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Molecular epigenetics, chromatin, and NeuroAIDS/HIV: Translational implications

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Abstract:
We describe current research that applies epigenetics to a novel understanding of the immuno-neuropathogenesis of HIV-1 viral infection and NeuroAIDS. We propose the hypothesis that HIV-1 alters the structure-function relationship of chromatin, coding DNA and non-coding DNA, including RNA transcribed from these regions resulting in pathogenesis in AIDS, drug abuse, and NeuroAIDS. We discuss the general implications of molecular epigenetics with special emphasis on drug abuse, bar-codes, pyknons, and miRNAs for translational and clinical research. We discuss the application of the recent recursive algorithm of biology to this field and propose to synthesize the Genomic and Epigenomic views into a holistic approach of HoloGenomics.

Keywords: epigenetics; hologenomics; coding and noncoding DNA; HIV-1; AIDS; NeuroAIDS; molecular medicine paradigm-shift; translation-clinic

Background:
In the era of highly active antiretroviral therapy (HAART) many factors, including host genetics, HIV strain variation, and comorbidities, including drug abuse and co-infection with hepatitis C virus (HCV) contribute to the pathogenesis of AIDS and NeuroAIDS **[1, 2, 3]**. Drug abuse is considered by many to be an important cofactor in HIV pathogenesis and the issue of laboratory data support for the deleterious effects of abused drugs on peripheral and brain HIV-1 infection impacts on the approaches to combat these diseases. Over several decades, laboratory studies showed there are *in vitro* effects of drug abuse (e.g. cocaine, opiates, and amphetamines) on brain and immune cell function. However, there have been mixed findings in epidemiological studies designed to detect deleterious effects of abused drugs *in vivo* **[4]**. Some studies, for example, have demonstrated exacerbating effects of abused drugs on HIV peripheral and brain disease **[2, 5, 6]**. HIV-1 strain variation is also important for possible vaccine strategies against HIV-1 reservoirs in the peripheral and central nervous system (CNS) **[7]** and so the interactive effects of HIV strains, drug abuse, and host genetics **[1, 2, 3, 7]** impacts on the methods designed to combat AIDS/NeuroAIDS. Key issues that remain to be addressed are the effects on non-coding DNA and this could prove to be a productive avenue of basic and translational research in addition to those approaches that have focused on classical coding genes.

Deleterious effects may be transmitted through the chromatin within non-coding and coding DNA. Drug abuse and HIV infection appear to exert effects derived from and directed by promoter, non-coding inter-exon, and across intergenic sequences. Early studies showed that chromatin remodeling was involved in HIV-1 basal transcription as well as in response to TNF-α **[8]**. The HIV-1 Tat protein is essential for the initiation of HIV transcription from the integrated HIV genome (provirus) through exploitation of host proteins and recent evidence also points to pathways that lead to chromatin remodeling as a component of HIV transcription. Putative pathways studied include bromodomain-binding chromatin modifying complexes such as p300/CBP, p/CAF, cdk9/cyclin T, and SWI/SNF complexes that via chromatin/nucleosomes affect the proximal promoter and ORF regions of the HIV-1 genome **[9]**.

Susceptibility to HIV Associated Dementia (HAD) is governed by many immune-related genes including chemokines as discussed in **[10]** (and below), and the mechanisms involved

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may include mutated non-coding DNA sequences (e.g. chemokine and TNF-α untranslated regions UTRs)). We also discuss the Principle of Recursive Genome Function **[11]** in that it subsumes most of the epigenetic mechanisms discussed here.

Data sources for epigenetics

Since the beginning of the AIDS epidemic, many approaches have been utilized in HIV research. Although epigenetics is not a new field it has been relatively neglected in AIDS research. However, it is becoming increasingly prominent. Searches of the NIH CRISP indicate that in 2008, there are 300 NIH funded grants that focus on epigenetics, of which 10 are on epigenetics and HIV, 10 on epigenetics and AIDS, and 37 on epigenetics and the brain. Web-sites used in our studies included: NIH CRISP: http://crisp.cit.nih.gov/; OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM; OMIM Morbid map: http://www.ncbi.nlm.nih.gov/Omim/getmorbid.cgi.

NeuroAIDS description

The AIDS epidemic provides neuropathologists, neurologists, and neuroscientists with a multitude of neurological complications that require study and suggests the importance of epigenetics and the "beyond genome approach" in this field. In the opinion of many, the most interesting of these complications was and remains HAD. Even prior to the advent of HAART in 1996, HAD was problematic for neuropathologists. HIV-positive multinucleated giant cells were considered the hallmark of HIV encephalitis (HIVE) and HIVE was generally thought to be the neuropathologic substrate of HAD. However, prior to HAART, in some studies, only 50% of patients with HAD had HIVE at autopsy; furthermore, there was little correlation between the neurocognitive status of the patient and the amount of virus in the brain as determined by immunohistochemical detection of gp41 **[12]**. Conversely, there was a highly significant correlation between the number of activated macrophages and microglia in the brain and the severity of neurocognitive impairment.

NeuroAIDS and HAART

In the HAART era, it has been considered that HAD and the milder but still disabling syndrome of minor cognitive-motor disorder (MCMD) remain a significant problem, in part because of the increased longevity of those who suffer from these diseases **[13]**. However, the previously weak correlation between the amount of virus in the CNS and the presence of cognitive impairment has become even more tenuous. The reason for this apparent disconnect is uncertain. Some patients, whose neurocognitive dysfunction is progressive at death, still have no detectable virus in CSF or brain **[14, 15, 16]**. Moreover, it is likely that the CNS acts as a reservoir for low (undetectable) levels of virus since many of the drugs used in HAART poorly penetrate the blood-brain barrier with incomplete recovery **[17, 18]**. Furthermore, although some studies **[18]** emphasize the beneficial effects of HAART, the experience of other investigators is that for many HIV-1 infected individuals, exposure to HAART has modified but not eradicated the deleterious effects of HIV-1 on neurocognitive functioning **[16]**. How can very low or nonexistent levels of virus cause progressive damage found in some patients? Alternative explanations exist and one possibility is that the virus triggers cascades of neurotoxic and inflammatory events that are perpetuated even when virus may have been cleared. We propose the hypothesis that epigenetic mechanisms are involved in these processes.

NeuroAIDS and the new paradigm shift in Molecular Biology

Setting the stage for the important role of the interplay of epigenetics and genomics in the pathogenesis of neuropsychiatric diseases, including NeuroAIDS, requires a quantum paradigm shift in our basic understanding of molecular biology. This has been accomplished as described in detail by Pellionisz **[11]** who proposed a new information flow recursion process paradigm in all living systems. This theory is termed the Principle of Recursive Genome Function and its promulgation supercedes two dogmas or axioms concerning the genome that were central to biology and molecular medicine for the last 38 years. These axioms were fundamental flaws in the molecular approach to Translational Medicine and were 1. Ohno's promulgation of the concept that the 99% of the genome that is non-coding is garbage or junk DNA and 2. Crick and Watson's contention that there is no flow of information from proteins to RNA and DNA **[19, 20, 21]**. The work of McClintock (transposons), Baltimore and Temin (reverse transcriptase), and Prusiner (prions), became counter-examples and demonstrated the limiting nature of these two hypotheses.

From the viewpoint of the Principle of Recursive Genome Function, we now discuss one of these findings, the groundbreaking work done in the 1940's by Barbara McClintock **[22]**. This work led to the concept of mobile elements in the genome that can influence subsequent gene expression. This work was inconsistent with the axioms and hypotheses generated by Ohno, Crick, and Watson and actually predate their work and hypotheses by a decade. In fact, McClintock's work remained at odds with the molecular biology establishment for some forty years and was finally recognized with McClintock's Nobel Prize in 1983 **[23]**. McClintock's insight was her appreciation of the ability of the genome to respond to various insults including X-rays and insect parasitism mediated by transposable elements that were components of the non-coding part of the genome, including reiterated sequences. McClintock points out in her 1983 Nobel lecture **[23]**, the importance of effects exerted by non-coding portions of the genome on the coding portions of the genome. Furthermore, she remarks on her findings that some prior genome-level event takes several generations to result in full

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phenotypic expression. Her pioneering work was a cardinal, yet for many years largely ignored precursor of the Pellionisz principle and further supports the plasticity and utility of genome function of both coding and non-coding portions of the genome in their interplay with the environment.

Another important central case supporting the importance of a holistic approach inclusive of non-coding DNA and epigenetics is mRNA interference (miRNA), an evolutionarily conserved process by which eukaryotic cells regulate coding DNA expression from their non-coding DNA utilizing small, double-stranded RNAs to degrade target mRNAs in a sequence-specific manner. Moreover, antisense transcripts are involved in the regulation of miRNA and result in both activation and inhibition of mRNA transcription **[24]**. Transcriptional and post-transcriptional gene silencing by miRNAs can thus lead to degradation of cellular, as well as viral RNAs, including RNA from HIV-1 in the CNS. Additionally, this understanding of fundamental mechanisms provides a rational basis for double stranded small inhibitory RNAs (siRNAs) transfected into specific cells to target particular RNAs. Using hairpin motifs, siRNAs are expressed intracellularly using molecular therapeutic vectors. This potent translational evidence-based epigenetic intervention has been envisaged as a technology to counter, for example, HIV-1-induced encephalopathy **[25]**. In addition, the miRNA silencing machinery has been shown to have an important molecular physiological role in HIV-1 infection by regulating viral replication as well. This epigenetic process is associated with chromatin assembly, repair, and remodeling and it was shown to be dependent upon histone acetyl-transferase Tat cofactor **[26]**.

Molecular signature concepts and variations

The concept of nucleic acid and amino acid molecular signatures has been used in molecular biology to predict virus associations with NeuroAIDS as reviewed **[27]**. This approach was extended to information at the DNA level and the term recently used is 'bar-code'. Bar-codes are DNA thematic signature sequences that are hypothesized to represent epigenetic functions related to cell and organ specificity, viral evolution, and disease pathogenesis. It follows as well that DNA bar-codes may play a central role in evolutionary biology and species specificity **[28]**. A recent analysis of human genomes supports this approach and identifies many possible such bar-codes in the fundamental genomic modulation in the cellular responses to viral infections **[29]**. Additionally, the bar-code approach is useful to identify multiple disease markers **[30]**.

Related to and perhaps conceptually overlapping with bar-codes are 'pyknons', short blocks of DNA sequences in non-coding segments of the human genome. The term 'pyknons' is derived from the Greek (πυκνοσ) "piknos" meaning serried, dense, or frequent **[31]**. More than 380,000 non-overlapping regions of the human genome contain 60-80 nucleotide-length pyknons. The cell processes associated with these pyknons are likely to include cell communication, transcription, and regulation of transcription, signaling, and transport. It was suggested that pyknons subsume 40% of miRNAs identified to date. It is therefore inferred that pyknons may be involved in gene regulation or silencing as part of the normal cell's control of transcription.

Thus, short segments of non-coding DNAs that include bar-codes and pyknons and that produce miRNAs play an important role - not yet fully elucidated - in fundamental molecular processes **[32]**. In addition, these advances greatly improved the understanding, development, and use of siRNA technology to mediate mechanisms to silence genes related to HIV/NeuroAIDS **[24]**.

Studies on gene expression also revealed possible epigenetic processes at work in NeuroAIDS. Briefly, gene expression was analyzed in cultured human neurons treated with cocaine and HIV-1 proteins Tat and envelope (Env) **[33]**. This serves as a model for Neuropsychiatric disease including Dementia in NeuroAIDS due to HIV-1 infection compounded by drug abuse. At a $p < 0.0005$, of 12,500 genes examined, 35 genes were significantly expressed. The functions of these genes included control of transcription, immune-communication, and signaling. The gene locations were mapped and found not stochastically arranged on the human chromosomes. Furthermore, the 35 genes were in proximity to genes known to be associated with neuropsychiatric diseases. Several hypotheses resulted from this work including that the expression of the genes identified was perturbed by the abused drug, cocaine, and HIV-1 proteins, Tat and Env; that these genes were influenced in transcriptionally isolated groups; and that transcription overload (coerced expression due to cocaine and HIV-1 proteins) may result in damage to the chromosome's organization and control of the chromatin transcription machinery (chromatin remodeling). It should be noted that an association of HIV and host-related genes was found in additional studies **[34, 35]**.

Drug abuse and NeuroAIDS Epigenetics

Drug abuse and HIV infection appear to exert effects derived from and directed by promoters, non-coding inter-exon stretches of DNA, and across intergenic DNA sequences. Briefly, experimental evidence and hypotheses support the concept that deleterious effects may be transmitted through chromatin as well as non-coding and coding DNA. Moreover, it should be noted further that susceptibility to HAD is governed by several immune-related genes including chemokine genes and that these effects may also be propelled by intergenic or non-coding sequences including their promoter regions **[1, 2, 10, 11, 35, 36]**. These are examples of the involvement of non-coding DNA sequences (including chemokine and TNF-α UTRs) involved in NeuroAIDS. Epigenetics and risk for HIV-associated neurocognitive disorders (HAND) has been further reviewed by Levine and

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colleagues **[36]** with a focus on genetic polymorphisms including single nucleotide polymorphisms (SNPs) and variable number tandem repeats (VNTRs). Reviewed were polymorphisms in a variety of cytokines and their receptors (e.g., SDF, MCP-1, MIP-1α, MIP-1β, IL-2, CCR5, and TNF-γ) that have been shown to be associated with increased risk for HAND and/or progression to AIDS. In addition, recent findings indicate that polymorphisms in dopamine-related genes (e.g. DAT-1 and DARPP-32) may be associated with increased risk of HAND (A. J. Levine, *personal communication*, 2008). We also hypothesize that additional related genes with non-coding DNA mutations that are implicated in possible association with HAD and HAND include dopaminergic function, metabolism, and growth factors (e.g. DRD2, DRD4, COMT, DBH, BDNF, and ApoE). It is an expected result that all of these genes have polymorphisms in their non-coding portions and thus are in the epigenetic category that could relate to the risk and pathogenesis of HAD and HAND. This is because the occurrence of SNPs in the human genome is approximately 8-9 per 10 Kb of genome **[37, 38, 39]**. Above and beyond the prior discussion of the shortcomings of the Ohno, Crick, and Watson hypotheses in molecular biology, it is of great interest to note that the rate of SNPs in the genome is increased in miRNA domains compared to the ambient genome **[38]**.

Thus, the evolution and function of different parts of the genome has clearly occurred at differing rates, including parts of the non-coding portions as, clearly unanticipated in the two prior hypotheses in molecular biology. Current work on genetic and biochemical pathways demonstrates the importance of non-coding DNA and epigenetics and epigenetics-based drugs for translational success in the clinic.

Conclusion:

In conclusion, AIDS and NeuroAIDS are extremely complex diseases that, once initiated by HIV-1 infection, may be propelled by events that take place at the level of intergenic and non-coding DNA, and transmitted through the chromatin as well as at the classic level of genes and signaling. We propose that research on the epigenetic effects on intergenic DNA and chromatin be expanded greatly. Moreover, the new Principle of Recursive Genome Function provides a more holistic approach for the application and translation of molecular biology into the clinic. After all, the defeat of disease requires addressing the entirety of the human genome and epigenetic factors that interact with each other as well as with the environment. Thus, the discussion presented here makes a compelling case for emerging HoloGenomics and its impact on host and viral interactions that determine the natural history, effect of treatment, and prognosis of AIDS and NeuroAIDS.

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