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Invited review

Transcriptome profiling in neurodegenerative disease

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ABSTRACT

Changes in gene expression and splicing patterns (that occur prior to the onset and during the progression of complex diseases) have become a major focus of neurodegenerative disease research. These signature patterns of gene expression provide clues about the mechanisms involved in the molecular pathogenesis of neurodegenerative disease and may facilitate the discovery of novel therapeutic drugs. With the development of array technologies and the very recent RNA-seq technique, our understanding of the pathogenesis of neurodegenerative disease is expanding exponentially. Here, we review the technologies involved in gene expression and splicing analysis and the related literature on three common neurodegenerative diseases: Alzheimer's disease, Parkinson's disease and Huntington's disease.

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1. Introduction

Neurodegenerative disease occurs when a threshold number of neurons lose their ability to function, become unable to respond to changes in their internal and external environments, lose projection connections and ultimately degenerate. Whereas brain structure and function have been the ongoing focus of much biological research for centuries, the complex structure of the brain and its nuclear, laminar and cellular heterogeneity have made it extremely difficult for researchers to distinguish vulnerable neurons from those that are unaffected as a neurodegenerative disease progresses. Biological techniques that were initially developed to investigate chemical phenotypes, neuronal connectivity and the structure of the brain have recently been successfully applied to the study of neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD). The significant variations that have been observed in morphology, physiology, connectivity and function of the neurons affected by these diseases suggest that complex gene expression networks coordinate the function and survival of the neurons that these diseases affect. Because these networks are extremely vulnerable to the disturbances of homeostasis, such disturbances have become a major focus of disease research.

With the completion of the Human Genome Project in 2003, it became apparent that it is, in fact, the intricate network of gene regulation, rather than the number of genes themselves (approximately 23,000 protein-coding genes) that is responsible for the complexity of humans (International Human Genome Sequencing Consortium, 2004). Based on this finding, it is reasonable to assume that knowledge of the precise changes in gene expression profiles within affected neuronal cells will provide valuable information regarding possible causes or consequences of the onset and progression of a neurodegenerative disease and will also add to the current understanding of disease pathology.

Over the past few years, there has been rapid advancement in technologies for gene expression analysis, most notably in high-throughput technologies such as microarrays and RNA-Seq. Microarrays have been widely used in research of neurodegenerative diseases and have provided much of what we know today regarding the transcriptional profiles associated with various neurodegenerative disorders. RNA-Seq is a newer technology that offers even more reliability, accuracy and reproducibility (see the following sections for details).

In this report, we will review the current literature directed at understanding the dynamics of gene expression profiles in neurodegenerative diseases including AD, PD and HD, with reference to current expression analysis technologies and how they have been applied to research. The technologies focused on include microarrays, RNA-Seq and real-time quantitative PCR.

2. High-throughput technologies for RNA detection, quantification and verification

2.1. Microarrays

Microarray technology has proven to be an extremely valuable tool for quantifying large numbers of messenger RNA transcripts simultaneously; its use has resulted in an exponential increase in the amount of available information regarding gene regulation and its effects on cell physiology. The principle of microarrays involves numerous parallel hybridization reactions between labeled target cDNA (reverse-transcribed mRNA extracted from a cell line or tissue of interest) and 'probe' DNA that have been immobilized to a solid surface.

Microarray technology has advanced in recent years, due mainly to advances in array robotics, surface technology, labeling protocols and a massive increase in genome sequence data (Schulze and Downward, 2001). Today, commercially available microarrays can probe for 15,000–30,000 different human mRNA types and allow for genome-wide gene expression profiling (Altar et al., 2009). Specialized microarrays have also been developed that can identify other characteristics of transcripts, for example, the use of probes that bridge exon junctions to detect alternative splicing patterns (Clark et al., 2002).

The two most commonly used array platforms are cDNA and oligonucleotide microarrays. cDNA arrays contain spots (100–300 μm in size) of probe DNA at defined locations; the probe DNA usually consists of PCR products amplified from cDNA libraries or clone collections. Using such arrays, two-sample comparisons can be performed simultaneously using differential dyes, such as cyanine 3 (Cy3) and cyanine 5 (Cy5). This technique is particularly useful in disease gene profiling; in such profiling, control and disease samples are labeled differentially. Although this approach is time- and cost-effective, a more statistically powerful and reliable method involves pairing each sample with a common reference sample (Jurata et al., 2004; Altar et al., 2005; Konig et al., 2004). This particular strategy helps minimize inaccuracies that occur due to background noise, such as dye bias and printing inconsistencies between arrays. The cDNA arrays are washed to remove excess sample that has not hybridized, and the slide is imaged using either a phosphorimager (for radioactively labeled probes) or a laser scanner (for biotinylated/fluorescently labeled probes). Signal strength is expressed in terms of the total hybridization signal strength of the array, which minimizes error due to differences in hybridization efficiency across the array platforms. cDNA arrays are particularly useful for quantifying low levels of RNA that require amplification because although the detection of such RNA has a 3′ bias, the entire 3′ end is included in the cDNA array (Altar et al., 2005).

Oligonucleotide arrays contain short 20–25mers that are synthesized and deposited using either photolithography (Affymetrix) or inkjet technology (Rosetta Inpharmatics). These techniques ensure precise probe deposition on the slides, reducing variation among arrays (Lockhart et al., 1996), and also allow for identification of single base mismatches due to the strong specificity of the short oligonucleotide probes. Oligonucleotide arrays have been a popular resource in neurological research because they span entire genomes, produce reliable data and are easy to process (Prabakaran et al., 2004; Hakak et al., 2001). Specialized types of oligonucleotide arrays have been designed for specific purposes, such as tiling and exon arrays that are useful for investigating alternative splicing. Tiling arrays use overlapping probes of a specific portion of the genome, whereas exon arrays use probes specifically designed for exon detection (Kapur et al., 2007; Mockler and Ecker, 2005). Recent advances in microarray technology include the Illumina BeadArray Technology, which utilizes 3-μm silica beads coated in hundreds of thousands of copies of oligonucleotide probes. The beads are assembled on either fiber optic bundles or planar silica slides, and hybridization on the bead surfaces is detected after the beads are presented with target DNA. Illumina arrays offer many advantages, including quality control, high redundancy and increased flexibility due to the ease of manufacturing (Dunning et al., 2007). Unlike cDNA arrays, oligonucleotide arrays perform poorly when used to detect underexpressed genes because of reduced sensitivity for samples with a 3′ bias (Jurata et al., 2004).

Oligonucleotide and cDNA arrays offer a high-throughput approach to transcriptome research at a relatively low cost. Microarray technology, however, possess a number of limitations due to its use of indirect signal detection by hybridization. These limitations are: (i) microarray technologies depend heavily on known genome sequence data; (ii) they rely on hybridization reactions, which can present inaccuracies due to non-specific hybridization; and (iii) they have small dynamic ranges due to back-