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Advances in Epigenetics and Epigenomics for Neurodegenerative Diseases

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Abstract In the post-genomic era, epigenetic factors—literally those that are "over" or "above" genetic ones and responsible for controlling the expression and function of genes—have emerged as important mediators of development and aging; gene-gene and gene-environmental interactions; and the pathophysiology of complex disease states. Here, we provide a brief overview of the major epigenetic mechanisms (ie, DNA methylation, histone modifications and chromatin remodeling, and non-coding RNA regulation). We highlight the nearly ubiquitous profiles of epigenetic dysregulation that have been found in Alzheimer's and other neurodegenerative diseases. We also review innovative methods and technologies that enable the characterization of individual epigenetic modifications and more widespread epigenomic states at high resolution. We conclude that, together with complementary genetic, genomic, and related approaches, interrogating epigenetic and epigenomic profiles in neurodegenerative diseases represent important and increasingly practical strategies for advancing our understanding of and the diagnosis and treatment of these disorders.

Keywords Alzheimer's disease · Amyotrophic lateral sclerosis · Chromatin · DNA methylation · Epigenetics · Epigenomics · Histone modification · Huntington's disease · Neurodegenerative disease · Next generation sequencing · Non-coding RNA · Parkinson's disease

Introduction

A decade has passed since the completion of the Human Genome Project [1], yet the mysteries of neurodegenerative diseases remain largely unsolved. Efforts aimed at elucidating the origins of familial and sporadic forms of neurodegenerative

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diseases, including positional cloning, genome-wide association, and candidate gene studies, have identified some causative genes and disease-associated genetic risk factors with varying, but typically small, effect sizes [2]. However, a genetic basis has not emerged for the most common forms of the majority of these diseases, implying that complementary and alternative strategies for probing the underpinnings of these disorders are necessary. Among these shifting paradigms are efforts focused on studying the role of epigenetics in the molecular pathophysiology of neurodegenerative diseases.

Recent studies have implicated dynamic cell- and tissue-specific epigenetic processes in regulating genomic structure and function during development, adult life, and aging including the mediation of gene expression and gene-gene and gene-environment interactions, such as responses to diet, physical and chemical exposures, and behavioral and social factors [3]. Here, we provide a brief overview of these distinct but highly coordinated mechanisms, which include DNA methylation, histone modifications and chromatin remodeling, and non-coding RNA (ncRNA) regulation. Genetic or acquired defects in the epigenetic apparatus and/or the accrual of epigenetic "lesions" throughout life lead to subtle changes in the expression and function of individual genes and gene networks that reduce cellular phenotypic plasticity—the ability to respond appropriately to intracellular and environmental cues—increasing cellular vulnerability to injury and death [4]. Emerging observations have, in fact, revealed that epigenetic dysregulation is one of the hallmarks of complex disease states, such as cancer [5], autoimmunity [6], and neurodegeneration [3]. Thus, we highlight recent evidence demonstrating the interconnected relationships that exist between epigenetic processes and neurodegenerative disease–causing genes and related pathogenic mechanisms as well as the multilayered profiles of epigenetic dysregulation that have been found in neurodegenerative diseases.

Studying dynamic epigenetic mechanisms that can act in a cell- and tissue-specific manner is not trivial, and large-scale studies of neurodegenerative disease epigenomics have not been performed. Nonetheless, innovative methods and technologies, such as those that leverage next-generation sequencing platforms, are reducing operational and economic barriers to defining neurodegenerative disease-specific epigenomic signatures. We consider examples of these tools and techniques, which have the potential not only for uncovering neurodegenerative disease mechanisms but also for advancement into the clinical arena promoting early diagnosis and the development of personalized prevention and treatment strategies. Diagnostic tools and tailored therapeutic agents targeting specific epigenetic factors and processes have already reached the bedside for selected indications, such as cancer [7–9].

Principles of Epigenetics

DNA Methylation

DNA methylation describes the covalent modification of cytosine residues in cytosine/guanine-rich regions (ie, CpG islands) that are found in gene regulatory elements (eg, promoters) as well as other genomic sites (eg, intergenic regions and repetitive elements) [3]. DNA methylation status is responsible for regulating transcriptional activity at individual gene loci and more globally. Higher levels of DNA methylation are typically associated with transcriptional repression, although activation has also been reported. DNA methyltransferase (DNMT) enzymes catalyze DNA methylation by transferring methyl groups from S-adenosylmethionine to cytosine residues. Factors implicated in demethylating DNA have been identified more recently (ie, DNA excision repair, cytidine deaminase, and Gadd45 proteins) [10]. Methyl-CpG-binding domain proteins bind specifically to methylated DNA and recruit additional transcriptional and epigenetic regulators to these sites.

Chromatin

Chromatin describes the packaging of genomic DNA, histone proteins, and associated factors within the cell nucleus [3]. The basic unit of chromatin is the nucleosome-DNA approximately 147 bp in length enfolding a histone protein octamer, including two of each of the classic histone proteins (ie, H2A, H2B, H3, H4), and linker histones (ie, H1). A series of nucleosomes form secondary and tertiary structures representing varying degrees of condensation including, for example, loosely packaged euchromatin and densely packaged heterochromatin. Changes in chromatin conformations along this continuum can, in turn, modulate the accessibility of regulatory and functional genomic regions to other nuclear factors including those that mediate transcription and DNA replication and repair. Chromatin can be dynamically modified and/or rearranged at the level of the nucleosome by post-translational modifications of histones, replacement of canonical histones by variant histones, and nucleosome repositioning and also at the level of higher-order structures by chromatin remodeling and nuclear compartmentalization [11].

Specific histone modifications are catalyzed in a reversible manner by enzymes with opposing functions, such as histone acetyltransferases (HATs)/deacetylases (HDACs) and histone methyltransferases/demethylases. The spectrum of possible histone modifications includes acetylation and methylation as well as phosphorylation, sumoylation, ADP-ribosylation, and others. Individual histone modifications and their

combinations can be associated with particular genomic elements, such as promoters, enhancers, and the bodies of genes, and with orchestrating specific functions including transcriptional activation or repression and DNA replication and repair. Combinations of histone modifications are thought to form "codes" demarcating functional genomic regions [12]. Transcriptional and epigenetic factors with particular protein domains (eg, Tudor, PHD fingers, chromodomains, and bromodomains), including those that are involved in nucleosome remodeling and repositioning as well as higher-order chromatin remodeling (eg, Polycomb Group [PcG] and Trithorax Group), bind to these histone modifications [13].

Non-coding RNAs

ncRNA regulation describes the actions of RNA molecules derived from the genome but not translated into protein [3]. In humans, 98.5% of genomic sequences are non-coding. These non-coding sequences are largely transcribed, forming diverse classes of ncRNAs, which are more abundant than their protein-coding RNA counterparts [14••]. Transfer RNAs and ribosomal RNAs are two well-known classes of ncRNAs. Many additional classes of ncRNAs have also now been recognized. ncRNAs are classified as those that are "short" or "long" (> 200 nucleotides). Classes of short ncRNAs include microRNAs (miRNAs)—the best studied among these—as well as endogenous short-interfering RNAs, PIWI-interacting RNAs, 3' untranslated region-derived RNAs, and many other emerging classes [15]. Classes of long ncRNAs (lncRNAs) include, but are not limited to, long intergenic ncRNAs and enhancer-like RNAs as well as those that are encoded in the genome in antisense, intronic, and overlapping configurations relative to protein-coding genes [16]. These diverse classes of ncRNAs are associated with distinct biogenesis and effector pathways and have a broad spectrum of functional roles that can include the mediation of DNA methylation, histone modification and higher-order chromatin remodeling, transcription and RNA post-transcriptional processing (eg, alternative splicing), transport, and translation [17]. For example, miRNAs are 21–24 nucleotide ncRNAs derived from the sequential processing of longer transcripts by the Drosha and Dicer endoribonucleases. Mature miRNAs bind to complementary regulatory sequences in target messenger RNAs (mRNAs) preventing the translation of these mRNAs or sequestering them for storage or degradation via the RNA-induced silencing complex [18]. By contrast, lncRNAs are more flexible molecules that can recruit transcriptional and epigenetic regulatory factors (eg, transcription factors, histone-modifying enzymes, and chromatin remodeling complexes) to specific genomic sites, form nuclear subdomains associated with RNA post-transcriptional processing, mediate nuclear-cytoplasmic transport of proteins, and control local translation in synaptic compartments [16, 19].

Epigenetic Dysregulation in Alzheimer's and Other Neurodegenerative Diseases

Profiles of DNA methylation, histone modifications and chromatin remodeling, and ncRNA expression are dynamically regulated during development, adult life, and aging in a tissue- and cell type–specific manner that is highly environmentally sensitive and activity-dependent in the nervous system [3]. Here, we highlight how specific neurodegenerative disease–causing genes can be regulated by these epigenetic phenomena and also how these genes may interact with various epigenetic factors and might, themselves, be involved in modulating epigenetic pathways. Furthermore, we draw attention to the nearly ubiquitous profiles of epigenetic dysregulation that have been found in peripheral and centrally derived tissues from patients with, and animal and in vitro models of, neurodegenerative diseases and to the broad range of studies using gene manipulation paradigms and pharmacologic agents (eg, HDAC inhibitors) to target epigenetic pathways that have demonstrated these strategies have the potential to reverse epigenetic abnormalities and mitigate neurodegeneration [20].

Our understanding of the roles played by myriad epigenetic factors in different neurodegenerative diseases is still evolving, and whether epigenetic dysregulation is involved generally in pathogenesis or rather represents a final common pathway remains unknown. Importantly, the majority of these observations are limited to studies interrogating a single epigenetic mechanism, acting on one gene or a limited number of genes. Future technologies will allow real-time high-resolution genome scale profiling, providing a more complete view of dynamic epigenetic processes within a single cell and between individual cell types within and across different tissue samples and elucidating regulatory and functional relationships between epigenetic processes and disease-causing genes and gene networks and related pathways.

Alzheimer's Disease

Epigenetic processes have many potential roles in the pathophysiology of Alzheimer's disease (AD) [21]. AD-related genes are subject to regulation by DNA methylation. For example, amyloid precursor protein (*APP*) and microtubule-associated protein tau (*MAPT*) gene promoter regions are subject to age-dependent alterations in DNA methylation in the human cerebral cortex that can influence

the transcriptional activity of these genes and potentially mediate ß-amyloid deposition [22, 23]. Further, tissues from AD patients and controls show differential profiles of DNA methylation specifically for AD-related genes, such as *APP* [24], and also more generally [25]. One particularly interesting study examined a set of monozygotic twins discordant for AD and demonstrated significantly reduced levels of DNA methylation in neurons of the temporal neocortex in the AD twin [26]. Another analysis of DNA methylation performed using postmortem brain samples and lymphocytes found evidence of age-specific epigenetic drift in late-onset AD, significant interindividual epigenetic variability associated with genes involved in ß-amyloid processing (ie, presenilin 1 *[PSEN1]* and apolipoprotein E *[APOE]*) and DNA methylation (ie, methylenetetrahydrofolate reductase *[MTHFR]* and *DNMT1*), and the presence of a complex DNA methylation profile at the *APOE* gene including a hypomethylated CpG-poor promoter and a fully methylated 3′-CpG-island associated with the ε4 haplotype [27]. Even in peripheral tissues, such as blood, genes including telomerase reverse transcriptase (*TERT*) exhibit DNA methylation profiles that can differentiate AD patients from controls [28]. DNA methylation and DNMT enzyme abnormalities are similarly present in various models of AD. For example, DNMT activity is perturbed in brain tissues derived from a monkey model of AD, induced by lead exposure during development that leads to the aging-related emergence of neuropathologic features of AD (eg, amyloid plaques) [29]. This striking observation implies that developmental injury is linked to late-onset disease through an epigenetic mechanism. In another model system, murine cerebral endothelial cells, ß-amyloid itself seems to induce global DNA hypomethylation, suggesting the presence of feed-forward dysregulation [28].

Abnormalities in histone and chromatin regulation are also implicated in AD, but they are less well characterized particularly in human tissue. Nonetheless, as in the case of DNA methylation, evaluation of histone modifications and histone-modifying enzyme levels in postmortem AD brain regions has revealed significant differences (eg, H2AX phosphorylation and HDAC6 expression), including those that are found in AD discordant monozygotic twins (ie, H3K9me3) [21]. Corresponding changes in histone-modifying enzyme activity have been shown in model systems, and intriguingly, AD-related genes have the capacity to modulate these enzymes. For example, *APP* forms a complex with lysine acetyltransferase 5 (KAT5/Tip60), a HAT enzyme [30], and *PSEN1* activity influences the function of another HAT enzyme, CREB-binding protein (CBP/p300) [31], which is linked to neurodegeneration [32, 33]. Numerous studies have focused on using HDAC inhibitors and other chromatin-modifying agents in AD models to reverse these abnormalities and even to rescue cells from neurodegeneration [20].

Various miRNAs have been linked to AD through studies that predict and validate regulatory relationships between miRNAs and AD-related genes and pathogenic mechanisms and also studies that profile differential tissue-, regional-, and stage-specific miRNA expression in brain, cerebrospinal fluid, and blood from AD patients and animal models [34]. lncRNAs may also play a role in regulating AD-related genes, as antisense transcripts have been identified embedded within the *APP, MAPT,* ß-site APP-cleaving enzyme 1/2 *(BACE1/2),* anterior pharynx defective 1 homolog A *(APH1A),* and basigin *(BSG/CD147)* gene loci [35]. *BACE1-AS* modulates the expression and stability of *BACE1,* and its levels are elevated in specimens derived from AD patients and AD mice, suggesting that the other antisense transcripts may have similar roles and might be deregulated in AD [36].

Huntington's Disease

Multiple layers of epigenetic dysregulation are implicated in the molecular pathophysiology of HD [37]. The Huntingtin (HTT) protein can directly and indirectly modulate the activity of chromatin regulatory factors. For example, HTT promotes PcG repressive complex 2-mediated H3K27 trimethylation, which is critical for transcriptional repression [38•]. HTT indirectly controls the nuclear-cytoplasmic trafficking of the RE1-silencing transcription factor (REST), a master epigenetic regulatory factor that aberrantly accumulates in the nuclei of cells from HD tissues [39]. REST regulates the expression of both protein-coding genes and ncRNAs, and not surprisingly, miRNA levels are perturbed in the brains of HD patients and HD mice [40, 41]. Further, 80% to 90% of miRNAs in the adult human brain exhibit length and sequence variations, and REST is implicated in modulating the expression of many of these so-called miRNA isoforms (isomiRs), which are significantly deregulated in the frontal cortex and striatum of HD patients [42••]. In addition to miRNAs and isomiRs, an lncRNA transcribed from the Human Accelerated Region 1 genomic locus is also targeted by REST and deregulated in the striatum of HD patients [43•]. Intriguingly, studies performed using peripheral lymphocytes collected from patients with HD have shown that REST activity is increased in these cells, suggesting that this could serve as a peripheral signature for HD [44].

Parkinson's Disease

Like genes associated with AD and HD, genes associated with PD are regulated by epigenetic mechanisms and can also modulate the function of various epigenetic factors.

For example, analyses of the α-synuclein *(SNCA)* gene in substantia nigra, putamen, and cortex specimens derived from patients with idiopathic PD have revealed significantly decreased levels of DNA methylation compared with controls [45, 46]. These observations suggest that decreased DNA methylation leads to an increase in SNCA expression, which promotes PD pathogenesis. ncRNA regulation may also play a role in regulating SNCA. An miRNA, *miR-7,* has been shown to repress SNCA expression and even its toxicity [47]. SNCA is also predicted to be regulated by other miRNAs and by lncRNAs, including antisense transcripts derived from the *SNCA* genomic locus [35]. There is further evidence that suggests SNCA can modulate the function of epigenetic factors. In animal models, SNCA interacts with DNMT1 and can influence the subcellular localization of this DNA methylation enzyme [48•]. Furthermore, when SNCA is targeted to the nucleus, as seems to be the case with certain PD-causing mutations (ie, A30P and A53T), it can bind directly to histones, reduce histone H3 acetylation, and inhibit HAT enzyme activity [49]. Many other miRNAs are also implicated in the pathophysiology of PD through potentially bidirectional interactions with various PD-related factors [50], such as leucine-rich repeat kinase 2 (LRRK2), which negatively regulates miRNA-mediated translational repression [51••].

Amyotrophic Lateral Sclerosis

Epigenetic pathways may also play a role in the pathogenesis of ALS. For example, variants of the elongator protein 3 *(ELP3)* are associated with sporadic ALS by linkage analysis, and genetic manipulation of *Elp3* leads to neurodegeneration [52]. Intriguingly, this factor is implicated in DNA demethylation [53•], has HAT enzyme activity including the promotion of H3K14 and H4K8 acetylation [54], and influences nucleosome positioning [55]. Moreover, ELP3 plays a role in modulating the transcriptional elongation of members of the SSA subfamily of HSP70 genes (*SSA3/4*) through H3 acetylation in the coding regions of these genes [56]. These observations demonstrate how a factor linked to ALS can be important in mediating numerous epigenetic processes, suggesting that genetic variations in this factor may perturb its regulatory functions.

In addition, 43-kDa TAR DNA-binding domain protein (TDP-43) and fused in sarcoma/translated in liposarcoma (FUS/TLS)—factors linked to both sporadic and familial forms of ALS—are generally involved in regulating many aspects of RNA metabolism [57]. These roles are relevant for protein-coding RNAs and ncRNAs. In fact, both TDP-43 and FUS/TLS associate with components of the Drosha and Dicer complexes, which are respectively involved in the earlier and later stages of miRNA biogenesis [58•, [59]. Moreover, TDP-43 knockdown leads to the dysregulation of various miRNAs [60]. Notably, changes in ncRNA expression levels may act as powerful mechanisms for promoting compensatory responses that mitigate neurodegenerative disease processes. For example, *miR-206* is a skeletal muscle-specific miRNA that is strongly upregulated in mouse models of ALS [61••]. It slows the progression of the disease by sensing motor neuron injury and enhancing regenerative responses at neuromuscular synapses.

Methods and Technologies for Epigenetics and Epigenomics

Recent studies, such as the Encyclopedia of DNA Elements Project, have revolutionized our understanding of genomic architecture and the mechanisms responsible for modulating its organization and function [62]. Even the fundamental concept of a gene itself has evolved. It is now clear that, rather than discrete genomic units, genes and gene boundaries and associated regulatory regions are remarkably complex with flexible features that include multiple potential transcription start sites, alternative promoter and enhancer elements, splicing initiation and donor sites, and independently regulated 3′ untranslated regulatory regions. In addition, each nucleotide can potentially give rise to multiple distinct, overlapping, and interleaved transcripts that are regulated distinctly. Sophisticated multilayered epigenetic mechanisms mediate the activity state of each nucleotide and also coordinate real-time gene-gene, inter-allelic, and gene network interactions and communications [3].

Given these rapidly evolving perspectives, it has become apparent that the study of complex disorders, such as neurodegenerative diseases, requires advanced methods and technologies having appropriate resolution for characterizing the interplay between genetic and epigenetic pathogenic processes. There is a spectrum of techniques currently available for characterizing locus-specific epigenetic modifications. Many of these approaches can also be coupled with hybridization (eg, microarray) and existing and next-generation ultra high throughput sequencing technologies to define genome-wide and even whole genome epigenomic profiles. Each strategy offers distinct advantages and disadvantages in terms of sample preparation, speed, genomic coverage and resolution, and cost-effectiveness.

DNA Methylation

Techniques for detecting DNA methylation are the most highly developed and include those based on 1) bisulfite conversion, 2) enrichment of methylated DNA, and 3)

methylation-sensitive and -insensitive restriction endonuclease digestion. Bisulfite conversion is the most conventional among these and considered to be the gold standard because of its very high resolution—1 bp. Treatment of DNA with sodium bisulfite converts unmethylated cytosine residues into uracil, and subsequent analyses that target specific regions of interest, or less biased procedures, can be used to determine the methylation status of particular cytosines. In fact, the methylation status of the entire genome can be deciphered by combining bisulfite conversion with sequencing [63]. However, because of the high costs currently associated with very large amounts of sequencing, various approaches that have been developed focus on performing more limited sequencing of genomic regions selected semi-randomly (eg, reduced representation bisulfite sequencing [64]) or in a more targeted fashion (eg, using genomic site–directed "padlock probes" [65]).

Alternatives to bisulfite conversion have been developed because this approach typically requires large quantities of sample DNA that can degrade significantly with chemical treatment, can be limited by incomplete conversion of every single unmethylated cytosine to a uracil, and cannot discriminate between methylcytosine and hydroxymethylcytosine. DNA methylation can also be assessed by techniques based on enrichment of methylated DNA and subsequent analysis of these enriched sequences using various methods (eg, microarrays and sequencing). Enrichment can be performed with immunoprecipitation using an antibody that selectively recognizes methylated cytosines (ie, methylated DNA immunoprecipitation) or with affinity purification utilizing methyl-binding protein domains as ligands (eg, methylated DNA capture by affinity purification or methylated-CpG island recovery assay) [66]. DNA methylation can also be determined with techniques based on methylation-sensitive and -insensitive endonuclease digestion. Treatment with methylation-sensitive and -insensitive isoschizomers, restriction enzymes that recognize the same sequence, followed by comparative analysis of the resulting fragments utilizing various methods (eg, microarrays and sequencing), allows the determination of the methylation state of restriction sites. The HELP (HpaII tiny fragment enrichment by ligation-mediated PCR) assay is a prime example of this approach [67].

Recent studies comparing DNA methylation mapping methods and technologies have demonstrated that they generate highly concordant results, suggesting these diverse approaches are complementary to each other [68, 69].

Chromatin

There is a range of techniques available for assessing the presence of histone modifications and of proteins associated with particular structural and functional chromatin states as well as for measuring chromatin accessibility and nucleosome dynamics.

These methodologies are primarily based on enriching for genomic sequences associated with particular chromatin states utilizing chromatin immunoprecipitation (ChIP), a powerful paradigm used for identifying DNA-protein interactions. ChIP can be performed with an antibody that recognizes an epitope, such as a specific histone modification, and characterization of ChIP-enriched DNA sequences can then be used to construct a map defining the distribution of the histone modification throughout the genome. This technique has recently been used to define the profiles of 38 different histone modifications that are present in human $CD4^+$ T cells [70, 71]. Several protocols for ChIP assays and associated analysis of enriched DNA have been developed via approaches that are coupled with microarrays (ie, ChIP-chip) and sequencing with older and newer sequencing platforms, including so-called ChIP-serial analysis of chromatin occupancy (ChIP-SACO) and ChIP-sequencing (ChIP-Seq), respectively. However, the utility of these methods is not limited to identifying profiles of histone modifications. Antibodies that recognize various DNA- and chromatin-binding proteins including histone-modifying enzymes, chromatin remodeling complexes, and non-histone proteins associated with particular structural and functional chromatin states (eg, transcription factors and RNA polymerases) can similarly be used to create maps of their distributions throughout the genome.

There are also complementary techniques that can be used to determine chromatin accessibility and nucleosome dynamics. For example, chromatin accessibility can be detected by taking advantage of the hypersensitivity of open chromatin to deoxyribonuclease I (DNase I). Thus, DNase I digestion and sequencing of the resulting DNA fragments (DNase I–Seq) can define genomic regions of accessible chromatin [72, 73]. Sonication followed by sequencing (Sono-Seq) can similarly be employed because open chromatin is also hypersensitive to shearing by sonication [74]. FAIRE–Seq (formaldehyde-assisted isolation of regulatory elements followed by sequencing) is an approach that utilizes the differential solubility of open chromatin in the aqueous phase of phenol-chloroform extraction to enrich for DNA fragments associated with open chromatin and, thus, to define genomic maps of chromatin accessibility [75].

In addition, there are methods for characterizing nucleosome positioning and turnover. For example, nucleosome positioning can be detected by taking advantage of the capacity for nucleosomes to prevent associated DNA from being digested by micrococcal nuclease. Thus, micrococcal nuclease digestion and sequencing of the preserved DNA fragments (MNase-Seq) can be used to define maps of

nucleosome localization throughout the genome [76, 77]. By contrast, nucleosome turnover kinetics can be dynamically monitored by metabolically labeling newly synthesized histones with azidohomoalanine, a methionine analogue, and an affinity tag, such as biotin. Purification of labeled nucleosomes and associated DNA followed by microarray or sequencing analysis allows the rates of nucleosome turnover across the genome to be estimated based on the extent of newly synthesized histones incorporated at each site. This method has been termed CATCH-IT (covalent attachment of tags to capture histones and identify turnover) [78•].

Non-coding RNAs

Innovative approaches, particularly those employing combinations of ultra high throughput sequencing platforms and increasingly sophisticated computational and bioinformatics tools, have already revolutionized the study of DNA and RNA by enabling whole-genome, whole-exome, and whole-transcriptome analyses. In fact, the recent discovery of various classes of ncRNAs has been driven largely by the adoption of ultra high throughput RNA sequencing (RNA-Seq). Even more powerful next-generation technologies will dramatically lower barriers to performing these studies by reducing the sample quantities, time, and financial and other resources required for sequencing. These approaches will provide the means to interrogate transcriptional activity in a single cell with very high resolution. For example, emerging nanotechnology-based sequencing platforms allow the passage of one nucleic acid molecule through a nanopore and utilize various methods (eg, chemical and electrical) to detect the identities of the nucleotides. In comparison with existing techniques, nanopore sequencing has distinct advantages, such as relatively inexpensive protocols for sample preparation and straightforward readouts that eliminate the need for enzymes, cloning, and amplification [79].

In addition to sequencing, other modalities are also becoming available for studying the life cycles of RNA molecules, such as ncRNAs, in mammalian cells including approaches for imaging and analyzing RNA transcription, post-transcriptional processing, and trafficking. For example, real-time imaging of transcription in living cells can capture the act of transcription including the kinetics of RNA polymerase movement, the association of transcription factors, and the progression of the polymerase on the gene. Some promising approaches use nanoparticles [80] or antisense oligonucleotides, such as those labeled with radionuclides [81] or so-called molecular beacons, unimolecular stem-loop structures that very efficiently couple target recognition with specific fluorescent signals [82]. These strategies can even be coupled with existing imaging modalities such as positron emission tomography or MRI to noninvasively image cerebral RNAs in live animals [81, 83].

Conclusions

The study of human diseases was transformed by the advent of genetics and genomics, and it is now poised for the next stage in this revolution because of recent advances in epigenetics and epigenomics [84]. Our understanding of the pathogenesis of many complex disorders has already begun to evolve from a gene-centric view to one that also accounts for chromatin structure and function on a whole-genome scale. Major international efforts, such as the Human Epigenome Project, have even been launched to define these epigenomic states [85]. Large initiatives and more modest ones like those that we have highlighted will no doubt continue to uncover integrative profiles of epigenetic and epigenomic dysregulation both in models of, and in samples derived from patients with, neurodegenerative diseases.

There are many questions yet to be answered regarding the contributions of these peripherally and centrally derived tissue signatures to the pathobiology of neurodegenerative diseases and their interplay with genomic, transcriptomic, proteomic, metabolomic, and other factors. Nonetheless, molecular diagnostic tools permitting the rapid, accurate, and relatively inexpensive characterization of the epigenome and its dynamic variations are emerging, and they offer both basic scientists and translational researchers the potential to identify novel biomarkers for predicting disease risk, onset, progression, and response to treatment. Moreover, epigenomic tools can also be coupled with emerging methods and technologies, such as combined "high-throughput-high-content cellular screens," which aim to contextualize data gathered from diverse approaches into functional biological and brain networks that can be utilized in the discovery of truly personalized therapeutic targets and compounds [86].

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- • Of importance
- •• Of major importance

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