Epigenetic Modifications and Neurologic Disease

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The study of *epigenetics* is opening new fields of biomedical research in neurologic function and disease. Epigenetics refers to “modifications [of the genome] that result in heritable changes in gene expression that are independent of changes in the genetic sequence “ (Probst et al., 2009). In this process, cells with identical genomes acquire distinct phenotypes, as in normal and abnormal development, cell differentiation, susceptibility to or pathogenesis of disease, neoplasia (Narayan & Dragunow, 2010) and synaptic activity-dependent neuron tagging in long-term memory (Lesburgueres et al., 2011). Both endogenous cellular factors and exogenous environmental factors may induce normal or abnormal regulation of gene expression This essay will point out the central biochemical processes and a few examples with implications for neurologic disease.

Gene expression is dependent on relaxation of the dense chromatin structure or nucleosomes, which consist of a protein core of histone octamers (a tetramer of H3 and H4 and two dimers of H2A and H2B) about which a length of DNA (about 146 base pairs) is wound tightly in two turns “like a thread around a spool” (Alberts, 2007). Modifications of these chromatin, nucleosome and DNA structures through actions of cytoplasmic factors regulate the differential expression of genes. The totality of these cellular factors impacting on the gene has been labeled the ‘epigenome’ (Qureshi & Mehler, 2010a).

The three levels of reactions modifying gene expression are (1) DNA methylation, (2) modifications of the histone cores, and (3) RNA-based regulatory paths, including noncoding (nc)RNAs such as microinterfering (mi)RNAs. (See Chapter 27 for details of transcription.)

Transfer of methyl groups from S-adenosylmethionine

Transfer of methyl groups from S-adenosylmethionine (SAM)to cytosine in DNA, catalyzed by DNA n-methyltransferases (DNMTs), occurs on cytosine residues in CpG-rich regions of the genome. Cytosine methylation interferes with binding of transcription factors to DNA, which may lead to decreased expression. However, these methylated cytosines also recognize methylCpG-binding proteins (MeCP1 and 2), which may recruit coactivator and/or corepressor factors. Activation or repression of a given gene may thus affect negative or positive transcription factors with respect to other genes (Narayan & Dragunow, 2010; Qureshi & Mehler, 2010b). For example, elevated levels of DNA methylation found in ischemic rodent brain are thought to promote neural cell death.

DNA methylation regulates diverse cellular processes, including genome stability, genomic imprinting and X-chromosome inactivation in females, and these processes are particularly relevant to normal embryologic development and regenerative mechanisms (Qureshi & Mehler, 2010a). The role of methylation in the nervous system is pleiotropic. For example, B12 and folate are essential cofactors for regeneration of methionine, precursor to SAM, and both are essential for normal DNA methylation. Deficiencies of B12 and folate can be factors in depression and other behavioral disorders as well as in producing neural tube defects (see Spina Bifida, see Ch. 28). Dietary supplements of B6, B12 and folate to raise methionine levels and DNA methylation have dramatically reduced the incidence of spina bifida and may be useful in treatment of depression, while their use in treatment of AD gave mixed results (see Narayan & Dragunow, 2010).

Modification of histones within the nucleosome

Modification of histones within the nucleosome occurs at lysines in their N-terminal. Histone modifications on lysines may be acetylation, methylation, phosphorylation,

ubiquitination, ADP ribosylation, carbonylation, SUMOylation, glycosylation or biotinylation. These reactions also may have net positive or negative effects on expression of a given gene depending on which class of histones is modified and the position of these modifications. There is, in essence, a complex histone code since there are four isoforms, and for the most studied H3 isoform, there are three alleles. For H3 alone, about 150 different modifications have been shown (Garcia et al., 2007).

Histone acetylation and phosphorylation generally facilitate activation of a gene by decreasing attraction between histone and DNA, thereby relaxing nucleosome structure and allowing freer access of transcription factors. Histone phosphorylation adds a negative charge to the molecule, which results in a repulsion between the histone and DNA. Phosphorylation of the histone is itself subject to complex regulation by various protein kinases and phosphatases that are ultimately responsive to extracellular signals.

Histone acetylation of a lysine residue neutralizes its otherwise positive charge, decreases its affinity for DNA and relaxes the nucleosome/chromatin structure. The balance between acetylation and deacetylation derives from the activities of histone acetyl transferase (HAT) and histone deacetylases (HDACs), of which 18 different isoforms are categorized into four classes in human (Mai, 2007). Some HAT and the HDACs act also on cytoplasmic substrates other than histones, such as tubulin (see Ch. 6). Histone acetylation is usually activating; when the gene activator CREB is phosphorylated, it recruits HAT to activate gene transcription, an activating effect that is antagonized by HDAC (see in Ch. 27).

Thus, considerable attention is focused on investigating HDAC inhibitors as potential pharmacologic tools in therapy (Fischer et al., 2010). Frequently used HDAC inhibitors are valproic acid (VPA), trichostatin A, sodium or phenyl butyrate and curcumin, with newer more

selective inhibitors continually being introduced. While this research is still in its early stages, some encouraging results have been reported in brain ischemia and traumatic brain injury (see references in Gibson & Murphy, 2010), mouse models of spinocerebellar ataxia 3 (Chou et al., 2011) and Huntington's disease HD (Thomas et al., 2008). VPA has been used in exploratory clinical trials in humans with some positive effects in fragile X syndrome (Torrioli et al., 2010) and X-linked adrenoleukodystrophy (ALD) (Fourcade et al., 2010). In ongoing research, evidence for epigenetic factors at all three levels mentioned above has been found in Alzheimer's disease (Coppede & Migliore, 2010) and in a variety of developmental diseases involving epilepsy in humans, mental retardation, X-linked syndromes, particularly fragile X syndrome, and thalassemia (Qureshi & Mehler, 2010c). The variety of histone isotypes, the diverse array of chemical modifications and the numbers of enzymes involved indicate the complexity in finding specific drugs to target a specific desired effect (Narayan & Dragunow, 2010; Qureshi & Mehler, 2010a; Dietz & Casaccia, 2010).

Microinterfering RNAs

Microinterfering RNAs (miRNA) represent the third class of epigenetic modifiers in the epigenome. They are the best characterized of the ncRNAs. These single-stranded, noncoding RNA segments of 19–25 nucleotides in length are derived in the cytoplasmic processing of noncoding RNA translocated from the nucleus (see Ch. 27). In the cytoplasm, miRNAs interfere with the translation into proteins of many mRNA molecules. Evidence for miRNA-induced dysregulation of gene expression has been described in Tourette's syndrome, fragile X mental retardation, HD, AD and schizophrenia (see Narayan & Dragunow, 2010; Qureshi & Mehler, 2010c for comprehensive reviews). Mutations in mitochondrial ncRNAs are implicated in mitochondrial encephalopathies. Differentially expressed profiles of miRNAs found in ischemic

brain correlate with their target mRNAs, suggesting that miRNAs regulate various processes in postischemic brain. For a discussion of the complex subject of RNA-based epigenetic pathways in stroke, see in Qureshi and Mehler (2010b).

Enzymatic aberrations related to polyQ-htt

In HD, a number of enzymatic aberrations related to polyQ-htt (huntingtin) may lead to dysregulation of gene expression (Ch. 48). The long expansions of glutamine residues in polyQ-htt have been reported to sequester and decrease CREB binding protein/HAT activity, thereby decreasing DNA acetylation and CREB activation This conclusion is supported by results of experiments with *Drosophila* (see in Pallos et al., 2008). There is also evidence for increases in transglutamination (TG2) activity induced by the long expansions of glutamine producing increased protein cross-linking transaminations on lysine residues that may interfere with transcriptional regulation (see box, Ch. 48). Buckley, et al. (Buckley et al., 2010) have adduced evidence that levels of BDNF mRNA in cells *in vitro* and in HD mouse model brains are positively responsive to wild type htt but not to polyQ-htt and that BDNF levels are reduced in postmortem HD brain.

The nascent understanding of epigenetic programming is leading to development of new pharmacologic tools for investigating normal and pathologic neurobiological processes as well as for therapy of neurologic disease, such as HD and other neurodegenerative and developmental diseases (Narayan & Dragunow, 2010; Fischer et al., 2010; Qureshi & Mehler, 2010c).

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