EPIGENETIC DYSREGULATION IN NEURODEGENERATIVE DISEASES AND POTENTIAL TREATMENT

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy

School of Pharmacy
Brac University
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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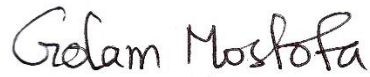

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Approval

The thesis/project titled “Epigenetic Dysregulation in Neurodegenerative Diseases and Potential Treatment” submitted by Golam Mostofa (18146021) of Spring, 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

This study does not involve any kind of animal trial or human trial.

Abstract

Epigenetics holds significant relevance in the contemporary neuroscience and has been associated with the pathogenesis of different neurodegenerative disorders. Epigenetic modifications including DNA methylation and histone acetylation have been widely studied in context of transcriptional regulation and dysregulation. In Alzheimer's disease, presenilin 1 expression is significantly increased from DNA hypomethylation, thereby increasing the amyloid β peptide deposition. In Parkinson's disease, decreased methylation increases the expression of α-synuclein and leads to SNCA protein accumulation. In Huntington's disease, mutant Huntingtin reduces the functioning of histone acetyltransferases, resulting in the transcriptional dysregulations. In this literature review, the role of epigenetic deregulation in the development of neurodegenerative disorders was discussed followed by an insight on the potential of epigenetics-based treatment for these neurodegenerative disorders.

Keywords: Epigenetics; Dysregulation; Methylation; Acetylation; Alzheimer's disease; Parkinson's disease; Huntington's disease; Epidrugs

Dedication

Dedicated to my family

Acknowledgement

To begin with, I would express my deepest gratitude to Allah (SWT) for blessing me with good health, patience, courage, and knowledge without which I would never have made it this far.

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List of Acronyms

3′UTR	3 Prime Untranslated Region
3C	Chromosome Conformation Capture
3D	3 Dimensional
5-hmC	5-hydroxymethylcytosine
5-mC	5-methylcytosine
ACTB	Actin Beta
AD	Alzheimer’s Disease
AICD	APP Intracellular Domain
APOE	Apolipoprotein E
APP	Amyloid Precursor Protein
Aβ	Amyloid Beta
BACE-1	Beta-Secretase 1
BACE1-AS	BACE1 Antisense RNA
BBB	Blood Brain Barrier
BCL2	B-cell Lymphoma 2
BDNF	Brain Derived Neurotrophic Factor
bp	Base Pair
CAG	Cytosine-Adenine-Guanine

Cas	CRISPR Associated Protein
CBP	CREB Binding Protein
ChIP	Chromatin Immunoprecipitation
CLU	Clusterin Gene
CNS	Central Nervous System
Co-A	Coenzyme A
CpG	5'-Cytosine-phosphate-Guanine-3
CREB	cAMP Response Element Binding
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
dCas	Dead Cas
DNA	Deoxyribonucleic Acid
DNMT	DNA Methyltransferases
DNMTi	DNA Methyltransferase Inhibitor
DRD2	Dopamine Receptor 2
EGCG	Epigallocatechin-3-Gallate
ERK1	Extracellular Signal Regulated Kinase 1
fAD	Familial AD
FISH	Fluorescent *in situ* Hybridization
GADD45	Growth Arrest and DNA Damage Gene

GDNF	Glial Cell Derived Factors
GRIN1	Glutamate Ionotropic Receptor NMDA Type Subunit 1
GSK-3'	Glycogen Synthase Kinase 3
H3K14	Lysines 14 on Histone H3
H3K27	Lysine 27 on Histone H3
H3K27ac	Acetylation at Lysine 27 in Histone H3
H3K4me3	Trimethylation at Lysine 4 in Histone H3
H3K9	Lysines 9 on Histone H3
H3S10	Serine 10 on Histone H3
H4K16ac	Acetylation at Lysine 16 on Histone H4
HAT	Histone Acetyltransferases
HATi	Histone Acetyltransferases Inhibitor
Hcy	Homocysteine
HD	Huntington’s Disease
HDAC	Histone Deacetylase
HDACi	Histone Deacetylase Inhibitor
HDM	Histone Demethylase
HDMi	Histone Demethylase Inhibitor
Hi-C	High Throughput Chromosome Conformation Capture

HMT	Histone Methyltransferases
HMTi	Histone Methyltransferase Inhibitor
HSP70	Heat Shock Protein 70 kD
HTT	Huntingtin
KDM1A	Lysine Demethylase 1A
KDM1B	Lysine Demethylase 1B
KDMs	Lysine Demethylases
lcRNA	Long Noncoding RNA
LOAD	Late Onset Alzheimer's Disease
LRRK2	Leucine Rich Repeat Kinase 2
LSD1	Lysine Specific Demethylase 1
MAPT	Microtubule Associated Protein Tau
mHTT	Mutant Huntingtin
miRNA	MicroRNA
mRNA	Messenger RNA
ncRNA	Noncoding RNA
NFT	Neurofibrillary Tangles
NGS	Next Generation Sequencing
NUDT15	Nudix Hydrolase

Nurr1	Nuclear Receptor Related Protein 1
PAH	Proteins Binding to Acetylated Histones
PARK	Parkin/ Parkinson Juvenile Disease Protein 2
PARP1	Poly [ADP-ribose] Polymerase 1
PD	Parkinson's Disease
PENK1	Proenkephalin
PHF	Paired Helical Filaments
PINK1	PTEN Induced Kinase 1
PKC	Protein Kinase C
PMH	Proteins Binding to Methylated
PMT	Protein Methyltransferases
PP2A	Protein Phosphatase 2A
PS	Presenilin
qRT-PCR	Quantitative Reverse Transcription Polymerase Chain Reaction
REST	Repressor Element 1 Silencing Transcription Factor
RNA	Ribonucleic Acid
SAH	S-Adenosyl-Homocysteine
SAM	S-Adenosyl-Methionine
sAPPα	Soluble Peptide APPα

sAPPβ	Soluble Peptide APP β
SIRT	Sirtuins
SN	Substantia Nigra
SNCA	Alpha Synuclein
SNP	Single Nucleotide Polymorphism
SNpc	Substantia Nigra Pars Compacta
TdCyd	4′-thio-2′-deoxycytidine
TET	Translocase Dioxygenases
TNF-α	Tumor Necrosis Factor Alpha
TXNRD1	Thioredoxin Reductase 1
US FDA	United State Food and Drug Administration
α-synuclein	Alpha Synuclein
α-tubulin	Alpha Tubulin

Chapter 1

Introduction

1.1 Background

The term epigenetics was initially introduced by Conrad Waddington to define a conceptual solution to a process that occurs as a fundamental consideration in developmental biology. An individual has the same DNA sequence in almost all cells of the body. For instance, a person's liver cells, and muscle cells have exactly the same DNA but the gene product these two types of cells produce differ vastly (Coppedè, 2014). Certain level of biological process must exist that operate above ('epi') the genes, and such that identical genes can be differentially translated into different cell types and define cell functions (Hwang et al., 2017). Therefore, as conclusion, in the 1940s, Waddington defined epigenetics as the study of different interactions among genes and their products that result in bringing phenotype into the being (Landgrave-Gómez et al., 2015).

Epigenetics involves the heritable modification into gene expression without changing the DNA sequence, and thereby playing a significant role in embryonic development and cell differentiation (Coppedè, 2021). Epigenetic changes include DNA methylation, hydroxymethylation; nucleosome positioning; histone acetylation, deacetylation, phosphorylation, ubiquitination and non-coding RNAs e.g., microRNAs, long non-coding RNAs which construct a complex network of covalent modifications to DNA cytosine bases and histone proteins that then interacting with different cellular proteins resulting in altered gene expression (Figure 1). Epigenetic modification affects chromatin compaction and changes the bindings of histone proteins to the DNA promoter regions of adjacent nucleosomal core,

thereby affecting the availability and capability of transcriptional factors to control gene expression (Z. Xu et al., 2012).

The role of epigenetics in human disease was first identified in oncology, and now has expanded to many other areas such as neurology, immunology and metabolic disorders (Z. Xu et al., 2012). Recent research has shown that epigenetic regulations can also play important roles in the neurological system, such as regulating neuronal gene expression, genome stability and DNA damage (Rudenko & Tsai, 2014). The importance of epigenetic alterations in neurological disorders including Alzheimer's disease, Huntington's disease, Parkinson's disease, epilepsy and multiple sclerosis is becoming rapidly evident (Gangisetty & Murugan, 2016). The available therapeutic options for neurological disorders do not stop the progression of disease, but rather prevent the condition from getting worse for a limited time (Coppedè, 2014).

Epigenetic based drugs have demonstrated unexpected therapeutic effects in neurological disorders, leading researchers to study the epigenetic process in neurodegenerative disorders (Z. Qi et al., 2021). Studies of neurodegenerative diseases in animal models have shown the possible significance of epigenetic based drugs in relieving cognitive symptoms and preventing or limiting motor symptoms, leading to the basis for a possible implementation in human pathology (Coppedè, 2021). The United State Food and Drug Administration (US FDA) authority has approved numerous histone deacetylation and DNA methylation inhibitors, and for years these have been in clinical uses. Histone methylation and the role of microRNA in regulating gene expression have recently received attention as potential targets for therapy (Kelly et al., 2010).

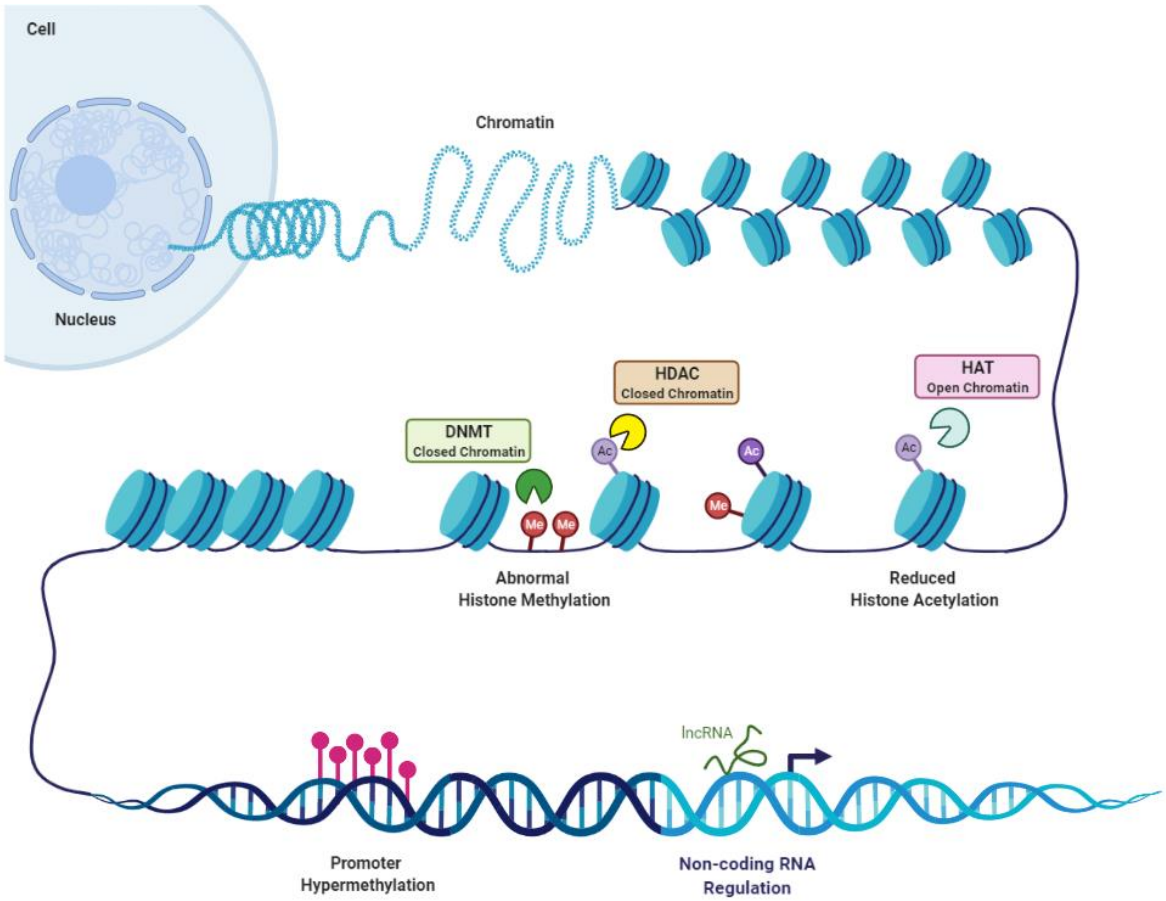

Figure 1: Schematic representation of the epigenetic modification in genome (Adapted from Conboy et al., 2021). DNA methylation, histone methylation, acetylation, and non-coding RNAs are some major epigenetic mechanisms that regulate gene expression and suppression.

1.2 Objective of the Study

The role of epigenetics in maintaining the homeostasis of the nervous system and in the emergence of neurodegenerative diseases is a significant development of modern neuroscience. To correlate with the development in the field of epigenetics, the objectives of this literature review are:

- To summarize various mechanisms associated with epigenetic regulation of genes.
- To discuss the significance of maintaining epigenetic signatures throughout the lifetime.
- To provide an overview on the effect of epigenetic dysregulation in the pathology of neurodegenerative disorders.

- To highlight the potential of epigenetics-based treatment approach in neurodegenerative diseases.

1.3 Rationale of the Study

Over the last few decades, it has become more and more evident that epigenetic modifications play an important role in the complex neural functions that we have. Epigenetics may be the missing piece in the puzzle of how genes and the environment interact with each other, giving us possible clues about the causes of some neurodegeneration disorders. Understanding how epigenetic changes contribute to neurodegeneration will help to learn more about the diseases' biology and hopefully, uncover new treatment targets. It will be then easier to predict, diagnose, and treat these common neurodegenerative disorders if we know how epigenetic changes play a role in their development. Through a detailed understanding on the epigenetic modifications that cause these diseases, new epigenetic therapeutic targets are being looked at which would pave way in the near future to treat these disorders.

Chapter 2

Methodology

To conduct the literature review, followed by the selection of the topic, an outline was prepared to present the information in a systematic manner. The information relevant to the topic was collected from secondary research articles indexed in databases like PubMed, Scopus, and Google Scholar using the following keyword ‘Epigenetic Dysregulation’, ‘Neuroepigenetics’, ‘Neurodegenerative Disorders’, ‘Alzheimer’s Diseases’, ‘Parkinson’s Diseases’, ‘Huntingtin Diseases’, ‘Epigenetic Therapy’, ‘Epigenetic Drug’. Then the articles were analyzed to collect the required information according to the prepared outline, followed by the compilation, paraphrasing and citation of the articles. Software including ChemDraw for drawing the chemical structure of the drugs, BioRender for drawing the figures and diagrams, Mendeley for generating in-text citation and bibliography have been used in this review.

Chapter 3

Epigenetics

3.1 Epigenetic Modifications

Epigenetic processes are the controlling mechanisms that regulate the structure and function of the genome in accordance with the external stimuli. DNA methylation, histone modification, chromatin remodeling and non-coding RNA are the core mechanisms of epigenetic regulation (Qureshi & Mehler, 2013). Epigenetic mechanisms regulate the expression of genes by modifying the structure of chromatin or by revealing binding sites for regulatory subunits for transcription (Kofink et al., 2013). Such dynamic and highly connected mechanisms are involved in the execution of cell specific genomic processes including gene expression, transcription, RNA processing, translation, DNA repair, genomic imprinting, maintaining genomic integrity and X chromosome inactivation (Qureshi & Mehler, 2014).

3.1.1 DNA Methylation and Hydroxymethylation

DNA Methylation

In DNA methylation, a methyl group is chemically transferred to the carbon fifth position on the cytosine residue of a pyrimidine ring in the CpG background. DNA methyl transferases (DNMTs) utilize the S-adenosyl methionine (SAM) as a source of methyl to catalyze the covalent addition of a methyl group into the cytosine base (Figure 2) (Gangisetty & Murugan, 2016). This is most common in CpG islands, which are sequences of dense CG dinucleotide repetitions that, when methylated, can alter in gene expression by affecting the binding of transcription factors to the promoter region. A group of enzymes termed as DNA methyltransferases facilitated the DNA methylation modification. There have been descriptions of five DNA methyltransferases enzymes that are required for the establishment

and subsequent maintenance of DNA methylation which includes DNMT1, DNMT3A, DNMT3B, DNMT3L, and DNMT2, DNMT3a and DNMT3b are responsible in the *de novo* methylation to establish DNA methylation pattern during early development and DNMT1 maintains the methylation pattern over subsequent cell generations during DNA replication. In contrast to DNMT3a, DNMT3b and DNMT1, DNMT2 are required in RNA methylation (Bertogliat et al., 2020; Z. Xu et al., 2012).

DNA Hydroxymethylation

In DNA hydroxymethylation, translocase dioxygenases (TETs) are the enzymes that oxidize 5-methylcytosine (5-mC) into 5-hydroxymethylcytosine (5-hmC) (Figure 2). 5-hydroxymethylcytosine (5-hmC) is typically detected in high amounts in euchromatin and is frequently involved in transcriptional activity. The translocase dioxygenases (TETs) enzymes are directed into the methylated CpGs region of the genome and have been demonstrated to prevent the methyltransferase interaction with DNA, and further facilitate the demethylation by oxidizing 5-hmC to produce an unmethylated DNA by base excision mechanism (Bertogliat et al., 2020).

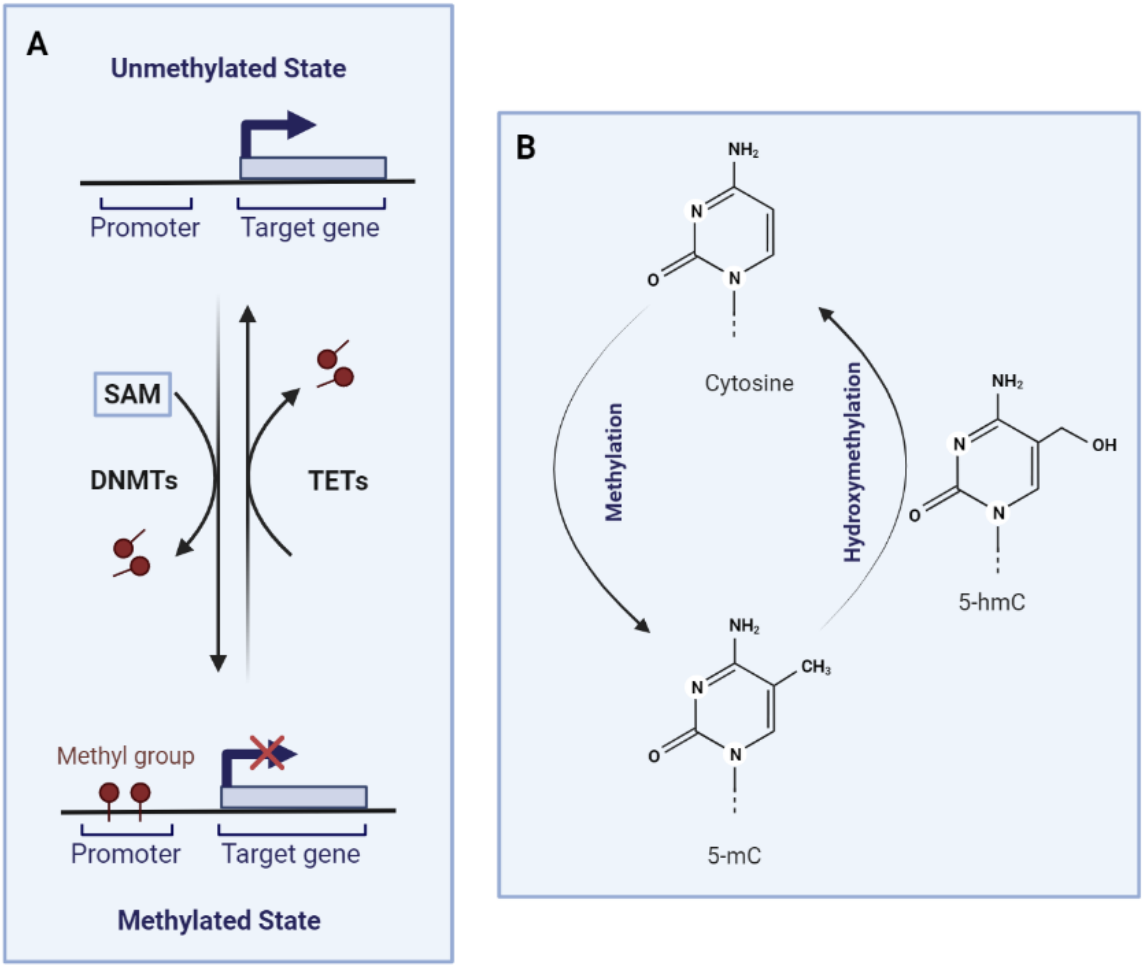

Figure 2: Methylation and hydroxymethylation of the DNA (Adapted from M. Xu et al., 2021). A) DNA methylation of the promoter region of a gene catalyzed by DNMTs inhibit the transcription activity and in hydroxymethylation, TETs catalyze the removal of the methyl group from the promoter region and restore the gene transcription. B) DNA methylation involve covalent addition of a methyl group at the fifth carbon on the cytosine base and alternatively, in DNA hydroxymethylation, the methyl group from the fifth carbon is removed to establish a demethylated cytosine base.

3.1.2 Histone Modifications

Nucleosomes, the fundamental building blocks of chromatin, are composed of DNA wrapping around the histone proteins, which are required for DNA structure and function. DNA is densely packed with histone protein octamers, each of which includes two copies of H2A, H2B, H3, and H4. In the brain, chromatin remodeling is characterized by post-translational histone modification. The N terminal tail of histones contains specific amino acids residues such as lysine, arginine, serine, and threonine that can be modified by acetylation, methylation,

phosphorylation, ubiquitination, and sumoylation. According to the location of the residue and the type of modification, these changes can lead to transcriptional activation or repression, depending on how they are formed. Thus, these makes up the histone code (Bertogliat et al., 2020; Gangisetty & Murugan, 2016).

Histone Acetylation and Deacetylation

Histone acetylation controls the positive transcription of genome. Acylation on the histone lysine residues is a well-characterized epigenetic modification. The acetylation on the histone H3 and H4 promotes open conformation of chromatin, which in turn activates gene expression. Histone acetyl transferases (HATs) and histone deacetylases (HDACs) dynamically control the acetylation of the histone residues. The histone acetylation is mostly facilitated by the histone acetyl transferases (HATs) in which this enzyme catalyze the acetyl group transfer from acetyl co-A to histone lysine residues (Figure 3). These transferred acetyl groups neutralize the positive charge on histones, thus enabling open configuration of chromatin for transcriptional activation to occur. Alternatively, histone deacetylation is the mechanism by which histone deacetylases (HDACs) enzyme catalyze the removal of acetyl groups from lysine residues at the conserved tails of the histone proteins, thus changing the charge from negative to positive. This leads to a close interaction between negatively charged DNA and positive charged histones, thereby facilitating the formation of the closes compact chromatin structure which is involved with transcriptional repression (Gangisetty & Murugan, 2016).

Histone Methylation and Demethylation

Histone methylation, based on the modified amino acid residue, is currently linked to various processes, including transcriptional activation and repression and most frequently found on arginine and lysine residues (Landgrave-Gómez et al., 2015). It mainly occurs at arginine and lysine residues as mono-methylation, di-methylation, or trimethylation. The methylation

modification of H3 and H4 amino acid side chains is mainly catalyzed by histone methyltransferases (HMTs) enzyme utilizing SAM as a methyl donor (Figure 3). The extent, symmetry, and position of histone methylation determine whether or not a gene is suppressed or expressed in a given situation. Despite the fact that methylation of the histones does not alter the overall shape of chromatin, but theses do make easier for additional proteins to regulate gene expression. Histone demethylation also associated in a major role on regulating how genes are expressed. Lysine-specific demethylase1 (LSD1) is the first known histone demethylase enzyme that can act on the mono- and di-methylations of DNA (Gangisetty & Murugan, 2016).

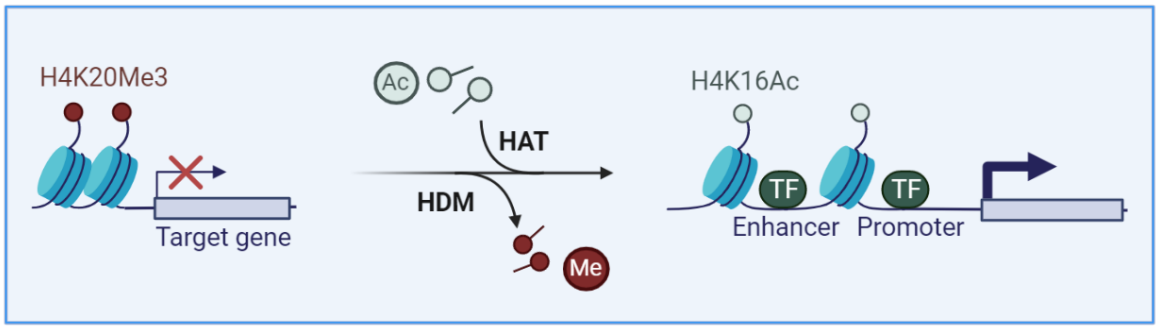

Figure 3: Histone acetylation and methylation on lysine residues (Adapted from Bannister & Kouzarides, 2011). Histone acetylation performed by HATs leads to opening of chromatin that allow transcription factors to bind on the promoter region and induce target gene expression. Conversely, histone demethylation, performed by HDMs leads to condensation of chromatin, thereby prevent the binding of transcription factor and repressing the target gene expression.

3.1.3 Non-coding RNA

Non-coding RNAs (ncRNAs) are different functioning RNAs that do not translate in proteins, instead, regulate the expression of different genes at the transcriptional and post-transcriptional level, which is why these are important to study (Figure 4). The ncRNAs that are linked to epigenetics include micro, short-interfering, circular, long non-coding and PIWI-interacting RNA, among others. MicroRNAs (miRNAs) and long noncoding RNAs (lncRNAs) are most studied two types of ncRNAs because of their roles in diseases of the central nervous system (CNS) (Bertogliat et al., 2020).

MicroRNA (miRNA) regulates the post-transcriptional expression of messenger RNA (mRNA) by binding to their 3' untranslated region (3' UTR) and degrading or silencing transcripts. The tendency of a single miRNA to function on multiple genes results in a highly complex epigenetic environment, which opens up numerous therapeutic possibilities for human disease (Bertogliat et al., 2020).

Long non-coding RNA (lncRNA), which are RNA transcripts with a length greater than of 200 nucleotides, are widely present in the brain. lncRNAs have been shown to be involved in chromatin remodeling, frequently directing different modifying proteins to particular histones or DNA sites and thus controlling gene expression. Additionally, lncRNA can involve in the formation of antisense transcripts, resulting in masking the genes and preventing the sequences from miRNA degradation (Bertogliat et al., 2020).

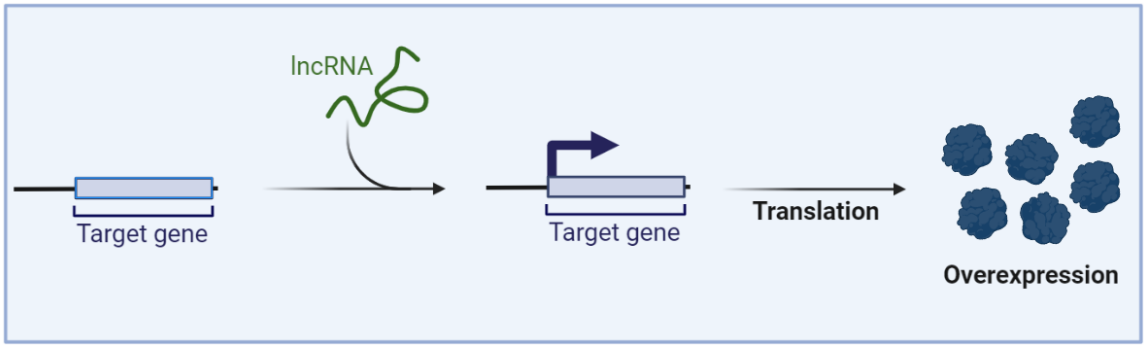

Figure 4: Non-coding RNA (ncRNA) mediated gene regulation (Adapted from Kumar et al., 2020). Non-coding RNAs including lncRNA can interact with genes or gene product and influence expression of certain proteins.

3.2 Epigenetic Regulation across Human Lifespan

Epigenetics is at the juncture from where genes and the environment interact together. It controls how genes are expressed based on the needs of the organism, which will be influenced by both endogenous factors e.g., synaptic alterations, hormonal changes, response to medication and exogenous factors e.g., physical exercise, dietary habits, stress, environment modifications (Figure 5) (Cacabelos & Teijido, 2018). Epigenetic changes are defined by a

dynamic and adaptable responses to intra- and extracellular stimulations, which can be mediated through cell-cell interaction, surrounding cells, individual physiology, or the surrounding environment exposed by the organism. Epigenetic modifiers including growth factors, cytokines, hormonal level and also stress-response and neurotropic factors are few of the causes that are influenced by the environment. In response of these epigenetic changes, enzymes add or remove epigenetic marks on the DNA and histone proteins which causes the chain reaction of changes affecting cellular function either temporarily, permanently, or with heritable modification (Kanherkar et al., 2014).

Epigenetic expression, in many ways, can be considered as genomic "software," regulating embryogenesis and individual development after birth. Studies have revealed that epigenetic modifications continues to remain 'plastic' throughout all stages of brain development and aging, and such dynamic modification continues even in neurons and other postmitotic brain elements (Jakovcevski & Akbarian, 2012). A highly modifiable epigenome allows to adapt to the changing environment and to "learn" from the incidents in life. During different phases of life, specific groups of genes are activated or suppressed by epigenetic modification, and it is these genes that are responsible for the growth and maturation of the person through a series of combined events with the environmental input. It is possible that any type of epigenetic factor affecting the transcription of genes during different phases of lifespan would lead to an impairment in the regulation mechanism, which may have a lifelong influence on the person.

While these type of adaptability toward environmental situations is advantageous, these also enables vulnerabilities to incorporate and exhibit adverse and disease-causing effects on both individual and hereditary scales. For the majority of genes, complete reprogramming is required shortly after fertilization to establish an epigenetic "clean slate" that permits different cells originating from the embryo to emerge with a cell-specific gene expression patterns and maintain appropriate differentiation. A mistake made at this critical period may result in an

aberrant phenotypic expression in the child. Many processes, including learning and physical growth continue to be influenced by internal body signals, but eventually other different external environmental and social impacts start to take place (Kanherkar et al., 2014).

Investigations on genomic imprinting have demonstrated how diet changes DNA methylation patterns and how epigenomic responses to environmental factors can affect disease development. Important nutrients like as folic acid, B vitamins, and s-adenosyl methionine (SAM) formed the methyl group which facilitates the formation of essential epigenetic changes for gene silencing. Folate and methionine deficiencies, which are often associated with cellular functions and source the methyl groups required for methylation, can cause growth factor genes to express alternatively. Study on mice offsprings on the impact of maternal folic acid supplementation demonstrated that group feeding folic acid has a different global DNA methylation level in comparison to group receiving a relatively low dose of folic acid supplements, crucial in the pathogenesis of autism spectrum disorder (Kanherkar et al., 2014).

Encounter with pesticides, poisons, pollutants, and chemical substances can cause methylation of genes and lead to the transmission of diseases in offspring years following *in utero* exposure. Heavy metals such as nickel, cadmium and arsenic are prevalent environment pollutants that are capable of altering DNA methylation as well as histone acetylation. For that reason, these have been implicated with various different diseases such as cancer, autoimmune diseases and neurological disorders among many conditions. Metals may catalyze oxidation reactions that lead to degradation of biological macromolecules, producing free radicals and inducing epigenetic alteration, according to possible mechanisms. Several recreational drugs, such as alcohol, nicotine, cocaine, opiates and amphetamines alter the epigenome by modifying methylation level in brain areas including the nucleus accumbens, which is the primary pleasure reward center. Smoking affects epigenetic modifications, such as alterations in DNA methylation, which change gene expression. Smoking cigarettes, for example, demethylate the

metastatic genes of lung cancer cells by inhibiting DNMT3B expression. Ethanol is reported for causing site-specific histone acetylation, methylation, and phosphorylation, and also DNA hypomethylation because of reducing tissue SAM level (Kanherkar et al., 2014).

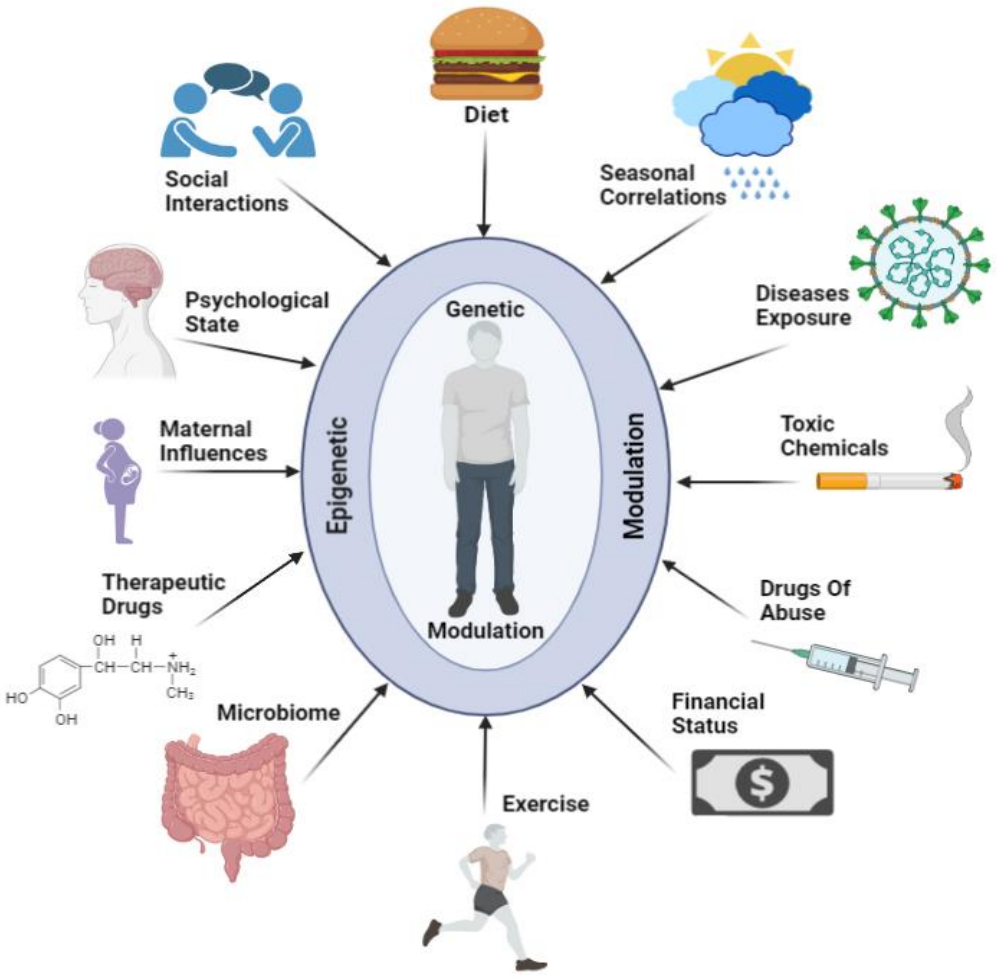

Figure 5: Epigenetic modification through different factors (Adapted from Kanherkar et al., 2014). Different factors including diet, disease exposure, toxic chemicals, therapeutic drugs and maternal health can induce epigenetic modifications and thereby affect gene transcription.

3.3 Epigenetic Modification and Diseases

Epigenetics has broadened scientific understanding of human disease by bringing additional explanation to disease pathogenesis that cannot be characterized by genetic or environmental factors exclusively (Oh & Petronis, 2021). Major epigenetic processes including DNA methylation, demethylation, histone modification, and non-coding RNA (ncRNA) are responsible for initiating and maintaining epigenetic regulation of gene expression profile. The

fact that different diseases develop when some wrong types of epigenetic marks are incorporated at the wrong moment or in the wrong position indicates the significance of epigenetic changes in maintaining the normal development in human biology (Portela & Esteller, 2010). There are many genes that encode epigenetic regulators which, if mutated, can cause a wide range of diseases (Murgatroyd, 2016). Changing the way genes are expressed, which is regulated by epigenetics, can cause autoimmune disorders, cancers, and several other diseases (Zhang et al., 2020).

It is possible for epigenetic modification to occur through either a direct or an indirect pathway. Direct modification appears to happen whenever a factor directly changes the configuration of epigenetic enzymes—either by binding to and preventing those from performing the usual function, by damaging to malfunction or by upregulating the expression. Then, the bioavailability of epigenetic enzymes changes, which leads to the wrong types of epigenetic tags being added to enhancers and promoters on a genomic scale. A direct effect of these changes could be effective across the entire genome, not affecting any particular gene but resulting in a randomly changed epigenome. Direct modification also can happen whenever a factor affects a biochemical pathway, resulting in changing availability of different substrates, intermediates, by-products, or any different component involved in the biochemical pathway that is required to form epigenetic tags. It then causes change in the availability of epigenetic tags, resulting in non-specific modification in epigenome. The second major mechanism by which factors might affect epigenetic expression is through indirect mechanisms. The indirect mechanism suggests that acute exposure from a factor alters cellular signaling networks, resulting in modified expression of receptors and growth factors and, all which affect the functioning of transcription factors. With prolonged exposure from the factors, the transcriptional and genetic regulatory proteins, results in change in gene expression, and

hence, influence the enzymes that add or remove epigenetic tags from the chromatin (Kanherkar et al., 2014).

3.4 Molecular Profiling of Epigenetic Pattern

Numerous conventional techniques are available for determining the estimated or exact methylation content of DNA. Bisulfite conversion has been the most common technique and is often considered as the gold standard due to its relatively high resolution—1 bp. In epigenetic regulation, 5mC is very common form of DNA methylation, and bisulfite technique depends on treating genomic DNA with the sodium bisulfite, which eventually result in the deamination of unmethylated cytosines to uracil while keeping the methylated cytosines unchanged, allowing 5mCs to be differentiated from unmethylated cytosines through the genomic DNA. Additionally, DNA methylation can be determined using techniques that require enrichment of methylated DNA and then analyzing the enriched sequences using a different technique like microarrays and sequencing. For the enrichment of methylated DNA, immunoprecipitation with antibody (i.e., methylated DNA immunoprecipitation) can be performed that can recognize methylated cytosines selectively (Li, 2021; Qureshi & Mehler, 2011).

Different techniques are available for assessing histone modifications and proteins associated with chromatin configuration, as well as assessing the nucleosome dynamics and chromatin accessibility. These methods are based mostly on enrichment of genomic sequences that is correlated with specific chromatin configurations using chromatin immunoprecipitation (ChIP), an effective technique for determining interactions between DNA and protein. In ChIP, an antibody recognizes a specific histone modification which work as an epitope. Then, the DNA sequences which is present in ChIP-enriched DNA can be analyzed to construct a map showing the histone modification is throughout the genome. When ChIP-sequencing combined with 3C-based techniques such as Hi-C, 3D chromatin architecture may be

projected, showing the correlation between histone modification patterns and the 3D spatial configuration of chromosomes of the cell (Li, 2021; Qureshi & Mehler, 2011).

Non-coding RNAs (ncRNAs) have been identified and studied in a number of biological samples, mostly in body fluid and neural tissue. Microarrays or next-generation sequencing (NGS) are currently used in large-scale profiling of ncRNAs. Additionally, fluorescent *in situ* hybridization (FISH) can be applied to analyze the origin of RNA in tissue samples specifically. During the initial discovery stage, techniques such as qRT-PCR and northern blotting were used for validating primary findings (Snijders et al., 2018).

Chapter 4

Epigenetic Dysregulation in Neurodegenerative Diseases

4.1 Epigenetic Dysregulation in Alzheimer's Disease

4.1.1 Pathogenesis of Alzheimer's Disease

The amyloid-cascade hypothesis indicates that amyloid beta (Aβ) initiates the pathogenic pathway in Alzheimer's disease (AD) that results in neurodegeneration and accumulation of amyloid peptide. The amyloid precursor protein (APP), a transmembrane protein, is synthesized by different types of cells. In the CNS, APP can be cleaved sequentially following two separate pathways. The first, non-amyloidogenic pathway begins with the cleavage of APP by α-secretase, producing an extracellular secreted protein termed as sAPPα and a membrane-bound C-terminal 83 amino acid fragments known as C83. Then γ-secretase cleavage the C83 and leading to formation of a different secreted fragment called as p3 and leaves the membrane-bound APP intracellular domain (AICD). In the second, amyloidogenic pathway, β-secretase, or BACE-1 (β-site APP cleavage enzyme 1) first cleavages APP, producing extracellular secreted product called sAPPβ and the membrane-bound C-terminal 99 amino acid fragments called C99. C99 is then processed by γ-secretase, leading to the extracellular secretion of Aβ and same membrane-bound APP intracellular domain (AICD). Increased production or decreased clearance of Aβ, which is normally soluble, causes Aβ to self-assemble into oligomers form and subsequently into highly regular amyloid fibrils that form the plaques. The length of Aβ peptide generated by γ-secretase cleavage of C99 ranges between 37 to 42 amino acids. The longer forms (Aβ40 and particularly Aβ42) are more likely to self-aggregation and fibril formation than the shorter versions. Soluble Aβ and amyloid plaques both are toxic, and assumed to cause effect on cellular activities by secondary occurrences for instance

hyperphosphorylation of the tau protein, which leads to the formation of neurofibrillary tangles (NFTs), excitotoxicity, oxidative stress, and inflammation (Soria Lopez et al., 2019).

Alzheimer's disease has been also linked with mutations in the APP and presenilin (PS) genes PS1 and PS2. PS1 and PS2 genes are associated with γ-secretase proteins which is related to the production of Aβ peptide from APP. Any mutations in the PS genes cause the production to shift towards Aβ42, which is the longer isoform of Aβ that is more likely to aggregate than the shorter isoform Aβ40, by increasing the production of Aβ42, or reducing the production of Aβ40. Certain mutations in the PS and APP genes appear to have a significant role in disease pathogenesis, particularly the e4 allele of the apolipoprotein E (APOE) gene, which confers the highest risk in the population. Another gene associated to Alzheimer's disease, is the CLU gene, which considered to cause the aggregation and clearance of Aβ. Apart from that, dysfunctional tau is also being associated with the pathogenesis of AD. In Alzheimer's disease, tau, a microtubule-related protein which facilitates assembly of microtubule, is being hyperphosphorylated. As a result, it dissociates from microtubules and aggregates, causing disorganizing cytoskeletal, neuronal dysfunction, and cell death that is implicated in the pathology in Alzheimer's disease (Lardenoije et al., 2015).

4.1.2 DNA Methylation in AD

The significant component of senile plaque is amyloid β protein (Aβ). Aβ is produced by the sequential proteolytic degradation of amyloid precursor protein (APP) by β-secretase (BACE) and γ-secretase (presenilin). Aβ40 and Aβ42 is the result of γ-secretase cleavage where Aβ42 has been associated in neurotoxicity. The APP, presenilin 1 (PS1), and BACE genes are all associated in familial AD (fAD). The APP protein was found to be significantly increased in AD patients, and it was established that the promoter site of the APP gene is hypomethylated, that appears to increase Aβ synthesis. *In vitro*, BACE and PS1 gene expression was increased

following folate deficiency-induced hypomethylation and returned to regular levels when folate deficiency was combined with SAM supplementation. These observations could indicate that a reduction in the DNA methylation level of the APP, PS1, and BACE genes promotes subsequent transcription. When combined, these factors increase Aβ production and deposition, leading to the development of senile plaque. Additionally, Aβ has exhibited to cause genome-wide hypomethylation on *in vitro* cell cultures. Furthermore, the Aβ-induced genome-wide hypomethylation may have functional impact for other critical AD causing genes, leading to additional synergisms. For example, hypomethylation enhances the expression TNF-α and caspase-3, thus increasing the production of TNF-α and caspases resulting in enhancement of Aβ expression, potentially leading to the emergence of new adverse cycles (J. Wang et al., 2013).

Neurofibrillary tangles, which are bundles of intraneuronal paired helical filaments (PHF) are another pathological feature of Alzheimer's disease. Usually, the main structural element of NFTs is normally constituted cellular microtubules, but in AD, which changes to an abnormal phosphorylated structure known as tau protein. Tau, which is a phosphoprotein of the microtubule associated family, whose primarily responsible for maintaining the stability of microtubules. When tau is hyperphosphorylated in AD, it forms aggregates that reduce the ability to bind microtubules, resulting in degeneration of cytoskeleton and neuronal death. Hyperphosphorylated tau led to the generation of NFTs. This phosphorylation reaction is dependent on the cytoplasmic methylation reaction. There is evidence that a high homocysteine (Hcy) level causes hyperphosphorylation of tau, NFT formation and SP generation by inhibiting the methyltransferases and hypomethylating the protein phosphatase 2A (PP2A), that dephosphorylate the phosphorylated tau (J. Wang et al., 2013).

Clusterin gene (CLU) is the third most commonly linked LOAD risk gene, according to studies. The evidence suggests that CLU gene possesses a CpG-rich methylation region in the promoter

site and does have a major effect on the pathogenesis of AD by Aβ aggregation, clearance, thereby deposition. CLU levels have been identified to be higher in the cerebrospinal fluid and brain of patients with AD. Plasma CLU level was recently found to be linked to brain atrophy, rapid clinical progression and baseline disease severity in AD patients (J. Wang et al., 2013).

4.1.3 Histone Modifications in AD

Histone modifications are significant in neuronal development and also in the pathogenesis of Alzheimer's disease. Histone acetylation dysregulation has been linked to a variety of abnormalities in signaling, proliferation, neuronal plasticity, apoptosis, inflammation, and immunity. In the postmortem brains of patients with AD, 4162 different acetylated marks have been found in H3K27. Many of these acetylation was found in genes linked to AD pathology including APP, PSEN1, and PSEN2. Reduced histone acetylation was discovered in the temporal lobes of AD patients. In particular, reduced levels of acetylated lysine 16 on histone H4 (H4K16ac) has been found in the brain of AD patients which was involved in causing DNA damage and accelerated aging in comparison to aged healthy controls. In animal studies, areas associated with plasticity displayed reduced acetylation of lysine 27 on histone H3 (H3K27). Additionally, increased levels of histone deacetylases (HDACs), particularly Class I HDACs (HDAC2 and HDAC3), the enzymes that catalyzing the removal of acetyl groups and suppress transcriptional activity by compacting chromatin, have been identified in certain brain areas involved in neuroplasticity, memory and learning in AD patients, and are involved in reduced cognitive and synaptic activity. HDAC6 levels were observed to be increased in the hippocampus and cortex of patients with AD, and also in animal models of the disease. The HDAC6 enzyme has been involved in tubulin acetylation, with tau phosphorylation and degradation as well as in the inflammatory processes. Reduced level of HDAC6 enzyme result in increased clearance of tau, which may lead to and reduction of tau aggregation and neuronal survival. However, overexpression of HDAC6 enzyme lowers the acetylation level of α-

tubulin, which impairs microtubule stability, vesicular transport, and mitochondrial transport. HDAC4 could also be significant in nerve function because increased levels cause apoptosis and inhibition prevents neuronal cell death. Class III HDACs, also known as sirtuins, are associated with the pathogenesis and play an essential part in memory and synapse formation. In AD patients, SIRT1 levels were further found to be lower in the parietal cortex of the brain. These changes have been linked to the accumulation of Aβ and tau, along with tau acetylation of lysine 28, which resulting in significant tau aggregation. Along with acetylation, AD has been linked with changing histone methylation level in postmortem brains of patients with AD, higher trimethylation of lysine on histone H3 (H3K9) were observed which is involved in gene silencing, as well as condensing of heterochromatin structure. Additionally, higher phosphorylation of serine on histone H3 (H3S10) has also been found in the neurons and astrocytes of the hippocampus, indicating DNA damage (Nikolac Perkovic et al., 2021).

4.1.4 Non-coding RNAs in AD

Increasing APP expression causes a rise in Aβ production and, that lead to the pathogenesis of Alzheimer's disease. APP has been investigated extensively for miRNA target gene, and different miRNAs that control directly the APP mRNA expression have been found through *in vitro* studies. MiR-101 has been identified to be prevalent in the anterior temporal cortex of the human brain, providing evidence that it has a significant function in the regulation of APP expression and Aβ production in AD. Inhibiting miR-101 overexpression lead to a significant decrease in APP and Aβ load in neurons of the hippocampus. MiR-16 also target APP in order to possibly impact the progression of AD mice, and miR-16 overexpression both *in vitro* and *in vivo* resulted in decreased expression of APP protein. miR-124 expression was decreased in patients with AD, showing the significance of miR-124 pathway for regulating APP splicing in neurons. Further research revealed that miR-124 controlled the BACE1 expression, which is highly associated with cell death caused by Aβ neurotoxicity. Additionally, suppressing or

overexpressing of miR-124 could increase or decrease BACE1 expression, accordingly (J. Wang et al., 2013).

The rate-limiting enzyme in Aβ production, β-secretase BACE1, is important for normal synaptic, cognitive, and emotional functions. miRNAs including miR-9, miR-29a/b-1, miR-29c, miR-107, miR-298, -485-5p as well as lcRNAs including BACE1-antisense (BACE1-AS) have been demonstrated to regulate BACE1 mRNA expression. BACE1 levels as well as Aβ level was decreased by overexpressing transient miR-29a/b-1. miR-107 was identified to be downregulated at different stages in the AD pathogenesis, indicating that miR-107 might contribute significantly to accelerating AD pathogenesis through regulating BACE1. Finally, miR-298, miR-328, and miR-195 have been shown to be inversely associated with the BACE1 protein production and reduce the level of Aβ in neuronal cultured cells by blocking BACE1 translation. Similarly, miR-125 expression was lowered while BACE1 expression was higher in neuronal cultured cells (J. Wang et al., 2013).

Several studies have discovered that miRNAs contribute significantly to the metabolic activities of Tau, contributing significantly to many neuropathological characteristics of AD. The miR-15 class might affect the expression of extracellular signal-regulated kinase 1 (ERK1) in mouse neural cells, which is a direct tau kinase, and decreasing miR-15 levels may have caused to hyperphosphorylation of neuronal tau. GSK-3', another tau kinase protein, is essential for Aβ synthesis and NFT development. miR-26a directly suppresses the GSK-3' mRNA in smooth muscle, which is modulated by BDNF. And miR-26a expression is changed in AD. It is well known that SIRT1 could deacetylate tau, and so its deficiency promotes Tau acetylation and leads to the deposition of hyperphosphorylated Tau in AD. All three miRNAs, miR-9, -34c, and -181c, have been designed to strongly suppress SIRT1 mRNA and to show related dysregulation through AD brain (J. Wang et al., 2013).

4.2 Epigenetic Dysregulation in Parkinson's Disease

4.2.1 Pathogenesis of Parkinson's Disease

Patients with Parkinson's disease (PD) are mainly affected with motor complications including tremor, bradykinesia, rigidity, and gait disturbances. These motor complications are further followed with psychiatric complications including, cognitive dysfunctions such as dementia, sleep disturbances, and autonomic impairments. The cause of these psychiatric complications is associated with the malfunctioning neurotransmitters of serotonergic, cholinergic and noradrenergic systems (Lardenoije et al., 2015).

The major pathological cause of Parkinson's disease is decreasing of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Clinical and pathological correlative research has demonstrated that moderate to severe loss of dopaminergic neurons within the substantia nigra pars compacta area is associated with motor complications, bradykinesia and rigidity in particular, in the advanced phase of Parkinson's disease. A common hallmark of neurodegenerative diseases is the aggregation of misfolded protein which is also true for Parkinson's disease. Lewy pathology is associated with the critical feature of Parkinson's disease. Lewy bodies are protein aggregation found in the cytoplasm that are primarily composed of α-synuclein, parkin, and ubiquitin. In a misfolded state, the protein is insoluble and form clumps with each other to create intracellular inclusions in the cell body (Lewy bodies) and processes (Lewy neurites) of neurons, which are difficult to remove. Lewy pathology is not confined to the brain only but also affects the spinal cord and peripheral nervous system, which includes sympathetic ganglia, vagus nerve, enteric nervous system, cardiac plexus, salivary glands, sciatic nerve, cutaneous nerves, and adrenal medulla (Kalia & Lang, 2015).

Synuclein alpha (SNCA), the gene that express the presynaptic protein α-synuclein, is among the main risk genes for Parkinson's Disease; overexpression of SNCA gene can only lead to familial Parkinsonian symptoms by point mutations and also multiplications. Along with SNCA, Parkinson disease 16 (PARK16), microtubule-associated protein tau (MAPT), and leucine-rich repeat kinase 2 (LRRK2) are identified as risk genes, in specific SNCA and MAPT SNPs imparting the most risk (Lardenoije et al., 2015).

4.2.2 DNA Methylation in PD

Numerous factors, particularly those associated in one-carbon metabolism, can affect the process of DNA methylation. Folates and vitamins are required for homocysteine to be transformed to methionine and subsequently to SAM, a universal methyl donor. After donating a methyl group, SAM is converted to S-adenosylhomocysteine (SAH) and then to homocysteine. In an animal model of Parkinson's disease, inadequate folate level and increased homocysteine are being observed to be damaging to dopaminergic neurons. Furthermore, vitamin B12 and folic acid level have been observed to be lower in some Parkinson's patients. Therefore, dysregulation of one-carbon metabolism could lead to abnormal DNA methylation, which may lead to the pathogenesis of Parkinson's disease. An increased homocysteine level has long been assumed to be a potential risk for several neurodegenerative diseases, including Parkinson's Disease. Higher level of homocysteine causes oxidative stress, which results in the generation of radicals, dysfunction in mitochondria and cell apoptosis ultimately. Increased homocysteine levels may potentially result in higher mutation level as a result of oxyradical-linked DNA damage (Feng et al., 2015).

DNA methylation regulates the activity of alpha-synuclein (SNCA), which is one of the most critical risk genes for Parkinson's disease. In patients with Parkinson's disease, hypomethylation has been observed in intron 1 of SNCA which may lead to its overexpression.

Additionally, PD patients had significantly lower levels of methylation at CpG-2 locations in the SNCA promoter in compared with controls. Moreover, decreased nuclear DNMT1 level has been observed in brains of SNCA animal model as well as in the postmortem brain with PD thus providing insight into the molecular process that cause decreased methylation in SNCA. DNMT1 is most often located within the nucleus of neurons and when SNCA accumulates and aggregates in neurons, SNCA affect the normal nuclear passage of DNMT1, leading to the delocalization of DNMT1 in the subcellular areas. Moreover, with decreased level of DNMT1, global hypomethylation of genes was found in brains of patients with PD (Feng et al., 2015; Rathore et al., 2021).

The microtubule-associated protein tau (MAPT), that encodes tau protein and helps in the stabilizing the axonal cytoskeleton, is one of the major risk factors not just in Alzheimer's disease but also in Parkinson's disease. Hypermethylation in the MAPT gene could be associated in the pathology of Parkinson's disease by modifying the expression of MAPT gene and thus affecting the stability of the axonal cytoskeleton. Moreover, tumor necrosis factor alpha (TNF-α), an inflammatory response factor, is also associated in the pathogenesis of Parkinson's disease (PD). In PD patients, the TNF-α promoter has identified to be hypomethylated, showing an increased sensitivity of neurons to TNF-mediated inflammation located in the SNpc area (Feng et al., 2015).

4.2.3 Histone Modifications in PD

PD is defined by the aggregation of SNCA within presynaptic nerve terminals of dopaminergic neurons in substantia nigra (SN) of the brain. The neurotoxic effects of SNCA could be mediated by altering with the acetylation level of histones. An enriched pattern of histone 3 lysine 27 acetylation (H3K27ac)-enriched has been also discovered at the SNCA locus (Feng et al., 2015; Rathore et al., 2021).

In a new study, it was found that acetylation levels on histone H2A, H3, and H4 were observed to be increased in dopaminergic neurons collected from PD patients compared to healthy controls. Furthermore, research findings have shown that α-synuclein accumulation promotes histone H3 hypoacetylation. It has been observed that while histone acetylation controls α-synuclein production, α-synuclein can also control histone acetylation through the feedback mechanism. Thus, in Parkinson's disease, synuclein accumulation can cause neurotoxicity and ultimately cell death by concealing histone proteins and thus preventing histone acetylation, chromatin compaction, and gene expression. Histone deacetylase 6 is critical in forming misfolded protein aggregates. This enzyme is present in the Lewy bodies of PD pathogenesis and associate in the neuroprotection of dopaminergic neurons. Thus, the histone deacetylase 6 could promote the formation of synuclein inclusions, protects dopaminergic neurons from α-synuclein toxicity and any mutations in histone deacetylase 6 related gene could lead to the aggregation of toxic synuclein oligomers (Feng et al., 2015; Rathore et al., 2021; Renani et al., 2019).

Environmental toxins such as dieldrin that are implicated with the pathogenesis of Parkinson's disease, were shown to increase H3 and H4 acetylation over the time. Another neurotoxin associated in the pathogenesis of Parkinson's disease, paraquat, is also linked with hyperacetylation (Feng et al., 2015).

Also, the decrease in dopamine levels discovered in PD patients is linked with lower levels of trimethylation at lysine 4 in histone H3 (H3K4me3). A study discovered that vulnerability in an area of the substantia nigra of brain was caused by hypomethylation within the TNF gene. In PD brain, increased level of TNF-α overexpression induce apoptosis in nerve cells (Renani et al., 2019).

Additionally, histone modifications also regulate a number of other genes associated with Parkinson's disease. When the PINK1 protein interacts with the transcriptional repressor HDAC3, the neuronal cells undergo phosphorylation, which increases deacetylation of the histone. Moreover, α-synuclein inhibits PARP1 protein significantly by initiating nitric oxide synthase and producing nitric oxide (Rathore et al., 2021; Renani et al., 2019).

4.2.4 Non-coding RNAs in PD

The effect of miRNA in the dopaminergic neurons revealed that miR133b is expressed specifically in these neurons and regulates the maturation and function of dopaminergic neurons in the midbrain. In Parkinson's disease, this miRNA was discovered to be significantly downregulated in the midbrain tissue of PD patients (Renani et al., 2019).

miR132 is another miRNA linked with dopaminergic neuron differentiation in the midbrain. In the animal model of PD, increasing miR132 expression decrease the production of the target protein, nuclear receptor related protein 1 (Nurr1). Nurr1 is a nuclear receptor transcription factor which plays a key function in the neuroendocrine regulation of the hypothalamic-pituitary-adrenal axis. Nurr1 is frequently present in the central nervous system, particularly in the substantia nigra, and study suggests that Nurr1 is required for dopaminergic neurons to develop, migrate, and survive (Renani et al., 2019).

Certain miRNAs can also regulate α-synuclein expression. miR7 is expressed at a 40-fold level higher in neurons than in other cells. miR7 reduces α-synuclein protein levels by binding to synuclein mRNA. Additionally, miR153, in combination with miR7, can control α-synuclein expression by 3′UTR and inhibit the activity related to mRNA. Also, miR-106a has been shown to increase levels of synuclein protein (Renani et al., 2019).

The transcription of microRNAs (miRNAs) in the brain cells of PD patients has revealed that miR34b and miR34c have been reduced in PD patients, which has been linked by a decrease in the transcription of two important PD genes, PARK2 and PARK7 (Renani et al., 2019).

miR205 is reported to be an important regulator of the LRRK2 gene, and existing research had revealed a considerable reduction in miR205 levels in the corpus striatum and frontal cortex in patients with PD. The inhibition of miR205 is also linked to increase in the expression of the LRRK2 gene. MiR205, in addition, has therapeutic effects, as it has been shown to decrease the expression of LRRK2 gene. In addition, several studies found that the levels of miR-10a, 10b, 212, 132, and 495 had altered in patients with PD in compared with controls group (Renani et al., 2019).

4.3 Epigenetic Dysregulation in Huntington's Disease

4.3.1 Pathogenesis of Huntington's Disease

Huntington's disease is an autosomal-dominant neurodegenerative disorder characterized by increasing number of cytosine-adenine-guanine (CAG) repeats in the coding region of the Huntingtin (HTT) gene. HD is caused by CAG repeat lengths that are longer than 40. CAG repeat lengths below 35 are usually thought to be harmless and lengths of 36-39 repeat demonstrate incomplete penetrance. The normal Htt protein is mostly a cytoplasmic soluble protein, but the polyglutamine-expanded mutant Htt (mHtt) and the N-terminal fragments are mostly found to form insoluble protein aggregates in the nucleus. The Htt/mHtt protein is present across the CNS and body, but the most significant neuropathological effects are found in the striatum. As the disease progresses, neuronal death occurs in different parts of the striatum. Chorea is the most identifying movement symptom of HD and defined by abnormal, involuntary movements that occur in unpredictable ways throughout the body. Psychological

symptoms such as anxiety, depression, social withdrawal, and impulsivity are frequently present and contribute significantly to the overall diseases (Thomas, 2019).

4.3.2 DNA Methylation in HD

In striatal cells of a mouse model with HD, the consequences of mutant HTT expression level in DNA methylation was investigated and found that the majority of region were hypomethylated, and the genes with altered methylation have been linked with neurogenesis, neuron differentiation, and neuronal signaling (Lardenoije et al., 2018).

An increased level of global methylation was found in tissue from the frontal and parietal cortex of HD patients in compared with the control group patients. *In vitro* mouse striatal cells with expressing full-length huntingtin (HTT) demonstrate that promoter methylation results in reducing the expression of multiple genes involved in controlling neuronal developmental, migration, and cell signaling (Bertogliat et al., 2020).

HD pathogenesis has been linked to transcriptional dysfunction. A genome-wide study in cell lines derived from mouse striatal neurons have demonstrates that presence of mHTT affect the methylation of transcriptional regulators in region with low CpG content (Mohd Murshid et al., 2020). The promoter locations of transcriptional regulatory factor, which include Ap-1, Sox2, Pax6, and Nes genes, are heavily methylated, thus significantly reducing their expression levels. Due to the direct involvement of these genes in neurodevelopment, DNA methylation-mediated impairment of hippocampal neurogenesis may be directly associated to cognitive decline in patient with HD (Lardenoije et al., 2018).

A correlation has been discovered between global hypermethylation and the pathogenesis of disease within the cerebral cortex region of brain. Reduction in 5-hmC level has also been observed genome-wide in a mouse model of HD, most notably in the striatum and cerebral cortex (Bertogliat et al., 2020).

4.3.3 Histone Modification in HD

The initial evidence linking mHtt and histone acetylation indicated that mHtt might bind to the histone acetyltransferase domain from the CREB-binding protein, resulting in hypoacetylation of histones and altered gene expression in the *in vivo* and *in vitro* model of HD (Thomas, 2019).

Furthermore, HD mouse models with impaired cognitive function had globally reduced acetylated histone H3. Reduced histone acetylation was observed at particular gene loci, most notably the promoter parts of genes known to have downregulated in HD, including Penk1, Drd2, Grin1, and Actb with hypoacetylation on the histones H3 and H4 implicated in the animal model. Various studies using genome-wide techniques identified numerous of loci in the striatum of animal model that that have been hypoacetylated particularly on histone 3 at lysines 9 and 14 (H3K9, H3K14) (Thomas, 2019).

A few studies on histone phosphorylation have found abnormal phosphorylation level on histone H3 in HD brain tissue. It was further shown that primary striatal neurons transfected with mutant form of HTT gene had reduced levels of histone H3 phosphorylation, particularly at serine 10. The transcriptional deregulation reported in HD has been linked to altered histone monoubiquitylation, which is controlled by mHtt (Thomas, 2019).

4.3.4 Non-coding RNAs in HD

Normally, it is hypothesized that HTT interacts with the repressor element 1 silencing transcription factor (REST), thereby reducing its nuclear translocation into neurons. Mutant HTT, on the other hand, cannot, resulting in increased nuclear REST levels. As REST regulates a number of genes, including several miRNAs, increased nuclear REST levels result in widespread dysregulation in gene expression. Among the miRNAs affected by REST, it indicates that neuron-specific miR-132 expression was decreased in HD patients, whereas miR-29a and miR-330 levels were enhanced. Additionally, miR-132 was downregulated in two

animal models of HD, as were miR-22, miR-29c, miR-128, miR-138, miR-218, miR-222, miR-344, and miR-674 (Lardenoije et al., 2018).

In vitro studies in STHdhQ111/HdhQ111 cells demonstrated the mutant HTT (mHTT) can decrease miR-125b, miR-146a, and miR-150 expression, however miR-146a suppression is an effect of higher level of cellular tumor antigen p53, which is the target of miR-125b and miR-150. Increased levels of p53 promote p65-mediated transcription factor suppression, thereby decreasing miR-146a expression. Since the HTT gene contains several p53 responsive factors, it has been hypothesized that higher p53 levels could result in enhanced HTT levels, mitochondrial malfunction, and subsequently neurodegeneration (Lardenoije et al., 2018).

Chapter 5

Epigenetics-Based Therapeutic Interventions in Neurodegenerative Diseases

5.1 Epidrugs: Targeting Epigenetic Modifications

The epigenomic flexibility has established a promising argument for exploring the reversion in epigenome by therapeutic interventions as a strategy for improving disease phenotypes (Ganesan et al., 2019). Epigenetic enzymes are essential for many cellular and biochemical functions throughout life, and their dysregulation leads to various diseases. Given that the modifications facilitated by epigenetic enzymes can be reversible, controlling the activity of such epigenetic modifying enzymes may be a promising therapeutic option for some of the disorders (Singh et al., 2018). Epigenetic drugs or epidrugs are chemical substances that alter the epigenetic marks in the DNA and histones by controlling the enzymes involved in epigenome modification, thereby, regulating chromatin remodeling and gene expression (Miranda Furtado et al., 2019).

Several epigenetic based drugs have been approved by the US FDA and are currently being used to treat different diseases (Miranda Furtado et al., 2019). Cytidine related compounds decitabine and azacitidine functions as DNA methyltransferase inhibitor (DNMTi) and was initially approved for the treating of myelodysplastic syndrome. Additionally, hydralazine and procainamide were approved for the treatment of hypertension and arrhythmia respectively. Vorinostat and romidepsin, two histone deacetylase inhibitors (HDACi), have been approved by the US FDA for treating cutaneous T-cell lymphoma whereas panobinostat and belinostat were approved for peripheral T-cell lymphoma and multiple myeloma treatment. DNMTi and HDACi compounds represent the therapeutic potential of the 'first generation' of epigenetic drugs available for clinical treatment. Significant advances has been made in the development

of the 'second generation' epigenetic drugs, which are novel molecular inhibitors of additional epigenetic enzymes and associated proteins, such as histone acetyltransferases (HATs), histone methyltransferases (HMTs), proteins binding to acetylated histones (PAHs) and proteins binding to methylated (PMHs) (Y. Qi et al., 2016). Moreover, newly developed drugs are being constantly evaluated for pharmacological action and cytotoxicity to understand their activity in the preclinical and clinical studies and develop safe and effective epigenetic therapy (Miranda Furtado et al., 2019). Some of the epigenetic drugs targeting different enzymes have been presented in Table 1 and Figure 6.

Table 1: List of different categories of epigenetic drugs (Adapted from Berdasco & Esteller, 2019; Y. Qi et al., 2016; Singh et al., 2018).

Category	Epigenetic Drugs
DNMTi	Decitabine, Azacytidine, Procainamide, Procaine, Epigallocatechin-3-gallate (EGCG), Zebularine, Hydralazine, Guadecitabine, 4′-thio-2′-deoxycytidine (TdCyd)
HATi	Curcumin, C-646, PU-139, Anacardic Acid, Garcinol
HDACi	Trichostatin A, Vorinostat, Tefinostat, Givinostat, Panobinostat, Chidamide, Belinostat, Entinostat, Romidepsin, Valproic acid, Plitidepsin, Tasquinimod, Sodium butyrate, CG-1521, SB939, CHR-2845, CHR-3996, CUDC-907, ACY 241, AR-42, KA2507, MPT0G009, ORY-2001, 4-phenylbutyrate, Pivanex, Resveratrol, Abexinostat, Resminostat, Dacinostat, Droxinostat, Tubastatin A, Abexinostat, Mocetinostat, Givinostat, Quisinostat, Pracinostat
HDMi	Pargyline, Clorgyline, Bizine, GSK2879552, GSK-J4, KDM5-C70, JIB-04, EPT-103182, Tranylcypromine. Phenelzine, INCB059872, ORY-1001, IMG-7289, ORY-2001
HMTi	Allantodapsone, BIX-01294, EPZ-6438, GSK126, GSK3235025, GSK332659, CPI360, DZNep, GSK343, EI1, UNC0638, EPZ004777, UNC0224, Tazemetostat, Ellagic acid, MAK683, Pinometostat, DS-3201b, GSK2816126, GSK3326595, CPI-1205

DNA Methyltransferase Inhibitors (DNMTi)

DNA methylation is regulated by DNMTs enzyme family, which comprises DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L. DNA methylation alterations (e.g., hypermethylation and hypomethylation) have mostly been linked to cancer, genetic mutations,

neurological, and immunological disorders. Therefore, DNMTs have gained much importance as a therapeutic target, with the discovery of many small-molecule inhibitors that specifically targets the DNMT family. The FDA has approved two DNMT inhibitors from the nucleoside-based cytidine chemotype: 5-aza-cytidine (Azacitidine or Vidaza) and 5-aza-2-deoxycytidine (Decitabine or Dacogen) for the treatment of myelodysplastic syndrome. These nucleoside analogs bind to DNA and form an irreversible complex with the DNMTs, causing the DNMT enzymes to degrade and resulting in decrease in DNA methylation. The number of chemical compounds tested as DNMT inhibitors has increased in recent years, and many of the significant non-nucleoside analogs that target DNMT are being investigated, including hydralazine, procainamide, epigallocatechin 3-gallate (EGCG), SGI-110, and RG108. The inhibitors from non-nucleoside oligonucleotide do not integrate into the DNA but instead bind with DNMTs directly, at either the 3′ UTR, resulting in transcriptional suppression, or at the enzyme active site, decreasing their enzymatic reactions (Prachayasittikul et al., 2017; Singh et al., 2018).

Histone Acetyltransferase Inhibitors (HATi)

Histone acetyltransferases (HATs) previously has been initially identified as tumor suppressor regulators and have since been correlated with a number of disorders, which include the cancer progression, viral infection, and some pulmonary diseases. Numerous basic lysine and arginine residues could be located in the N-terminal tails of histone proteins. To transform lysine residues to the associated acyl-lysine residue, histone acetyltransferase (HAT) enzymes utilize acyl coenzyme A donors. There are approximately 20 human HATs that range through nuclear as well as cytoplasmic distribution. The key reaction associated with HATs is the lysine acetylation, which is associated with increasing the side chain size, and these acetylation reactions occur in the histones as well as in the non-histone proteins on the nucleus and other cellular sites. The first step in designing and developing HATi is to select molecules that

closely features the lysine substrate and acetyl-coA complex that is formed in the active site of those enzymes during the acetylation reactions. This was performed by covalently connecting the acetyl-CoA cofactor to a lysine residue in the histone peptide substrate. Curcumin, anacardic acid and garcinol are three natural compounds that have been documented as HAT inhibitors (Ganesan, 2018; Prachayasittikul et al., 2017; Singh et al., 2018).

Histone Deacetylase Inhibitors (HDACi)

Deacylation reaction is the reversal of lysine acylation, which is performed using two separate groups of enzymes: zinc-dependent histone deacetylases (HDACs) and sirtuins (Sirts). Biologically, HDACs and sirtuins transform acyl-lysine residues back to their initial protonated lysine. This causes chromatin compaction and gene silencing in the nucleosome. Much of the involvement in the inhibition of such enzymes ensures reprogramming by reactivating previously suppressed pathways in DNA repair, immunomodulation, tumor suppression and apoptosis. There are around 18 HDACs defined in organisms, which have been classified into four structurally and phylogenetically different classes, which included class I, IIA, IIB, and III. These enzymes play a significant part in a different biological processes, such as differentiation, proliferation, apoptosis, and senescenc (Ganesan, 2018; Prachayasittikul et al., 2017; Singh et al., 2018).

Different HDACi chemotypes have been discovered, including those based on short chain fatty acids (e.g., valproic acid, sodium butyrate, phenylbutyrate, pivanex), cyclic tetrapeptides, and natural compounds, bicyclic depsipeptide (e.g., romidepsin) and those based on selective classes consisting of hydroxamic acids (e.g., vorinostat, panobinostat, belinostat, and dacinostat). The majority of compounds currently undergoing clinical trials are hydroxamic acid analogs. Hydroxamic analogs have been clinically effective in the first instance in

combination with the FDA-approved drug vorinostat (Prachayasittikul et al., 2017; Singh et al., 2018).

Histone Demethylase Inhibitors (HDMi)

Modification of histone methylation is considered to be essential in epigenetic regulation and has significant therapeutic possibility for diseases such as cancer and genetic disorders. Lysine demethylases (KDMs) are a group of oxidative enzymes that remove methyl lysine residues. There are two subfamilies of KDM demethylases with distinct function: LSDs and Jumonji C demethylases. Humans possess two LSD isoforms, including LSD1 (KDM1A) and LSD2 (KDM1B), which are dependent on the enzyme flavin adenine dinucleotide (FAD). The LSD1/KDM1 demethylases are sequentially and structurally similar to amine oxidases and monoamine oxidases and monoamine oxidases inhibitors such as tranylcypromine phenelzine, and pargyline also inhibit the HDM KDM1A (Ganesan, 2018; Prachayasittikul et al., 2017; Singh et al., 2018).

Histone Methyltransferase Inhibitors (HMTi)

Histone/protein methyltransferases (HMTs/PMTs) catalyze the transfer of methyl groups from SAM to the lysine or arginine side chains on the target protein. The binding pocket of SAM and amino acid contain structural characteristics that facilitate inhibitor interaction, making these enzymes suitable targets for small-molecule inhibitors. These small compounds are known to exert their effect by competing for binding sites in the enzyme with either the substrate or the cofactor SAM (Prachayasittikul et al., 2017; Singh et al., 2018).

BIX-01294, BIX-01338, GSK126, and Tazemetostat are just a few examples of SAM-competitive inhibitors epigenetic treatment. Additionally, numerous novel SAM-competitive inhibitors including EPZ6438, GSK2816126, CPI-1205 are identified and are currently

undergoing clinical trials for the hematological malignancy treatment (Prachayasittikul et al., 2017; Singh et al., 2018).

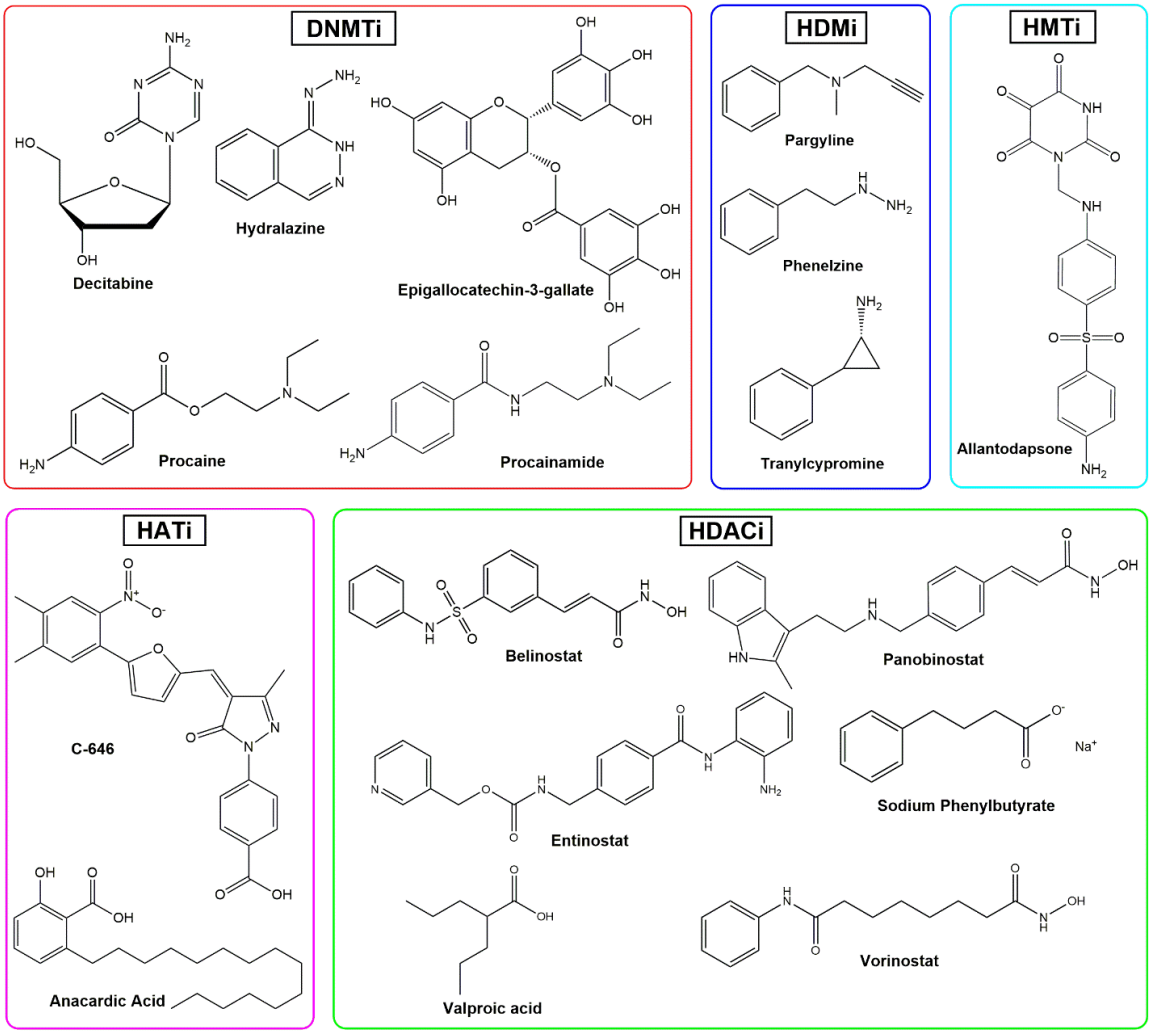

Figure 6: Chemical structure of different epigenetic drugs (Adapted from Prachayasittikul et al., 2017). Epigenetic drug consisting of DNA methyltransferase inhibitors (DNMTi), histone demethylase inhibitors (HDMi) histone methyltransferase inhibitors (HMTi), histone acetyltransferase inhibitors (HATi) and histone deacetylase inhibitors (HDACi) target specific epigenetic processes and influence the subsequent gene transcription.

5.2 Epigenetic Therapy in Alzheimer's Disease

The first line of available treatment for AD includes donepezil, rivastigmine, galantamine, Huperzine A which function as a cholinesterase inhibitor to increase the acetylcholine and improve the memory and cognitive impairment. Namzaric and memantine are used to control the abnormal glutamate levels that causes neuronal dysfunction (Solanki et al., 2016).

Moreover, many drugs that specifically targeting epigenetic machineries are in the development phase with significant clinical benefits.

The hypomethylation of genes that encodes for Aβ protein leads to overproduction of Aβ protein. The Dnmt3a2 DNA methyltransferase is reduced in the hippocampus in animal model, and hence Dnmt3a2 restoration recovers cognitive abilities. Methyl donor such as betaine, helps to improve memory impairments associated with AD. S-adenosylmethionine (SAM), another methyl donating compound, is discovered to be depleted in the cerebrospinal fluid sample from patients with AD. SAM decrease the formation of Aβ and tau phosphorylation by increasing the expression of PSEN1 and BACE1, hence improving cognitive function. Vitamin B12 supplementation increases the hypermethylation of TXNRD1 and NUDT15, the genes linked with improved cognitive functioning in patients with AD. Folic acid, another methyl donor, also improves the cognitive capabilities in patients. In this way, DNA methyltransferase or methyl donor could be used as an effective therapy for patients with AD (Xiao et al., 2020).

Hypermethylation is associated in the pathogenesis of AD and reducing methylation levels in certain genes could also be a potential therapeutic approach. Currently, DNMT inhibitors are used to treat hematopoietic malignancy. Also, DNMT inhibitors are being administered to treat some other neurodegenerative disorders, such as Friedreich's ataxia. While DNMT inhibitors have the prospect to be used in the treatment of AD, the limitation of gene specificity and safety is the key issue that needs to be solved before these can be used in AD patients. UNC0642, a potent chemical inhibitor of the methyltransferase activity of G9a/GLP, has been found on the histone H3 Lys 9 (H3-K9), therefore restoring the cognitive function by decreasing 5mC and increasing 5hmC. Suppressing the Tet1 gene, which is associated in the DNA methylation, improves cognitive abilities by oxidizing 5mC to 5hmC and decreasing methylation levels in the brain (Xiao et al., 2020).

Histone deacetylases (HDACs) suppress gene expression and are involved in learning and memory impairment by limiting the accessibility of transcription factors and suppress memory-related genes. In Alzheimer's disease, HDAC inhibitors (HDACi) are being identified as a promising treatment approach. Trichostatin A, an HDACi, increases H4 acetylation levels and improves contextual freezing ability in AD patients. Valproic acid was among the first HDACi to be discovered and has been shown to be beneficial in memory improvement. Histone deacetylase 2 (HDAC2) inhibitors have shown to improve memory by enhancing dendritic spine formation and development. Another HDACi, sodium phenylbutyrate, also improves memory by increasing neurotrophin production through the protein kinase C (PKC)-cAMP-response element-binding protein (CREB) pathway. M344, an HDACi, reduces Aβ expression and protects cognitive impairment by regulating numerous pathological pathways. A class II HDACi based on mercaptoacetamide and a class I and II HDACi based on hydroxamide both decrease Aβ levels and improve memory. In one genome-wide study, decreased PU.1 expression was linked with a decreased risk of AD. The high-throughput analysis of FDA-approved therapeutics suggests that vorinostat, an HDACi, reduces PU.1 expression and could be a potential treatment strategy for AD. Additionally, treatment with RGFP-966, a targeted HDAC3 inhibitor, increases cognitive performance and lowers Aβ and tau formation in neurons, adding further support for the importance of HDACi in AD patients. Thus, HDACi could be considered a possible therapeutic drug for Alzheimer's disease (Xiao et al., 2020).

Histone acetyltransferase (HAT) is required for the synthesis of CREB binding protein (CBP), which plays a significant role in memory. CBP expression could facilitate in the recovery of memory impairment in AD mice model, expression of CBP might promote in the recovery of memory deficits. Histone methyltransferases Inhibitors reduce hypermethylation histone protein and ameliorate synaptic impairments and cognitive functioning, indicating a potential therapeutic approach for AD (Xiao et al., 2020).

Noncoding RNAs also have a significant role in the pathology of AD. The RNA interference processes including short interfering RNA and short hairpin RNA have been used to downregulate the expression of APP, PSEN1, and PSEN2, which indicate a promising therapeutic process. To inhibit the target gene protein production in AD, microRNA analogues and anti-microRNAs have been developed. MicroRNA-384 analogues decrease APP and BACE1 expression, suggesting the microRNA-384 could be a major target in AD. Anti-microRNAs complement corresponding microRNAs and decrease their numbers to maintain homeostasis. MicroRNA-146a is elevated in the AD mice model, and anti-microRNA-146a therapy restores cognitive performance and controls the inflammatory response. Additionally, a variety of different microRNAs could be potential treating strategies in AD. MicroRNA-34c highly expressed in the hippocampus in AD and subsequent inhibition improves memory function in in-vivo studies. BACE1-AS lncRNA is linked with BACE1 protein expression and knocking out BACE1-AS with short interfering RNA improves cognitive performance in AD animal models. MicroRNA-29c, microRNA-124 and microRNA-339-5p reduce in vitro expression of BACE1 gene. In *in-vitro* study, microRNA-101 decreased the expression of Aβ and APP in neurons of hippocampus. In the *ex-vivo* model of AD, microRNA-153 reduces APP expression. As a consequence, non-coding RNAs could be potential therapeutic targets for AD therapy in coming future (Xiao et al., 2020).

To date, the majority of Alzheimer's disease clinical trials have focused on Aβ and tau protein. Numerous clinical trials are investigating epigenetic intervention for the treatment of Alzheimer's disease. Oral betaine was provided to eight AD patients; but, due to a lack of controls group and a limited sample size, the effectiveness of betaine could not be possible to determine. S-adenosylmethionine and nutriceuticals have been shown to improve neuropsychiatric activities in AD patients by about 30% as compared to normal groups. In a phase-I clinical trial, RDN-929, a selective HDACi, has been investigated for the treating of

AD patients. Phase-I clinical testing of EVP-0334, also known as FRM-0334, a HDACi with CNS-penetrating features has been completed. Because growing evidence indicates that epigenetics has a critical role in AD, treatments targeting epigenetics may represent potential advancements in the management of AD (Xiao et al., 2020).

5.3 Epigenetic Therapy in Parkinson's Disease

The current therapeutic options for Parkinson's Disease are levodopa and few dopamine agonists like tolcapone, ropinirole, apomorphine hydrochloride that increase the dopamine level and reduce the symptoms (Solanki et al., 2016). However, there are currently two major epigenetic therapies applicable, including through DNA methyltransferase inhibitors and histone deacetylase inhibitors.

PD has been characterized with a dysregulated SAM metabolism, and reduced DNA methylation has been associated with cognitive impairment. A possible mechanism for reversing this reduction might be to restore SAM levels through the administration of folates, or vitamin B12, methionine, choline. Through promoting hypomethylation, DNMT inhibitors like 5-aza-2′-deoxycytidine (5-aza-dC) could control the neuroprotective genes expression including tyrosine hydroxylase as well as the transcription of PD pathogenic genes including synuclein and UCHL1 (Renani et al., 2019).

Histone deacetylase inhibitors result in expanding chromatin structure, activating different neuronal growth factors, and preventing inflammation, providing with neuroprotection by decreasing the deacetylated histones. For example, intervention of histone deacetylase inhibitors influences the transcription of brain derived neurotrophic factors (BDNF), heat shock protein 70 kD (HAP70), glial cell derived factors (GDNF), synuclein, p21, and Gelosolin (Renani et al., 2019).

Vorinostat and Sodium butyrate and both function as histone deacetylase inhibitors and enhance acetylation in histones H3 and H4 in animal model with PD, indicating that these could be utilized to treat PD. Sodium butyrate, a short chain fatty acid, can efficiently crosses the blood–brain barrier (BBB) and inhibits both Class I and Class II histone deacetylases. Numerous studies have indicated that treating nerve cells with sodium butyrate promotes the production of anti-inflammatory molecules, including HSP70, and promotes neuroprotection. Sodium butyrate has been shown to promote acetylation of many histones in human dopaminergic neurons, such as lysine 5 on histones H2A and H2B, lysine 9 on histone H3, and lysine 8 on histone H4 (Renani et al., 2019).

Phenylbutyrate was originally approved to treat urea cycle disorders. Phenylbutyrate is formed by adding a phenyl group to the carbon on position 4 of sodium butyrate. Thus, hold a similar histone deacetylase inhibitory characteristic as sodium butyrate and has demonstrated great efficacy on preventing dopaminergic cell death in PD animal models. Additionally, phenylbutyrate can protect dopaminergic neurons from oxidative damage and α-synuclein neurotoxicity by boosting DJ1 protein expression. Thereby, phenylbutyrate can prevent α-synuclein accumulation and the increased incidence of motor symptoms and cognitive degeneration (Renani et al., 2019).

Vorinostat is a hydroxamate-based histone deacetylase that can elevate the production of neuroprotective proteins HSP70 and BCL2 in the PD animal models. Additionally, vorinostat has been shown to promote histone hyperacetylation and thereby releasing BDNF and GDNF factors from astrocytes for neuroprotection. Trichostatin A is also a less commonly used medicine, and a natural substance obtained from Streptomyces hygroscopicus. It was initially discovered as the antifungal antibiotic, but the histone deacetylase inhibiting activity was first documented in 1990 (Renani et al., 2019).

Trichostatin A functions as a histone deacetylase inhibitor of Class I and II and prevents dopaminergic neuronal death in PD by inhibiting the production of proinflammatory cytokines released by activated microglial cells. Trichostatin A also prevents from mitochondrial malfunctioning and apoptosis in nerve cells (Renani et al., 2019).

Apicidin, a histone deacetylase inhibitor from the cyclic tetrapeptide analog, exhibits high selectivity for histone deacetylases 2,3 and 8, but lower for histone deacetylase 8. Apicidin targets histone deacetylases 2, 3, which are the most prevalent histone deacetylases expressed in the substantia nigra region of the brain, and thus increases histone acetylation level, leading to increased neuroprotection, by increasing the expression of the HSP70 chaperone. Sirtuin 2 is a deacetylase that inhibits α-synuclein and reduce associated toxicity in animal model of PD (Renani et al., 2019).

Valproic acid (2-propylpentanoic acid) inhibits histone Class I and II deacetylases and can lead to a number of benefits on the brain by inhibiting these histone deacetylases. Besides increasing histone acetylation and reducing the levels of inflammatory proteins, valproic acid also enhances the expression of BDNF and GDNF, which are both important for neuroprotection. Valproate is known to be the most potential drug for the treatment of Parkinson's disease (PD) among the histone deacetylase inhibitors currently available (Renani et al., 2019).

Selegiline and Rasagaline, both monoamine oxidase inhibitors, may also contribute to restoring the decreased methylation of lysine 4 in histone H3 (Renani et al., 2019).

5.4 Epigenetic Therapy in Huntington’s Disease

For Huntington’s Disease, tetrabenzine is the only FDA-approved drug for treating the symptoms of HD, including involuntary writhing movements (Solanki et al., 2016). However, several epigenetic based drugs are in the pipeline of development which target specifically the pathology of Huntington’s Disease.

Methyltransferase inhibitors could be used therapeutically for treating HD and these drugs may function by interfering with histone acetylation. Histone methylation can be modified indirectly through binding to the GC with anthracyclines analogs including such chromomycin and mithramycin A, a type of antibiotic bacterial drugs with anticancer features. Chromomycin and mithramycin and have been demonstrated to limit the binding ability of transcriptional activators to the CpG-rich gene promoter, thereby inhibiting the gene transcription that initiate oxidative damage and apoptosis. In the HD mouse model, treating with mithramycin increases survival with improved motor function and significantly delaying neuropathological complication. Mithramycin intervention inhibits the increasing H3 methylation level in HD mouse model, indicating that such improved survival and neuroprotective change could be indicative to the restoration of silenced expression of genes essential for neuronal growth and functioning (Thomas, 2019; F. Wang et al., 2014).

DNA hydroxymethylation modification of is another focus of HD therapy. In HD mouse model, the total DNA hydroxymethylation level is considerably lower in the cortex and striatum regions, compared to control group, indicating that increasing DNA hydroxymethylation could prevent HD symptoms as well as provide a promising therapeutic target for the treatment of HD. Recently, some compounds were demonstrated to have an impact on genome-wide global DNA hydroxymethylation. In the *in-vitro* model, dimethyl sulfoxide can significantly enhance genome-wide and gene-specific DNA hydroxymethylation levels. This could lead to increasing the genes expression associate with DNA hydroxymethylation such as, TET and nucleotide excision repair (GADD45), or by inhibiting the genes transcription linked with DNA methylation such as Dnmt1, and Dnmt3b. Vitamin C has shown to increase the catalyzing efficiency of TET dioxygenases for oxidizing 5mC directly, indicating that this could also function as a cofactor component for TET enzymes. Vitamin C increases the 5mC oxidation products significantly, specifically 5-carboxylcytosine

and 5-formylcytosine, leading to a global reduction of 5mC in embryonic stem cells from mouse. Considering that the DNA hydroxymethylation level is markedly reduced both in the cortex and striatum parts of HD mouse brains, this is likely to be that drugs equipped with enhancing DNA hydroxymethylation could have therapeutic potential. More research is required to determine the therapeutic potential and clinical efficacy of these chemicals (F. Wang et al., 2014).

HDAC inhibitors can influence gene activation or suppression systemically by relaxing the DNA conformations. The first drugs to demonstrate potency for inhibiting HDAC in HD animal model are sodium butyrate and suberoylanilide hydroxamic acid. These major research findings provided the basis for initiating clinical trials of HDAC inhibitors in patients with HD. Knocking out HDAC2 gene or treating with sodium butyrate or suberoylanilide hydroxamic acid enhance memory formation in mice with HD. In *in-vitro* models of HD, polyglutamine reduces histone acetylation, and HDAC inhibitors have been demonstrated to lessen polyglutamine-induced toxicity. Sodium butyrates are the most advanced among all classes of HDAC inhibitors for clinical application and the bioavailability in the CNS is well documented. Sodium butyrate has a lower toxicity and has been shown to be tolerated well in animal and human studies. Sodium butyrate alters histone modifications, improves motor function and reduce neuropathologic implications, and significantly prolongs survival in animal mice model. Phenylbutyrate is a potential candidate for investigation since it is FDA authorized and there is adequate information available on pharmacology, pharmacokinetic, toxicity and dosing. Phenylbutyrate changes the structure of chromatin and improves motor impairments and neuropathological phenotypes in HD animal models. HDAC inhibitors have been used in clinical trials for HD patients due to their potential benefit on the overall neuropathology) and mortality in HD animal models. In the HD mice model, HDACi 4b therapy significantly corrected histone H3 acetylation and restored the level of mRNA

expression. Additionally, HDACi 4b significantly increased body mass and various factors of motor performance, as well as restored cognitive function in transgenic mice. Specifically, HDACi 4b administration modified gene networks involved in post-translational change, including protein phosphorylation and ubiquitination. HDACi 4b-mediated inhibition of kappaB kinase leads in the acetylation, phosphorylation, and clearance of the Htt protein by the ubiquitin-proteasomal and autophagic pathways. Studies demonstrated that targeting HDAC3 and HDAC1 with HDAC inhibitors were proven to be effective in reducing mhtt-induced neurotoxicity and improving mhtt-induced metabolic abnormalities. HDACi 4b and 136, two compounds with great efficacy for inhibiting HDAC3, were shown to be the most efficient in reversing the expression of certain genes involved with Huntington's disease (HD). These studies illustrate that HDACi 4b has significant biological effects as well as efficiency, which may make it a great therapeutic option for HD patients. Considering together, these research shows that HDAC inhibitors can ameliorate phenotypes either by upregulating surviving genes that have been repressed in HD or by suppressing prodeath genes that are upregulated in HD. Moreover, the specific mechanisms through HDAC inhibitors modify neuronal activity need to be investigated (Lee et al., 2013)

Given the fact that HD is resulting from mutations in exon 1 of the htt gene, genetic modification to suppress the expression of the pathogenic gene (mhtt) through ncRNAs has been suggested and is now being studied. The regular htt protein acts as a significant factor essential for neuronal function, whereas the mutant htt protein is neurotoxic. A major challenge in gene therapy would be eliminating the mutant allele with maintaining the expression of the regular allele, because of majority of HD patients have one mutant heterozygous htt allele and one regular-type htt allele at the locus. Therefore, developing noncoding small RNAs as nucleic acid therapy might have an effective approach for selectively suppressing mutant htt. In the transgenic mouse model of HD, RNA interference

with adeno-associated virus-small hairpin RNA (shRNA) relieves motor impairments and neuropathological phenotypes. The majority of RNA interference investigations, which have used adeno-associated virus-miRNA, adenovirus-shRNA, lentivirus-shRNA and cholesterol-conjugated siRNA have demonstrated the decreasing the aggregating mhtt, improves motor function, and reduces neuropathological impairments. Single-stranded siRNAs (ss-siRNAs) are 100 times more effective and 30 times more specific in inhibiting the allele of mhtt expression than unmodified RNA. In an animal model of HD, administering ss-siRNA through intraventricular infusion of specifically suppresses the mutant htt allele expression (Lee et al., 2013).

Chapter 6

Discussion and Future Scope

Starting with the brain development, formation of neuroplasticity, concurrent neurogenesis, progressive neurodegeneration, and transmission dependent cognitive processes are all directly associated with expression of specific genes at a specific time and are significantly controlled by epigenetic regulations. Neurodegenerative disorders are defined by a progressive decline in neuronal structure as well as function, followed by neuronal death. Different functional impairment can occur depending upon where in brain losing of integrity and neuronal death occurs, and these progressively intensify as the neurodegeneration spread. The fundamental pathology and localization of neurodegenerative processes typically differ among neurodegenerative diseases. The most prevalent are Alzheimer's disease, Parkinson's disease, and Huntington's disease, however amyotrophic lateral sclerosis and prion diseases are other commonly investigated examples of neurodegeneration. While the underlying pathogenesis of different neurodegenerative diseases is unclear, though in some cases, such as HD, have a definite genetic basis, others, such as sporadic AD and PD, have considerably more complex basis of genetics and disease development, presumably implicating gene-gene and gene-environment associations. Numerous research has investigated alternative explanations with epigenetic mechanisms appearing to be the most promising, in cases where genetics could not provide possible answers. Epigenetic dysregulations are currently drawing considerable attention as a potential reason for the development of accelerated aging and age-related neurodegenerative diseases. These deregulations occur as a consequence of the interaction between genetic and environmental factors. Despite the significant differences in epigenetic marks, some common changes occur that appear to bridge the apparently different pathophysiology of neurodegenerative diseases including AD, PD, and HD. For example, detailed evaluation of the demethylation dysregulations indicated that unique methylation

pattern in genes lead to the genetic predisposition of AD and PD, particularly APP, BACE, PS1, PS2, APOE for AD and SNCA, PARKIN16 for PD. Furthermore, in all three addressed diseases, there is aberrant histone acetylation, and in AD and PD specifically, there is a genome-wide deacetylation of histones is identified. Different modifications in histone 3, particularly the increased tri-methylation of H3K9 both in AD and HD, are some of the common modifications within those diseases. Finally, the dysregulation of different ncRNAs in three of the described diseases suggests that these play a significant part in the pathophysiology. In fact, altered expression of miR-132 and miR-29 is a characteristic change of not just the three age-related neurodegenerative diseases, but also of normal aging. miR-22, miR-26a, and miR-125 also exhibit a pattern of altered expression that is prominent within those diseases (Lardenoije et al., 2015; Singh et al., 2018).

Abnormal epigenetic changes have been implicated with a number of cognitive disorders, but interpreting these findings is complicated. In fact, it is still unclear whether epigenetic alterations are the root cause of the symptoms or are simply a reflection of other neural changes. Considering those epigenetic modification such as DNA methylation and histone acetylation may represent as an adjacent integration process for a number of different signaling pathways and second messenger system, it may be possible that epigenetic alterations are only one of several functional consequences of modified intracellular signaling. In concept, an absence of epigenetic function could be resulted from dysregulation in many different parts of neuronal biology, such as neurotransmitter release, receptor function, and molecular signaling pathway. Thus, continuing research to determine whether epigenetic modifications are the cause or consequence of specific cognitive impairments would improve the ability to treat and, in some circumstances, prevent those diseases. Even when a disorder is known to be caused by altered epigenetic mechanism, it is still uncertain how this alteration manifests through numerous of gene targets and functionally diversified brain areas. Thus, in addition to investigating the

epigenetic markers associated with a disease, it is essential to continue evaluating how these markers alter gene expression and function throughout entire brain systems (Day & Sweatt, 2012).

The use of drugs to control epigenetic mechanisms involves a number of additional challenges. Once an epigenetic target is discovered for a certain disease, different obstacles must be overcome before an epigenetic drug can be designed successfully including selectivity of enzyme isoform, selectivity of lysines and arginines histone substrate versus non-histone substrate, designing dual inhibitor, using combination of epigenetic drug and the multimeric complexes of epigenetic enzymes that complicated the translation of *in-vitro* potency to *in-vivo* efficacy. A significant limitation is that the available epigenetic drugs, such as HDAC and DNMT inhibitors, are not selective for specific areas of the brain, neuronal subgroups, or genes. The lack of selectivity has become a critical problem in the treatment of various diseases characterized by alterations in epigenetic expression at a specific number of gene sites. For example, schizophrenia has been associated with hypermethylation in the reelin gene. However, systemic or even region-specific application of DNMT inhibitors could also result in decreasing methylation of a considerable number of other genes in addition to reelin. Similarly, administration of HDAC inhibitors, which also have the potential to reverse hypermethylation in reelin gene, would lead to increases histone acetylation of unassociated genes. A similar challenge is that as DNA methylation and histone modifications act synergistically to regulate transcription, changing one mechanism will have a complex effect on other mechanisms consequently. Each of these challenges, in aspects of epigenetic treatments, will certainly lead to a range of adverse effects that would prevent clinical application. Thereby, more research will be needed in the future to develop more targeted epigenetic regulators. The HDAC enzymes are potential alternatives for developing more precise epigenetic regulators. Since these enzymes exist in several isoforms with distinct

expression levels across brain area with distinct biochemical function, therefore targeting one specific HDAC isoform may able to increase spatial and genetic specificity (Day & Sweatt, 2012). Finding drugs that can cross the blood-brain barrier (BBB) is one of the most challenging problems for epigenetic therapy. In general, molecules with less than 500 daltons and few than 8 pairs of hydrogen bonds can pass through the BBB. There are several strategies for overcoming this limitation, including disruption of BBB, intracerebral implantation, as well as intracerebroventricular infusion. Both DNMT inhibitors and HDAC inhibitors are two different classes of drugs that are capable of crossing the BBB. Several additional factors, besides that, including efficacy, delivery, toxicity, and patient factors, must be considered (Liu et al., 2018). As of 2018, the FDA has several drugs that primarily target epigenetic mechanisms (Table 2). Considering that many of these early drugs are authorized for cancer treatment, an increasing range of compounds targeting epigenetic modifications in several other diseases is currently under preclinical and clinical research (Figure 7) (Tzika et al., 2018).

Table 2: List of FDA-approved epigenetic drugs (Adapted from Berdasco & Esteller, 2019; Graça et al., 2016).

FDA Approved Drug	Commercial Name	Company	Drug Class	Treatment of Approval	Year
5-Azacytidine	Vidaza®	Celgene Corporation	DNMTi	Myelodysplastic Syndrome	2004
Decitabine	Dacogen®	Eisai	DNTMi	Myelodysplastic Syndrome	2006
Vorinostat	Zolinza®	Merck	HDACi	Cutaneous T-Cell Lymphoma	2006
Romidepsin	Istodax®	Celgene Corporation	HDACi	Cutaneous T-Cell lymphoma	2009
Belinostat	Beleodaq®	Spectrum Pharmaceuticals	HDACi	Peripheral T-Cells Lymphoma	2014
Panobinostat	Farydak®	Novartis	HDACi	Multiple Myeloma	2015
Chidamide	Epidaza®	Shenzhen Chipscreen Biosciences	HDACi	Peripheral T-Cells Lymphoma	2016

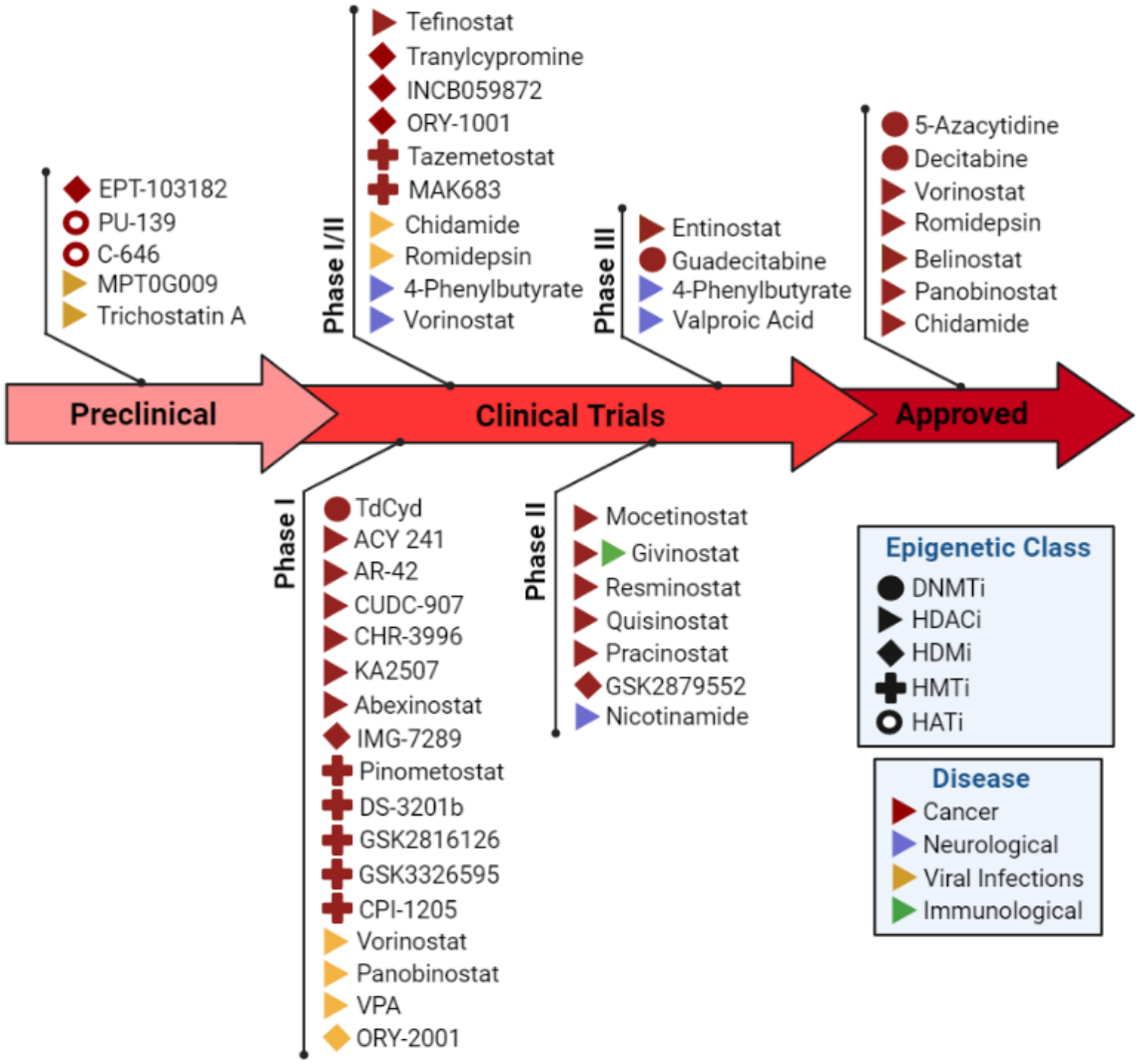

Figure 7: Epigenetic based drugs for different human diseases (Adapted from Berdasco & Esteller, 2019). Several epigenetic drugs from DNA methyltransferase inhibitors (DNMTi) and histone deacetylase inhibitors (HDACi) classes have been approved by the US FDA for treatment of haematological malignancies and further explored for other diseases including neurological, immunological and viral infections. Many different epidrugs from histone demethylase inhibitors (HDMi) histone methyltransferase inhibitors (HMTi), histone acetyltransferase inhibitors (HATi) classes are in the preclinical and clinical development phases targeting several human diseases.

Together with conventional chemical medicinal innovation, biological tools are essential for understanding the functional significance of inhibiting specific enzymes or their isoforms. The recently developed CRISPR/Cas method has created immense interest not only as a tool for correcting genetic mutations, but also as a tool of inactivating any given gene. Moreover, if a particular genetic function is lost due to epigenetic suppression, CRISPR/Cas could be

repurposed to specific effectors towards that genomic locus in order to re-express the suppressed gene. Specifically, this is accomplished by modifying the nuclease function of the Cas protein, which leads to a dead Cas (dCas). dCas is then recruited to the specific genomic site of by single guide RNAs and can be used thereby to shuttle the fusion protein to that site. When the catalytic site of an enzyme is fused in dCas, the system will be resulted then to rewrite the epigenetic signature at this specific position (epigenetic editing) (Ganesan et al., 2019).

Epigenetic profiling, like genetic and genomic testing, potentially gives clinically valuable information. Recent developments in high throughput sequencing technologies including such genome wide sequencing, combining with RNA profiling, chromatin immunoprecipitation, and bisulfite conversion have generated vast amounts of information which could be implemented to develop a comprehensive insight about epigenetic dysregulation that are frequent and specific to different disease states. These rapidly developing findings will certainly provide the foundation for the advancement of improved molecular diagnostics and personalized precision therapies, which may include site-specific epigenome editing (Kelly et al., 2010; Qureshi & Mehler, 2014).

While epigenetic interventions have not been authorized for treating the early neurogenesis of diseases, the strong correlation in between epigenetic dysregulation and neurodegenerative diseases indicates that pharmacological drugs targeting epigenetic modification including such DNA methylation and histone acetylation could be effective in treating these diseases. Nevertheless, considering that few certain types of epigenetic drugs have shown to improve different neuronal phenotypes, together with the early neuropathy associated with neurodegenerative diseases, therapeutic interventions targeting these epigenetic dysregulations, particularly in the early phase of the diseases, appear to be a potential therapeutic option (Kwon et al., 2017).

Chapter 7

Conclusion

The understanding of human diseases has been transformed over the time by the development of genetics and genomics and now advances to the next phase of this transformation through the expansions of epigenetics and epigenomics (Qureshi & Mehler, 2011). Recent discoveries in epigenetics have fundamentally changed the pathophysiological mechanisms associated with complex diseases. Thus far, evidence from different disease models varying from cell lines to post-mortem human brains indicated that epigenetic dysregulation plays a crucial role in neurodegenerative disorders. While the impact of epigenetics in brain disorders is continuously being investigated, it is evident that restoring epigenetic alterations could provide potential therapeutic benefits (Bertogliat et al., 2020). Different types of small molecular modulators have been identified for targeting the epigenetic enzymes involved in the dysregulation both as activators and inhibitors. These molecules, either individually or in combination, have the potential to open up a new field of neurotherapeutics in coming years. Besides, many existing drugs that have been used as neurotherapeutics can also be considered for targeting epigenetic enzymes for developing new drugs depending on their chemical structures (Singh et al., 2018). In conclusion, epigenetics may be providing the missing link to understand the complex etiology of neurodegenerative diseases and if the mechanism of these changes continues to be revealed, it may be plausible to intervene therapeutically by correcting the epigenetic dysregulation associated with these diseases (Marques et al., 2011).

References

Bannister, A. J., & Kouzarides, T. (2011). Regulation of chromatin by histone modifications. *Cell Research*, *21*(3), 381–395. https://doi.org/10.1038/cr.2011.22

Berdasco, M., & Esteller, M. (2019). Clinical epigenetics: seizing opportunities for translation. *Nature Reviews Genetics*, *20*(2), 109–127. https://doi.org/10.1038/s41576-018-0074-2

Bertogliat, M. J., Morris-Blanco, K. C., & Vemuganti, R. (2020). Epigenetic mechanisms of neurodegenerative diseases and acute brain injury. In *Neurochemistry International* (Vol. 133). Elsevier Ltd. https://doi.org/10.1016/j.neuint.2019.104642

Cacabelos, R., & Teijido, O. (2018). Epigenetics of brain aging. In *Conn's Handbook of Models for Human Aging* (Second Edi). Elsevier Inc. https://doi.org/10.1016/B978-0-12-811353-0.00074-9

Conboy, K., Henshall, D. C., & Brennan, G. P. (2021). Epigenetic principles underlying epileptogenesis and epilepsy syndromes. *Neurobiology of Disease*, *148*, 105179. https://doi.org/10.1016/j.nbd.2020.105179

Coppedè, F. (2014). The potential of epigenetic therapies in neurodegenerative diseases. *Frontiers in Genetics*, *5*(JUL). https://doi.org/10.3389/fgene.2014.00220

Coppedè, F. (2021). Epigenetic regulation in Alzheimer's disease: is it a potential therapeutic target? *Expert Opinion on Therapeutic Targets*, *25*(4), 283–298. https://doi.org/10.1080/14728222.2021.1916469

Day, J. J., & Sweatt, J. D. (2012). Epigenetic treatments for cognitive impairments. *Neuropsychopharmacology*, *37*(1), 247–260. https://doi.org/10.1038/npp.2011.85

Feng, Y., Jankovic, J., & Wu, Y. C. (2015). Epigenetic mechanisms in Parkinson's disease. *Journal of the Neurological Sciences*, *349*(1–2), 3–9.

https://doi.org/10.1016/j.jns.2014.12.017

Ganesan, A. (2018). Epigenetic drug discovery: A success story for cofactor interference. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1748). https://doi.org/10.1098/rstb.2017.0069

Ganesan, A., Arimondo, P. B., Rots, M. G., Jeronimo, C., & Berdasco, M. (2019). The timeline of epigenetic drug discovery: From reality to dreams. *Clinical Epigenetics*, *11*(1), 1–17. https://doi.org/10.1186/s13148-019-0776-0

Gangisetty, O., & Murugan, S. (2016). *Epigenetic Modifications in Neurological Diseases: Natural Products as Epigenetic Modulators a Treatment Strategy* (pp. 1–25). https://doi.org/10.1007/978-3-319-28383-8_1

Graça, I., Pereira-Silva, E., Henrique, R., Packham, G., Crabb, S. J., & Jerónimo, C. (2016). Epigenetic modulators as therapeutic targets in prostate cancer. *Clinical Epigenetics*, *8*(1), 1–24. https://doi.org/10.1186/s13148-016-0264-8

Hwang, J. Y., Aromolaran, K. A., & Zukin, R. S. (2017). The emerging field of epigenetics in neurodegeneration and neuroprotection. *Nature Reviews Neuroscience*, *18*(6), 347–361. https://doi.org/10.1038/nrn.2017.46

Jakovcevski, M., & Akbarian, S. (2012). Epigenetic mechanisms in neurological disease. *Nature Medicine*, *18*(8), 1194–1204. https://doi.org/10.1038/nm.2828

Kalia, L. V., & Lang, A. E. (2015). Parkinson's disease. *The Lancet*, *386*(9996), 896–912. https://doi.org/10.1016/S0140-6736(14)61393-3

Kanherkar, R. R., Bhatia-Dey, N., & Csoka, A. B. (2014). Epigenetics across the human lifespan. *Frontiers in Cell and Developmental Biology*, *2*(SEP), 1–19. https://doi.org/10.3389/fcell.2014.00049

Kelly, T. K., De Carvalho, D. D., & Jones, P. A. (2010). Epigenetic modifications as therapeutic targets. *Nature Biotechnology*, *28*(10), 1069–1078. https://doi.org/10.1038/nbt.1678

Kofink, D., Boks, M. P. M., Timmers, H. T. M., & Kas, M. J. (2013). Epigenetic dynamics in psychiatric disorders: Environmental programming of neurodevelopmental processes. *Neuroscience and Biobehavioral Reviews*, *37*(5), 831–845. https://doi.org/10.1016/j.neubiorev.2013.03.020

Kumar, S., Gonzalez, E. A., Rameshwar, P., & Etchegaray, J. P. (2020). Non-coding RNAs as mediators of epigenetic changes in malignancies. *Cancers*, *12*(12), 1–32. https://doi.org/10.3390/cancers12123657

Kwon, M. J., Kim, J. H., Kim, T. S., & Lee, S. B. (2017). Pharmacological intervention of early neuropathy in neurodegenerative diseases. *Pharmacological Research*, *119*, 169–177. https://doi.org/10.1016/j.phrs.2017.02.003

Landgrave-Gómez, J., Mercado-Gómez, O., & Guevara-Guzmán, R. (2015). Epigenetic mechanisms in neurological and neurodegenerative diseases. *Frontiers in Cellular Neuroscience*, *9*(FEB), 1–11. https://doi.org/10.3389/fncel.2015.00058

Lardenoije, R., Iatrou, A., Kenis, G., Kompotis, K., Steinbusch, H. W. M., Mastroeni, D., Coleman, P., Lemere, C. A., Hof, P. R., van den Hove, D. L. A., & Rutten, B. P. F. (2015). The epigenetics of aging and neurodegeneration. *Progress in Neurobiology*, *131*, 21–64. https://doi.org/10.1016/j.pneurobio.2015.05.002

Lardenoije, R., Pishva, E., Lunnon, K., & van den Hove, D. L. (2018). Neuroepigenetics of Aging and Age-Related Neurodegenerative Disorders. In *Progress in Molecular Biology and Translational Science* (1st ed., Vol. 158). Elsevier Inc. https://doi.org/10.1016/bs.pmbts.2018.04.008

Lee, J., Hwang, Y. J., Kim, K. Y., Kowall, N. W., & Ryu, H. (2013). Epigenetic Mechanisms of Neurodegeneration in Huntington's Disease. *Neurotherapeutics*, *10*(4), 664–676. https://doi.org/10.1007/s13311-013-0206-5

Li, Y. (2021). Modern epigenetics methods in biological research. *Methods*, *187*, 104–113. https://doi.org/10.1016/j.ymeth.2020.06.022

Liu, X., Jiao, B., & Shen, L. (2018). The Epigenetics of Alzheimer's Disease: Factors and Therapeutic Implications. *Frontiers in Genetics*, *9*(November), 1–10. https://doi.org/10.3389/fgene.2018.00579

Marques, S. C. F., Oliveira, C. R., Pereira, C. M. F., & Outeiro, T. F. (2011). Epigenetics in neurodegeneration: A new layer of complexity. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *35*(2), 348–355. https://doi.org/10.1016/j.pnpbp.2010.08.008

Miranda Furtado, C. L., Dos Santos Luciano, M. C., Silva Santos, R. Da, Furtado, G. P., Moraes, M. O., & Pessoa, C. (2019). Epidrugs: targeting epigenetic marks in cancer treatment. *Epigenetics*, *14*(12), 1164–1176. https://doi.org/10.1080/15592294.2019.1640546

Mohd Murshid, N., Aminullah Lubis, F., & Makpol, S. (2020). Epigenetic Changes and Its Intervention in Age-Related Neurodegenerative Diseases. *Cellular and Molecular Neurobiology*. https://doi.org/10.1007/s10571-020-00979-z

Murgatroyd, C. (2016). *Epigenetics*. 121–138.

Nikolac Perkovic, M., Videtic Paska, A., Konjevod, M., Kouter, K., Svob Strac, D., Nedic Erjavec, G., & Pivac, N. (2021). Epigenetics of Alzheimer's Disease. *Biomolecules*, *11*(2), 195. https://doi.org/10.3390/biom11020195

Oh, E. S., & Petronis, A. (2021). Origins of human disease: the chrono-epigenetic perspective.

Nature Reviews Genetics, *22*(8), 533–546. https://doi.org/10.1038/s41576-021-00348-6

Portela, A., & Esteller, M. (2010). Epigenetic modifications and human disease. *Nature Biotechnology*, *28*(10), 1057–1068. https://doi.org/10.1038/nbt.1685

Prachayasittikul, V., Prathipati, P., Pratiwi, R., Phanus-umporn, C., Malik, A. A., Schaduangrat, N., Seenprachawong, K., Wongchitrat, P., Supokawej, A., Prachayasittikul, V., Wikberg, J. E. S., & Nantasenamat, C. (2017). Exploring the epigenetic drug discovery landscape. *Expert Opinion on Drug Discovery*, *12*(4), 345–362. https://doi.org/10.1080/17460441.2017.1295954

Qi, Y., Wang, D., Wang, D., Jin, T., Yang, L., Wu, H., Li, Y., Zhao, J., Du, F., Song, M., & Wang, R. (2016). HEDD: The human epigenetic drug database. *Database*, *2016*, 1–10. https://doi.org/10.1093/database/baw159

Qi, Z., Li, J., Li, M., Du, X., Zhang, L., Wang, S., Xu, B., Liu, W., Xu, Z., & Deng, Y. (2021). The Essential Role of Epigenetic Modifications in Neurodegenerative Diseases with Dyskinesia. *Cellular and Molecular Neurobiology*, *0123456789*. https://doi.org/10.1007/s10571-021-01133-z

Qureshi, I. A., & Mehler, M. F. (2011). Advances in epigenetics and epigenomics for neurodegenerative diseases. *Current Neurology and Neuroscience Reports*, *11*(5), 464–473. https://doi.org/10.1007/s11910-011-0210-2

Qureshi, I. A., & Mehler, M. F. (2013). Understanding Neurological Disease Mechanisms in the Era of Epigenetics. *JAMA Neurology*, *70*(6), 703–710. https://doi.org/10.1001/JAMANEUROL.2013.1443

Qureshi, I. A., & Mehler, M. F. (2014). Epigenetic Mechanisms Underlying the Pathogenesis of Neurogenetic Diseases. *Neurotherapeutics*, *11*(4), 708–720.

https://doi.org/10.1007/s13311-014-0302-1

Rathore, A. S., Birla, H., Singh, S. Sen, Zahra, W., Dilnashin, H., Singh, R., Keshri, P. K., & Singh, S. P. (2021). Epigenetic Modulation in Parkinson's Disease and Potential Treatment Therapies. *Neurochemical Research*, *46*(7), 1618–1626. https://doi.org/10.1007/s11064-021-03334-w

Renani, P. G., Taheri, F., Rostami, D., Farahani, N., Abdolkarimi, H., Abdollahi, E., Taghizadeh, E., & Gheibi Hayat, S. M. (2019). Involvement of aberrant regulation of epigenetic mechanisms in the pathogenesis of Parkinson's disease and epigenetic-based therapies. *Journal of Cellular Physiology*, *234*(11), 19307–19319. https://doi.org/10.1002/jcp.28622

Rudenko, A., & Tsai, L. H. (2014). Epigenetic modifications in the nervous system and their impact upon cognitive impairments. *Neuropharmacology*, *80*, 70–82. https://doi.org/10.1016/j.neuropharm.2014.01.043

Singh, A. K., Halder-Sinha, S., Clement, J. P., & Kundu, T. K. (2018). Epigenetic modulation by small molecule compounds for neurodegenerative disorders. *Pharmacological Research*, *132*(April), 135–148. https://doi.org/10.1016/j.phrs.2018.04.014

Snijders, C., Bassil, K. C., & de Nijs, L. (2018). Methodologies of Neuroepigenetic Research: Background, Challenges and Future Perspectives. In *Progress in Molecular Biology and Translational Science* (1st ed., Vol. 158). Elsevier Inc. https://doi.org/10.1016/bs.pmbts.2018.04.009

Solanki, I., Parihar, P., & Parihar, M. S. (2016). Neurodegenerative diseases: From available treatments to prospective herbal therapy. *Neurochemistry International*, *95*, 100–108. https://doi.org/10.1016/j.neuint.2015.11.001

Soria Lopez, J. A., González, H. M., & Léger, G. C. (2019). Alzheimer's disease. *Handbook of Clinical Neurology*, *167*, 231–255. https://doi.org/10.1016/B978-0-12-804766-8.00013-3

Thomas, E. A. (2019). Epigenetic mechanisms in Huntington's disease. In *Chromatin Signaling and Neurological Disorders*. Elsevier Inc. https://doi.org/10.1016/b978-0-12-813796-3.00004-3

Tzika, E., Dreker, T., & Imhof, A. (2018). Epigenitics and metabolism in health and disease. *Frontiers in Genetics*, *9*(SEP), 1–8. https://doi.org/10.3389/fgene.2018.00361

Wang, F., Fischhaber, P. L., Guo, C., & Tang, T. S. (2014). Epigenetic modifications as novel therapeutic targets for Huntington's disease. *Epigenomics*, *6*(3), 287–297. https://doi.org/10.2217/epi.14.19

Wang, J., Yu, J. T., Tan, M. S., Jiang, T., & Tan, L. (2013). Epigenetic mechanisms in Alzheimer's disease: Implications for pathogenesis and therapy. *Ageing Research Reviews*, *12*(4), 1024–1041. https://doi.org/10.1016/j.arr.2013.05.003

Xiao, X., Liu, X., & Jiao, B. (2020). Epigenetics: Recent Advances and Its Role in the Treatment of Alzheimer's Disease. *Frontiers in Neurology*, *11*(October), 1–20. https://doi.org/10.3389/fneur.2020.538301

Xu, M., Zhu, J. Y., Liu, X. D., Luo, M. Y., & Xu, N. J. (2021). Roles of physical exercise in neurodegeneration: reversal of epigenetic clock. *Translational Neurodegeneration*, *10*(1), 1–15. https://doi.org/10.1186/s40035-021-00254-1

Xu, Z., Li, H., & Jin, P. (2012). Epigenetics-Based Therapeutics for Neurodegenerative Disorders. *Current Geriatrics Reports*, *1*(4), 229–236. https://doi.org/10.1007/s13670-012-0027-0

Zhang, L., Lu, Q., & Chang, C. (2020). Epigenetics in Health and Disease. In *Advances in Experimental Medicine and Biology* (Vol. 1253, pp. 3–55). https://doi.org/10.1007/978-981-15-3449-2_1