted life years
dcAdoMet	Decarboxylated AdoMet
DDT	Dichloro-diphenyl-trichloroethane
DFMO	DL-α-difluoromethylornithine
DHA	Dihydroartemisinin
DHFR	Dihydrofolate reductase
DHFR/TS	Dihydrofolate reductase/thymidylate synthetase
DHPS	Dihydropteroate synthetase
DIGE	Differential gel electrophoresis
DNA	Deoxyribonucleic acid
DS	Dapsone

EBA	Erythrocyte binding antigens
EDTA	Ethylenediamine tetra-acetic acid
eIF	Eukaryotic translation initiation factor
EMSA	Electrophoretic mobility shift assay
ESI	Electrospray ionisation
f	Forward primer
Fa-	Folic acid deficient
Fa+	Folic acid containing
FACS	Fluorescence activated cell sorting
FC	Fold change
FIC	Fractional inhibitory concentration
FIKK	A novel *Apicomplexa*-specific group of eukaryotic protein kinase-related proteins
FPP XI	Ferriprotoporphyrin IX
FTICR MS	Fourier transform ion cyclotron resonance mass spectrometry
g	Gram
G3PDH	Glyceraldehyde-3-phosphate dehydrogenase
GDH	Glutamate dehydrogenase
gDNA	Genomic DNA
GDP	Gross Domestic Product
GO	Gene ontology
h	hour
HA	Hyaluronic acid
HAART	Highly active antiretroviral therapy
HAT	Histone acetyltransferases
Hb	Hemoglobin
HC	Homocysteine
HCCA	4-hydroxy-α-cyanocinnamic acid
HDAC	Histone deacetylase
HDP	Hemoglobin derived products
HF	Halofantrine
HH4	Histone H4
HIV	Human Immunodeficiency Virus
HK	Hexokinase
HPI	hours post-invasion
HPLC	High-performance liquid chromatography
HPPK	Hydroxymethylpterin pyrophosphokinase
hPrx-2	human peroxiredoxin-2
HS	Homospermidine
Hsp	Heat shock protein
IC_{50}	Median Inhibitory concentration
ICAM	Intracellular adhesion molecule 1
ICAT	Isotope coded affinity tag
IDA	Information Dependant Acquisition
IDC	Intraerythrocytic developmental cycle
IEF	Iso-electrical focusing
IFN-γ	Interferon gamma
IL	Interleukin
Ile	Isoleucine
IMAC	Immobilised metal-ion affinity chromatography
iNOS	Inducible nitric oxide
IPG	Immobilsed polyacrylamide gel

IPT	Intermittent preventive treatment in pregnancy
iRBC	Infected red blood cell
IRS	Indoor residual spraying of insecticide
ITN	Insecticide treated nets
K	Thousand
kDa	Kilo daltons
L	Linear
l	litre
LC-ESI/MS	Liquid chromatography-electrospray ionisation/mass spectrometry
LDC	Lysine decarboxylase
LDH	Lactate dehydrogenase
LF	Lumefantrine
LLIN	Long lasting insecticidal nets
LOD	Limit of detection
LTα	Lymphotoxin alpha
M	Molar
MADIBA	Micro Array Data Interface for Biological Annotation
MALDI–TOF MS	Matrix assisted laser desorption/ionization time-of-flight mass
MAP	Malaria Atlas Project
MAQC	MicroArray Quality Control
MDG	Millennium development goal
MDL73811	5'-[(Z)-4-Amino-2-butenyl]methylamino]-5'-deoxyadenosine
mdr	Multi-drug resistance gene
Met	Methionine
mg	Milligram
MIAME	Minimum information about a microarray experiment
MIAPE	Minimum information about a proteomics experiment
MIM	Multilateral Initiative on Malaria
ml	Milliliter
MQ	Mefloquine
Mr	Molecular weight
mRNA	Messenger ribonucleic acid
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MSF	Malaria SYBR Green I-based fluorescence assay
MSP	Merozoite surface protein
MTA	5'-Methylthioadenosine
MTI	5'-Methylthioinosine
MudPIT	Multi-dimensional protein identification techniques
n/a	Not applicable
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NCBI	National Center for Biotechnology Information
ng	Nanogram
NKT	Natural killer T-cells
nm	Nanometers
NMR	Nuclear magnetic resonance
NTD	Neglected tropical disease
OAT	Ornithine aminotransferase
ODC	Ornithine decarboxylase
PABA	*p*-aminobenzoic acid
PAGE	Polyacrylamide gel electrophoresis

PBS	Phosphate-buffered saline
PCA	Perchloric acid
PEMT	Phosphoethanolamine N-methyltransferase
PEXEL	*Plasmodium* export element
Pf	*Plasmodium falciparum*
*Pf*3D7	*Plasmodium falciparum* choloroquine sensitive strain 3D7
*Pf*CRT	*Plasmodium falciparum* chloroquine resistance transporter
*Pf*EMP-1	Erythrocyte membrane protein-1
*Pf*HB3	Plasmodium falciparum pyrimethamine resistant
Pfmdr1	*Plasmodium falciparum* multiple drug resistant protein
*Pf*PR	*Plasmodium falciparum* parasite rate
*Pf*RBL	*Plasmodium falciparum* reticulocyte binding like
PG	Proguanil
Pgh	P-glycoprotein homologue
pi	Post invasion
pI	Isoelectric point
PK	Pyruvate kinase
PLP synthase	Pyridoxal-5-phosphate synthase
PMF	Peptide mass fingerprint
PNP	Purine nucleoside phosphorylase (uridine phosphorylase)
ppm	Parts per million
PPQ	Piperaquine
PQ	Primaquine
pt	Post treatment
PTM	Post-translational modifications
PVM	Parasite vacuolar membrane
PYR	Pyrimethamine
QN	Quinine
qRT-PCR	Semi-quantitative reverse transcription polymerase chain reaction
Q-TOF MS	Quadrupole-time-of-flight mass spectrometer
r	Reverse primer
R^2	Correlation coefficient of a regression line
RESA	Ring infected erythrocyte surface antigen
RIN	RNA integrity number
RNA	Ribonucleic acid
RP-HPLC	Reversed phase-high performance liquid chromatography
RPS4	Ribosomal protein S4
RQI	RNA Quality Indicator
rRNA	Ribosomal RNA
s	Second
SAGE	Serial analysis of gene expression
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SDX	Sulfadoxine
SELDI-TOF/MS	Surface-enhanced laser desorption ionisation-time-of-flight/mass
SEM	Standard error of the mean
SERCA	Sarco/endoplasmic reticulum calcium –dependent ATPase
SP	Sulfadoxine/Pyrimethamine combination therapy
SpdS	Spermidine synthase
SSH	Suppression subtractive hybridization
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
T	Treated
t_1	Time point 1
t_2	Time point 2

t_3	Time point 3
TEMED	N,N,N',N'-tetramethyl-ethylenediamine
THF	Tetrahydrofolate
TIM	Triosephosphate isomerase
Tm	Melting temperature
TNF	Tumor necrosis factor
Tris	Tris(hydroxymethyl)-aminomethane
TS	Thymidylate synthetase
Tt_1	Treated time point 1
U	Units
UN	United Nations
UNDP	United Nations Development Program
UNICEF	United Nations Children's Fund
US	United States
UT	Untreated
UTt_1	Untreated time point 1
UV	Ultraviolet
v/v	Volume per volume
Vhrs	Volt hours
VTS	Vacuolar transport signal
W	Watts
w/v	Weight per volume
WHO	World Health Organisation