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Functional genomics analysis of the effects of co-inhibition of the malarial S-adenosylmethionine decarboxylase/ornithine decarboxylase

by

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DECLARATION:

I, Anna Catharina van Brummelen declare that the thesis/dissertation, which I hereby submit for the degree *Philosophiae Doctor* at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE:.....

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Soli Deo gloria

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SUMMARY

Polyamines are ubiquitous components of all living cells and their depletion usually causes growth arrest or cytostasis, a strategy employed for treatment of West-African trypanosomiasis. In the malaria parasite, *Plasmodium falciparum*, polyamine biosynthesis is regulated by the uniquely bifunctional protein, S-adenosylmethionine decarboxylase/ornithine decarboxylase (PfAdoMetDC/ODC). The unique nature of this protein could provide a selective mechanism for antimalarial treatment.

To validate polyamine depletion and specifically PfAdoMetDC/ODC, as drug target for antimalarial therapeutic intervention, polyamine biosynthesis was completely restrained via the inhibition of both catalytic sites of PfAdoMetDC/ODC with DFMO and MDL73811. The physiological effects during the resulting cytostasis were studied with a comprehensive functional genomics approach. The study was preceded by various assays to determine the treatment dosage that would result in complete cytostasis, without non-specific chemical cytotoxicity. The results obtained revealed that the cytostatic mechanism with growth arrest of the treated parasites and normal progression of the untreated controls require special consideration for basic comparisons of response in terms of the assay methodology used and data analysis. This is particularly important when studying a multistage organism such as *P. falciparum*, which constantly develops and change during the intraerythrocytic developmental cycle, such that growth arrest compared to normal progression would result in significant differences merely due to stage. This critical principle was kept in mind throughout the investigation and was applied to the relative quantification of RNA, proteins and metabolites via a relative time zero approach as opposed to the standard parallel time point comparison.

Three independent functional genomics investigations, namely transcriptomics, proteomics and metabolomics were conducted, in which highly synchronised 3D7 parasite cultures were treated during the schizont stage and parasites were sampled during a time course at three time points (just before and during cytostasis). Transcriptome analysis revealed the occurrence of a generalised transcriptional arrest just prior to the growth arrest. To our knowledge this is the first time that transcriptional arrest as the preceding mechanism of cytostasis due to polyamine depletion, was demonstrated. However, despite the transcriptional arrest, the abundance of 538 transcripts was differentially affected and included three perturbation-specific compensatory transcriptional responses: the increased abundance of the transcripts for lysine decarboxylase and ornithine aminotransferase (OAT) and the decreased abundance of that for S-adenosylmethionine synthetase (AdoMet synthetase). Pearson correlations indicated more subtle effects of the perturbation on the proteome and even more so on the metabolome where homeostasis was generally maintained, except downstream to the enzymatic blockade at PfAdoMetDC/ODC. The perturbation-specific compensatory roles of OAT in the

regulation of ornithine and AdoMet synthetase in the regulation of AdoMet were confirmed on both the protein and metabolite levels, confirming their biological relevance.

The results provide evidence that *P. falciparum* respond to alleviate the detrimental effects of polyamine depletion via the regulation of its transcriptome and subsequently the proteome and metabolome, which supports a role for transcriptional control in the regulation of polyamine and methionine metabolism within the parasite. The study concludes that polyamines are essential molecules for parasite survival and that PfAdoMetDC/ODC is a valid target for antimalarial drug development.

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ABBREVIATIONS

^{1}H-NMR	Proton nuclear magnetic resonance
2D	Two-dimensional
2D-DIGE	Two-dimensional difference gel electrophoresis
2D-GE	Two-dimensional gel electrophoresis
2D-NMR	Two-dimensional nuclear magnetic resonance
2D-PAGE	Two-dimensional polyacrylamide gel electrophoresis
5mC	5-Methyl-2-deoxycytosine
5mC(P)	5-Methylcytidine
6mA	6-Methyl-2-deoxyadenine
A	Adenosine or average signal intensities (MA plot)
AcN	Acetonitrile
AdoHcy	S-adenosylhomocysteine
AdoMet	S-adenosylmethionine
AdoMet synthetase	S-adenosylmethionine synthetase
AdoMetDC	S-adenosylmethionine decarboxylase
AMA1	Apical membrane antigen 1
APAD	3-acetyl pyridine adenine dinucleotide
ApiAP2	Apicomplexan Apetala2
ATP	Adenosine triphosphate
BC	Before Christ
bp	Base pair
BSA	Bovine serum albumin
C	Cytidine
CD36	Cluster determinant 36
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
CO	Carbon monoxide
CpG	Cytosine Guanine dinucleotide with connecting phosphodiester bond
CPM	Counts per minute
CSA	Chondroitin sulphate A
C_t	Cycle threshold of the real-time amplification curve
Cys	Cysteine
DALY	Disability adjusted life years
dATP	Deoxyadenosine triphosphate
DAVID	Database for annotation, visualization and integrated discovery
dCTP	Deoxycytidine triphosphate
dcAdoMet	Decarboxylated S-adenosylmethionine
DDT	Dichlorodiphenyltrichloroethane
DELI	Double-site enzyme-linked LDH immunodetection
DEPC	Diethyl pyrocarbonate
DFMO	DL-a-difluoromethylornithine
dGTP	Deoxyguanosine triphosphate
DHFR	Dihydrofolate reductase

DHFR/TS	Dihydrofolate reductase/thymidylate synthase
DHPS	Dihydroopteroate synthase
DHPS/PPPK	Dihydroopteroate synthase/dihydroxymethylpterin pyrophosphokinase
DIGE	Difference gel electrophoresis
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphates
DPM	Disintegrations per minute
DTT	Dithiothreitrol
dTTP	Deoxythymidine triphosphate
dUTP	Deoxyuridine triphosphate
EDTA	Ethylenediamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
ESI	Electrospray ionization
EtOH	Ethanol
FACS	Fluorescence activated cell sorting
F-MES	Modified Falkow (medium)
FTICR	Fourier transform ion cyclotron resonance
G	Guanosine
GABA	Gamma-aminobutyrate or 4-aminobutyrate
gDNA	Genomic DNA
gff	General feature format
GO	Gene ontology
hpi	Hours post-invasion
HPLC	High-performance liquid chromatography
HRP	Histidine-rich proteins
hrp [a]	Horseradish peroxidase
hrp-conjugate	Anti-mouse horseradish peroxidase-conjugated secondary antibody
HRPII	Histidine- and alanine-rich protein 2
IC_{50}	Median inhibitory concentration
ICAT	Isotope-coded affinity tags
IDC	Intraerythrocytic developmental cycle
IEF	Iso-electric focusing
IFN	Interferons
IL	Interleukin
IPG	Immobilized pH gradient
iTRAQ	Isobaric tags for relative and absolute quantitation
KEGG	Kyoto Encyclopedia of Genes and Genomes
LB	Luria-Bertani (broth)
LC	Liquid chromatography
LC-ESI/MS	Liquid chromatography/electron spray ionization mass spectrometry
LDC	Lysine decarboxylase
LDH	Lactate dehydrogenase
LIMMA	Linear models for microarray data (software)
LOWESS	Locally weighted scatterplot smoothing

M	Log_2-ratios of transcript abundance
m/z	Mass/charge ratio
MALDI	Matrix assisted laser desorption/ionization
MAOBA	5'-Deoxy-5'-[N-methyl]-N-[2-(aminooxy)buthyl]amino]adenosine
MAOEA	5'-Deoxy-5'-[N-methyl]-N-[2-(aminooxy)ethyl]amino]adenosine
MDL73811	5'-{[(Z)-4-amino-2-butenyl]methylamino}-5'-deoxyadenosine
MDR1	Multidrug-resistance type 1 protein
MeOH	Methanol
MIAME	Minimum information about a microarray experiment
MOPS	3-(N-morpholino)propanesulfonic acid
MPMP	Malaria Parasite Metabolic Pathways
Mr	Molecular weight
mRNA	Messenger RNA
mRNP	Messenger ribonucleoprotein complexes
MS	Mass spectrometer/spectrometry
MS/MS	Tandem mass spectrometry
MSP1	Merozoite surface protein 1
MSRE	Methylation-sensitive restriction endonucleases
MudPIT	Multidimensional protein identification technology
NBT	Nitroblue tetrazolium
NMR	Nuclear magnetic resonance
NO	Nitric oxide
OAT	Ornithine aminotransferase
OAT_{met}	Methylated ornithine amino transferase DNA
ODC	Ornithine decarboxylase
ORF	Open reading frame
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
pdx1	Pyridoxal-5'-phosphate synthase
PES	Phenazine ethosulphate
PEXEL	*Plasmodium* export element
PfAdoMetDC/ODC	*P. falciparum* S-adenosylmethionine decarboxylase/ornithine decarboxylase
PfCRT	*P. falciparum* chloroquine-resistance transporter
PfEMP1	Erythrocyte membrane protein 1
PI	Propidium iodide
pI	Iso-electric point
PlasmoDB	*Plasmodium* database
PLP	Pyridoxal-5'-phosphate
PLS	Partial Least Squares
PMF	Peptide mass fingerprint/fingerprinting
PMT	Photon multiplier tube (fluorescent scanners)
ppm	Parts per million
PUMAdb	Princeton University Microarray database
Q	Quadropole
Q-TOF	Quadropole-time-of-flight mass spectrometer/spectrometry
r	Pearson correlation
R	Correlation coefficient of the regression line of data plotted on the same graph

Rifin	Repetitive interspersed family (genes)
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
SAGE	Serial analysis of gene expression
SDS	Sodium-dodecylsulphate
SERCA	Sarcoplasmic reticulum calcium-dependent ATPase
SRM	Single reaction monitoring (mass spectrometry)
SSC	Saline sodium citrate
SSP	Standard spot numbers
Stevor	Subtelomeric variable open reading frame (genes)
T	Thymidine or treated (sample)
t_0	Time zero
t_1	Time point 1
t_2	Time point 2
t_3	Time point 3
TAE	40 mM Tris, 20 mM glacial acetic acid, 1 mM EDTA (buffer)
T_m	Melting temperature
TNF	Tumour necrosis factor
TOF	Time-of-flight
tRNA	Transfer RNA
U	Units
UT	Untreated (sample)
UV	Ultraviolet
V	Volts
var	Variant (genes)
Vh	Volt hours
VTS	Vacuolar transport signal
WHO	World Health Organisation

a. HRP is the customary abbreviation for horseradish peroxide, but to distinguish from the abbreviated histidine rich protein, lowercase characters (hrp) were used.