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Profiling gene expression pattern changes in HIV-1-associated kidney disease

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Profiling Gene Expression Pattern Changes in HIV-1-Associated Kidney Disease

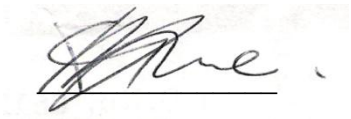
Keleabetswe Lerato Mpye

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Johannesburg, 2015

Declaration

I, Keleabetswe Lerato Mpye, hereby declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted for any other degree or examination at any other University.



Keleabetswe Lerato Mpye

This _**17th** ____ day of ____**June**____, 2015.

Abstract

HIV-1 infected individuals are at risk of developing clinically and morphologically diverse renal complications. Most of the ensuing renal complications are similar to those observed in non-HIV patients while some are limited to HIV-infected patients. Kidney diseases are amongst the top 10 leading causes of death in the general population and affect over 10 % of the adult population. Two to ten percent of HIV-1 infected patients ultimately suffer chronic kidney disease, and specifically HIV-associated nephropathy (HIVAN), characterized clinically by nephrotic range proteinuria and histologically by collapsing focal segmental glomerulosclerosis (FSGS). Genetic mutations and disruption of the podocyte foot process and actin-based cytoskeleton architecture through dysregulation of its principal element, actin, or its associated proteins, lead to proteinuria and nephrotic syndrome. The majority of these proteins are susceptible to proteolysis by a lysosomal cysteine protease, cathepsin-L. The current study set out to profile changes in gene expression in HIVAN relative to HIV negative FSGS, minimal change disease (MCD) and non-proteinuric healthy controls. Probing the presence and localization of cathepsin-L and dynamin in these proteinuric kidney diseases, we showed that cathepsin-L and dynamin expression is enhanced in HIVAN and FSGS, relative to the healthy controls and MCD. We further profiled the expression of the 3 isoforms of cathepsin-L and dynamin using Quantitative reverse transcriptase PCR. *CTSL-3* expression was similar between all disease groups whilst *CSTL-2* was reduced relative to the normal tissue. There was most variation in the expression of *CSTL- 1* between the disease tissues with down-regulation of *CSTL-1* in both HIVAN and FSGS relative to the control and MCD tissue. The expression of dynamin remained largely unchanged between the disease groups with the exception of *DYN-1* which was downregulated in MCD relative to FSGS. Given that dynamin expression was barely altered across the proteinuric states, compared to the healthy non-proteinuric controls, signifies that proteolysis of the actin-associated proteins synaptopodin and/or slit diaphragm-associated CD2AP by the cathepsins-L 2 and 3 was responsible for the proteinuria in HIVAN, FSGS and MCD. Our results show that FSGS manifests through attenuated CD2AP, up-regulated TGF-β1 and cathepsin-L activity and not via dynamin degradation. The attenuated mRNA expression levels of dynamin, CD2AP and synaptopodin in renal tissue of the proteinuric kidneys validate that podocyte injury, in the form of loss of foot process integrity and slit diaphragm permeability, manifests through the over-expressed cathepsin-L enzymatic activity. Many of the genes that were altered in proteinuric kidney diseases, as observed from the pathway analysis of the PCR array data, are

mediated by transcriptional activation of nuclear factor kappa B (NF-KB). Transcriptional mRNA expression levels, validated in real-time quantitative RT-PCR analysis illustrated that HIV-1 infection induces uncontrolled activation and induction of immunity and inflammatory responses, indicated by down-regulation of *SIGIRR*, and modulation of expression patterns of immunity and inflammatory response genes in proteinuric kidney diseases. Other genes that were down-regulated in HIVAN are involved in the MAPK JNK cellular signaling pathway and apoptosis. HIV-1 infection modulates expression patterns of the genes that are involved in immunity and inflammatory responses in proteinuric kidney disease, compromising the structure and function not only of the kidney but of the entire immune system.

This work is dedicated to:

The world's most beautiful and charismatic daughter, Agonnejaalo Mamotlopi, and my family who continuously support and encourage me in all I do.

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Research outputs

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List of abbreviations

°C	Degrees Celsius
Δ*G*	Change in Gibbs free energy
$2^{-\Delta\Delta Cq}$	Fold change
AIDS	Acquired immune deficiency syndrome
APS	Ammonium persulfate
ARV	Antiretroviral
C_t	Cycle threshold
C_q	Quantification cycle
CCL2	Chemokine (C-C Motif) ligand 2 or Monocyte chemoattractant protein 1
CCL3	Chemokine (C-C Motif) ligand 3 or Macrophage inflammatory protein 1-α
CCL4	Chemokine (C-C Motif) ligand 4
CCR5	Chemokine (C-C motif) receptor 5
CDKN1A	Cyclin-dependent kinase inhibitor 1A
CKD	Chronic kidney disease
CXCL12	Chemokine (C-X-C Motif) Ligand 12
CXCR4	Chemokine (C-X-C motif) receptor 4
Da	Daltons
DMEM	Dulbeco's modified eagle medium
DMSO	Dimethylsulphoxide
DTT	Dithiothreitol
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
Env	Envelope glycoprotein
ERK	Extracellular signal-regulated kinase
ESL	Endothelial surface layer
ESRD	End-stage renal disease
FSGS	Focal segmental glomerulosclerosis
GBM	Glomerular basement membrane
H_2O	Water

H_2O_2	Hydrogen peroxide
HAART	Highly active antiretroviral treatment
HCl	Hydrochloric acid
HIV-1	Human immunodeficiency virus type I
HIVAN	Human immunodeficiency virus-associated nephropathy
HIVICK	Human immunodeficiency virus immune complex kidney disease
HMGB1	High Mobility Group Box 1
IFN-γ	Interferon gamma
IHC	Immunohistochemistry
IL-1β	Interleukin-1 beta
IRAK	Interleukin-1 receptor-associated kinases
JNK	c-Jun NH2-terminal kinase
ℓ	Litre
LY96	Lymphocyte antigen 96
M	Molar
MAPK	Mitogen-Activated Protein Kinases
MAP3K1	Mitogen-Activated Protein Kinase Kinase Kinase 1
MAP4K4	Mitogen-Activated Protein Kinase Kinase Kinase Kinase 4
MAPK8	Mitogen-Activated Protein Kinase 8
μℓ	Microlitre
mℓ	Millilitre
NaCl	Sodium chloride
NPHS1	Nephrosis 1, congenital, Finnish type (nephrin)
NPHS2	Nephrosis 2, idiopathic, steroid-resistant (podocin)
NRTI	Nucleoside reverse transcriptase inhibitor
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PELI1	Pellino E3 Ubiquitin Protein Ligase 1
PI	Protease inhibitor
qPCR	Quantitative polymerase chain reaction

RAG	Recombination activating gene
REL	V-rel reticuloendotheliosis viral oncogene homolog (avian)
RNA	Ribonucleic acid
RPL13A	*Homo sapiens* ribosomal protein L13a
rpm	Revolutions per minute
RT	Reverse transcriptase
SDHA	*Homo sapiens* succinate dehydrogenase complex, subunit A, flavoprotein
SDS	Sodium dodecyl sulphate
SERPINA1	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin)
SIGIRR	Single Immunoglobulin and Toll-Interleukin 1 Receptor (TIR) Domain
SLPI	Secretory Leukocyte Peptidase Inhibitor
STAT1	Signal transducer and activator of transcription 1
SYNPO	Synaptopodin
TEMED	N, N, N', N'-Tetramethylethylenediamine
TGF-β1	Transforming growth factors beta 1
TLR	Toll-like receptor
TNFα	Tumor necrosis factor alpha
TNFSF10	Tumor necrosis factor (ligand) superfamily , member 10
TRAF6	Tumor necrosis factor receptor associated factor
µℓ	Micro litre
VDJ	Variable, diversity and joining
x g	Times gravity (relative centrifugal force)
YWHAZ	*Homo sapiens* tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide.

The IUPAC-IUBMB one and three letter notations for the amino acids have been used.