EPIGENETIC CHANGES IN ALZHEIMER'S AND HUNTINGTON'S DISEASE

A semi-quantitative analysis of histone changes

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ABSTRACT

Histone acetylation is an epigenetic mechanism that plays a critical role in the regulation of gene transcription, DNA damage repair and DNA replication. Histone acetylation homeostasis is maintained by a balance in the activity of histone deacetylases (HDAC) and acetyltransferases. Disruption in their activity can lead to malfunctioning cells, cell death and disease. There is increasing evidence suggesting that deregulation of histone acetylation and consequential effects leading to aberrant gene expression may influence neurodegenerative disease pathogenesis. To date, rodent models of Huntington's disease (HD) or Alzheimer's disease (AD) have generally exhibited evidence for histone hypoacetylation and reduced gene expression in disease pathogenesis. The use of HDAC inhibitors (HDACi) to restore histone acetylation levels and gene expression of down-regulated genes has shown promise in these models but so far not in the clinic. Furthermore the state of histone acetylation in human HD and AD brain has not been previously characterised. This discrepancy and lack of correlation in HDACi based therapeutic outcomes between lab and clinic formed the foundation of this current study.

Initially semi-quantitative image analysis methods were developed to quantify histone changes. These methods were then used to investigate histone changes in the inferior temporal gyrus and hippocampus of AD and the motor cortex, sensory cortex and cingulate gyrus of HD post mortem brains compared to age and sex matched neurologically normal control brains. Results obtained from semi-quantitative image analysis and Western blot analyses collectively demonstrated that histone H4 is markedly increased (independent of changes in total histone protein) in all regions of interest in HD brains compared to controls. Unlike HD however, histone acetylation increases in AD brains were significantly correlated with changes in total histone protein suggesting different mechanisms of epigenetic deregulation at work in these two diseases. These observations from the human brain highlight not only species differences but raise caution for current epigenome based therapeutic strategies and highlight the need for further investigation and characterisation of the human epigenome, in order to fully understand and develop potential epigenetic therapies to prevent and/or alleviate AD and HD pathogenesis.

I dedicate this thesis to 3 people:

To dear little Joseph Frederick O'Carroll (snow drop), who will always remind me of how precious life is.

To my brother, Praneet Ajit Narayan (Nitti), who I miss dearly, but who lives on in our memories and in the principles he taught me to live by.

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GLOSSARY

3-NP	3-nitropropionic acid
5-Aza	5-aza-2'-deoxycytidine
AcH3	Acetyl histone H4
AcH4	Acetyl histone H3
ACTB	Beta actin
AD	Alzheimer's disease
AICD	APP intracellular domain
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of Variance
AP-1	Activating protein-1
APOE	Apolipoprotein E
APP	Amyloid precursor protein
APP-CT	APP C-terminal fragments
ATCC	American type cell culture
Aβ	β-amyloid
BA	Brodmann's area
BSA	Bovine serum albumin
CA	Cornu ammonis
cAMP	Cyclic adenosine 3', 5'–monophosphate
CBP	Cyclic adenosine 3', 5' –monophosphate (cAMP) response element-binding protein (CREB) binding protein
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
ChIP	Chromatin immunoprecipitation
CN	Caudate nucleus
CREB	cAMP response element-binding protein
CSF	Cerebrospinal fluid
DNMT	DNA methyltransferase
dNTP	Deoxyribonucleotide triphosphate
ERK1/2	Extracellular-signal-regulated kinase 1 and 2
ESET	ERG-associated protein with SET domain
FBS	Foetal bovine serum
GABA	Gamma-aminobutyric acid
gDNA	Genomic DNA
GFAP	Glial fibrillary acidic protein
Gpe	Globus pallidum external segment
Gpi	Globus pallidum internal segment
HAT	Histone acetyl transferase
HCl	Hydrochloric acid
HDAC	Histone deacetylase
HDACi	Histone deacetylase inhibitors
HLA	Human leukocyte antigen
HPLC	High performance liquid chromatography
ICC	Immunocytochemistry
IHC	Immunohistochemistry
ITG	Inferior temporal gyrus
mAb	Monoclonal antibody

MALDI-TOF	Matrix-assisted laser desorption/ionization -Time Of Flight
MAPK	p38 mitogen-activated protein kinase
MBD1-3	Methyl binding protein
MeCP1,2	Methyl CpG binding protein 1,2
miRNA	Mirco RNA
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MSK1/2	Mitogen- and stress-activated protein kinase 1 and 2
MTHFR	5' 10' methylene tetrahydrofolate reductase
NAD	Nicotinamide adenine dinucleotide
NeuN	Neuronal N
NFT	Neurofibrillary tangles
NMDA	N-Methyl-D-aspartic
NMDAR	NMDA receptor
nt	Nucleotide
pAb	Polyclonal antibody
PANH3	Total histone H3
PANH4	Total histone H4
Pb	Lead
PCR	Polymerase chain reaction
PET	Positron emission tomography
PFA	Paraformaldehyde
PHF	Paired helical filaments
PKC	Protein kinase C
PS	Presenilin
Put	Putamen
PVDF	Polyvinylidene fluoride
RCF	Relative centrifugal force
REST	RE1 silencing transcription factor
RPL30	Ribosomal protein L30
RT	Room temperature
SIRT	Sirtuin
SNc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
STN	Subthalamic nucleus
TBP	Tata binding protein
TMA	Tissue microarray
TSA	Trichostatin A
UTR	Untranslated region
VPA	Valproic acid
WB	Western blot
Zn	Zinc