The Prospects of Epigenetics in Neuroscience

One of the known features of neuroprogenitors at early embryonic stages is their ploripotent property in generating diverse cell types during the brain development. Although the Darwinian theory of evolution could explain the origin of the species diversity based on random genetic mutations and natural selection, but it could not explain the cellular diversity in a single organism. In fact, after the discovery of DNA structure it became clear that the genetic material of all cells is the same in any specific organism. Waddington in1939 suggested that the same genetic materials may have diverse phenotypic outcome in different environments. He proposed the theory of epigenetic (means at the top of genetic) to explain the origin of cellular differentiation and the establishment of structural and functional characters of different cells in every organism (Waddington 1939). After decades advanced molecular techniques such as DNA methylation, histone modification, RNA editing and RNA interference (e.g. Micro RNA) were discovered to explore the molecular mechanisms of epigenetic regulation (reviewed in Abdolmaleky et al., 2004, 2008 and 2011a).

In mammals, DNA methylation is a simple reaction of addition of a methyl group to cytosines which are followed by guanine (CpG). However, recent reports indicate that adenine methylation is also common in embryonic cells (Lister et al., 2009) and in the adult brain cells, at least in a number of genes such as HTR2A promoter (Abdolmaleky et al., 2011b). While choline is the main source of methyl groups and s-adenosyl methionine (SAM) is the major methyl donor, several types of DNA methyltransferase (DNMT) are also involved in catalyzing DNA methylation reactions. Vitamin B12 and folic acid are also involved in remethylation of the demethylated Methionine (reviewed in Abdolmaleky et al., 2004 and 2008). Following DNA replication DNMT1 methylates the unmethylated DNA strand. DNMT3a and DNMT3b are considered to be de novo methyltransferases under the influence of intrinsic or extrinsic factors in the living cells (Bestor 2000; Kim et al., 2002). DNA methylation of the genes promoter regions promote binding of methyl-binding proteins to methylated DNA which generally inhibits transcription of that specific gene in the cells and the degree of DNA methylation defines and fine-tunes gene expression level (Bird 2002 and 2008). Although the established DNA methylome is erased during early embryonic stage to generate pluripotent cells for subsequent differentiation and tissue generation, the underlying mechanisms of remembering the tissue-specific DNA methylation pattern remained unknown (Bird 2002; Monk 2005; Russo 2006). The same is true for tissue-specific histone modifications mediated by histone acetyltransferases, histone deacetylases, histone methyltransferases and other enzymes/factors which regulate gene expression (Kouzarides and Berger 2007; Shilatifard 2008). RNA interference is among the lately discovered epigenetic mechanisms mediated by Micro RNAs (miRNAs) which bind to and degrade transcribed RNAs to prevent unnecessary protein synthesis (Park and Tang 2009). Regarding the complexity of epigenetic regulation, it should be noted that; while each miRNA can target hundreds of genes and each gene can be regulated by several miRNAs, the interplay of all epigenetic mechanisms determines common pattern of gene expression regulation (Vaissière et al., 2008).

Currently an extensive set of efforts is taking place to uncover other epigenetic marks and modifiers, as epigenetic aberrations become known as one of the major players in the pathogenesis of cancer, cardiovascular, neuropsychiatric and other complex diseases (Roach et al., 2011). Complex diseases tend to be multifactorial and/or polygenic in nature and most of the neurological and psychiatric disorders are considered to be complex and remained incurable in spite of huge progress in medical science in the last few decades. As pure genetic studies _using advanced high throughput techniques such as whole genome mutation_ analysis failed to find underlying pathogeneses of the complex neuropsychiatric diseases (e.g. Purcell et al., 2009), neuroscientists have been inspired to look for epigenetic aberrations; particularly because, most of these diseases do not show Mendelian pattern of heritability. Furthermore, they usually have episodic nature and often complete recovery/remission for many years (e.g. mood disorders and multiple sclerosis) which are not anticipated in genetic diseases. In fact, since the organic bases of DNA (genetic codes) are fixed and stable, genetic diseases often have an early age of onset and would follow chronic/progressive patterns. However, because epigenetic codes are flexible and dynamic, an episodic nature of illness presentation could be more compatible with an epigenetic dysregulation as the result of an intrinsic or extrinsic insult and/or environmental factors (Abdolmaleky et al., 2011a).

In order to conduct epigenetic analysis, scientists adopted almost all the current genetic technologies for epigenetic studies. For example, Bisulfite DNA sequencing is a modification of traditional sequencing after treatment of genomic DNA by bisulfite which converts unmethylated cytosines (but not methylated cytosines) to Uracil. The Uracil residues will be converted to Thymine with subsequent NAOH treatment (Frommer et al., 1992). Thus, one can design primers to amplify the modified DNA for sequencing or PCR analysis (Li and Dahiya 2002). Methylation specific PCR (MSP) is an adapted technique for amplification of unmethylated or methylated DNA modified by bisulfite, using primers specific for unmethylated or methylated DNA template in separate PCR reactions (Herman et al., 1996). The same DNA template and primers can be used for quantitative Meth-

ylation specific PCR analysis (qMSP) using real-time PCR technology (Fackler et al., 2004; Chan et al., 2004; Swift-Scanlan et al., 2006; Abdolmaleky et al., 2008b). Other advanced techniques such as microarray were also adopted for genome-wide DNA methylation, and histone acetylation or methylation and histone phosphorylation analyses. In these approaches, methylated DNA or acetylated, methylated and phosphorylated histones attached to the corresponding DNA will be precipitated with specific antibodies (Weber et al., 2005) bound to magnetic beads using magnet (e.g. active motif). Methylated DNA can be precipitated by methyl binding proteins, as well. Then, the precipitated DNA is purified, subjected to real-time PCR analysis or pre-amplification for the whole genome or promoter microarray analysis using affymetrix or other Chip analyzers. In general, these new methods of microarray analysis can detect epigenetic aberrations of all ~30,000 human genes in a short period of time. Such high throughputs methods are also used for expression analysis of almost 800 known micro RNAs.

Along with the development of these advanced techniques, normal and diseased tissue banks (e.g. Stanley Foundation, and the Harvard Brain Tissue Resources Center) have been established to provide well-characterized samples for concurrent research projects on different aspects of specific diseases. In the field of neuroscience, although neurosurgeons have been active in providing tumor tissues for cancer research, unavailability of the brain tissue in living clinical samples remains a dilemma in neurological and psychiatric diseases. Therefore, most of the epigenetic analyses have been done in human post-mortem brains or animals modeling human diseases. Another complexity in brain epigenetic analysis comes from structural and functional diversity of brain cells. For instance, the brain consists of neurons and non neuronal cell population. Neurons themselves are heterogeneous population of cells which compose six layers of the brain cortex with different cell types and thus diverse epigenetic marks. A clear-cut epigenetic analysis of each cortical layer requires a precise dissection of each layer which may not provide sufficient amount of cells required for most of the high throughput epigenetic analyses. Perhaps, only qMSP is sensitive enough to provide reliable data in promoter specific DNA methylation analysis in such heterogeneous tissues. Other methods including bisulfite sequencing and pyrosequencing cannot detect the presence of less than 5% DNA methylation which may represent complete methylation of one of the chromosomes in specific brain layers in the pool of un-dissected brain tissues (i.e. 1/24 of total cells assuming that the amount of white and gray matter is the same). Therefore insensitive methods may cause false negative results in epigenetic analysis.

Despite such limitations, recent epigenetic studies on post-mortem brain tissues have provided substantial amount of data in normal and diseased brains. There has also been significant progress in epigenetic research on other tissues during the last decade. For instance, in addition to hypermethylation of the expanded CCG repeats of FMR1 gene (fragile X mental retardation 1) in fragile X syndrome and DNA hypermethylation of the circadian genes of PER1 (period homolog-1) and CRY1(cryptochromes-1) in dementia, epigenetic dysregulation of RELN (reelin), MB-COMT (membrane-bound catechol-O-methyltransferase), MAOA (monoamine oxidase A), DAT1 (dopamine trasporter-1), DRD2 (dopamine receptor type-2), HTR2A (serotonin receptor type-2), 5-HTT (serotonin transporter), BDNF (brain-derived neurotrophic factor), GABAergic (gamma-amino butyric acid) and glutaminergic genes have been explored in the brain or blood of the patients with major mental diseases (reviewed in Abdolmaleky 2008a and 2011a). Among these genes the same epigenetic aberrations of HTR2A and MB-COMT were reported out of the DNA extracted from post-mortem brain as well as saliva of the patients with schizophrenia and bipolar disorder (Ghadirivasfi et al., 2011, Nohesara et al., 2011). These later findings suggest using of saliva epigenetic marks as peripheral biomarkers for diagnostic, prognostic or therapeutic purposes in living individuals that the brain tissue cannot be analyzed. While some of these genes are known targets of dysregulated miRNAs in mental diseases (Abdolmaleky et al 2011a), underlying etiology of these aberrations needs to be uncovered for preventive and therapeutic remedies. Therefore, much more works remain to be done in coming years to find the causes of epigenetic aberrations in order to prevent or treat diseased phenotypes.

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