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Review

Advances in microRNA experimental approaches to study physiological regulation of gene products implicated in CNS disorders

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ABSTRACT

The central nervous system (CNS) is a remarkably complex organ system, requiring an equally complex network of molecular pathways controlling the multitude of diverse, cellular activities. Gene expression is a critical node at which regulatory control of molecular networks is implemented. As such, elucidating the various mechanisms employed in the physiological regulation of gene expression in the CNS is important both for establishing a reference for comparison to the diseased state and for expanding the set of validated drug targets available for disease intervention. MicroRNAs (miRNAs) are an abundant class of small RNA that mediates potent inhibitory effects on global gene expression. Recent advances have been made in methods employed to study the contribution of these miRNAs to gene expression. Here we review these latest advances and present a methodological workflow from the perspective of an investigator studying the physiological regulation of a gene of interest. We discuss methods for identifying putative miRNA target sites in a transcript of interest, strategies for validating predicted target sites, assays for detecting miRNA expression, and approaches for disrupting endogenous miRNA function. We consider both advantages and limitations, highlighting certain caveats that inform the suitability of a given method for a specific application. Through careful implementation of the appropriate methodologies discussed herein, we are optimistic that important discoveries related to miRNA participation in CNS physiology and dysfunction are on the horizon.

Contents

Abbreviations: CNS, central nervous system; GOI, gene of interest; mRNA, messenger RNA; siRNA, short interfering RNA; miRNA, microRNA; pri-miRNA, primary microRNA; pre-miRNA, microRNA precursor; AD, Alzheimer disease; Aβ, amyloid-β; APP, amyloid-β precursor protein; UTR, untranslated region; RNP, ribonucleoprotein; miRNP, microribonucleoprotein; RISC, RNA-induced silencing complex; MRE, microRNA recognition element; CDS, coding sequence; LAMP, labeled microRNA pull down assay; IP, immunoprecipitation; RIP-Chip, ribonucleoprotein immunoprecipitation followed by microarray chip analysis; TAP-Tar, tandem affinity purification of microRNA targets; HITS-CLIP, high throughput sequencing of RNA isolated by crosslinking and immunopurification; CIMS, crosslinking induced mutation sites; PAR-CLIP, photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation; PAGE, polyacrylamide gel electrophoresis; LNA, locked nucleic acid; T_m, melting temperature; DIG, digoxygenin; RT-qPCR, reverse transcription quantitative real-time polymerase chain reaction; PAP, poly(A) polymerase; FFPE, formalin fixed paraffin embedded; ISH, in situ hybridization; FISH, fluorescent in situ hybridization; ELF, enzyme linked fluorescence.

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Introduction

The anatomical and cytoarchitectural complexity of the central nervous system (CNS) is a function of an equally sophisticated molecular framework. Derangements in tightly regulated molecular pathways underlying this complexity are not surprisingly linked to many CNS disorders and are currently a focus of intense investigation. As an example, there is growing evidence that genetic and biochemical abnormalities may be shared among some neurodevelopmental, neurodegenerative and neuropsychiatric disorders across the lifespan (Ray et al., 2011b; Sokol et al., 2011).

Gene expression is a critical node at which regulatory influences on molecular pathways are exerted. As such, fleshing out the full complement of regulatory schemes responsible for control of CNS gene expression should be a priority in the search for novel points of intervention against CNS disorders. Elucidating these regulatory networks not only provides a reference for exploring underlying etiologies but also expands the number of *functionally validated* drug targets, the paucity of which is a current impediment for CNS drug discovery (Krause and Chenard, 2008).

Insufficiency of current drug targets for CNS disorders: Alzheimer disease (AD) as an example

AD is illustrative of the need for an expanded set of functionally validated drug targets in CNS drug discovery. The disease itself is characterized by extracellular amyloid plaques consisting primarily of fibrillar amyloid-β (Aβ) peptide and intraneuronal neurofibrillary tangles composed of paired helical filaments of hyperphosphorylated tau (Nelson et al., 2009). Synaptic loss occurs early in disease and appears to correlate with cognitive decline better than other pathological findings (Terry et al., 1991). There are also functional alterations in memory networks in the early phase of disease (Sperling et al., 2010) and significant influences from multiple factors, including genetics and environment (Bertram et al., 2010; Lahiri et al., 2009). Despite the focus on disease-modifying therapies, there have been several recent clinical trial setbacks for phase three drugs directed against well known AD targets (Becker and Greig, 2010; Sambamurti et al., 2011; Schneider and Lahiri, 2009; The Lancet, 2010). Currently approved medications are not that effective and only modify symptoms; there is still no approved medication that alters the progression of the disease (Lahiri et al., 2003; Mangialasche et al., 2010; Raina et al., 2008; Schneider et al., 2011). New putative biomarkers, therapeutic strategies and targets are being tested (Alley et al., 2010; Bailey et al., 2011; Cogswell et al., 2008; Ray et al., 2011a) and are needed for successful intervention (Lahiri, 2011).

Regulatory networks controlling expression of gene products implicated in AD

Our aim has been to clarify the regulatory networks that control expression of gene products implicated in AD. The goal is to fully characterize the normal, physiological pathways with the expectation they might serve as novel therapeutic targets for modulating disease progression. Given the hypothesized centrality of the amyloid-β (Aβ) peptide to AD etiology (Hardy and Selkoe, 2002), we have focused our efforts on those gene products involved in Aβ production, especially the Aβ precursor protein (APP). The regulation of APP messenger RNA (mRNA) expression has been extensively investigated. The promoter structure is complex and consists of proximal and distal elements (Lahiri and Robakis, 1991; Pollwein et al., 1992; Quitschke and Goldgaber, 1992; Song and Lahiri, 1998) that mediate both constitutive and dynamic transcriptional expression (Ge et al., 2004; Lahiri and Nall, 1995). Regulatory elements in the genomic 5′-UTR can drive APP promoter activity (Maloney et al., 2004; Vostrov et al., 2010). Elements in the APP mRNA 5′-UTR also mediate post-transcriptional regulation (Lahiri et al., 2005; Rogers et al., 1999, 2002). The APP 3′-UTR contains several stability control elements