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Review article

The Yin and Yang of nucleic acid-based therapy in the brain☆

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ABSTRACT

The post-genomic era has unveiled the existence of a large repertory of non-coding RNAs and repetitive elements that play a fundamental role in cellular homeostasis and dysfunction. These may represent unprecedented opportunities to modify gene expression at the right time in the correct space *in vivo*, providing an almost unlimited reservoir of new potential pharmacological agents. Hijacking their mode of actions, the druggable genome can be extended to regulatory RNAs and DNA elements in a scalable fashion.

Here, we discuss the state-of-the–art of nucleic acid-based drugs to treat neurodegenerative diseases. Beneficial effects can be obtained by inhibiting (Yin) and increasing (Yang) gene expression, depending on the disease and the drug target. Together with the description of the current use of inhibitory RNAs (small inhibitory RNAs and antisense oligonucleotides) in animal models and clinical trials, we discuss the molecular basis and applications of new classes of activatory RNAs at transcriptional (RNAa) and translational (SINEUP) levels.

Contents

Abbreviations: 3C, chromosomal conformation capture; AAV, adeno-associated virus; AD, Alzheimer's disease; Ago2, argonaute2; ALS, amyotrophic lateral sclerosis; AntagoNATs, antagonist of natural antisense transcript; APP, amyloid precursor protein; ASOs, antisense oligonucleotides; Aub, aubergine; BACE1, β-amyloid precursor protein cleaving enzyme 1; BBB, blood brain barrier; BD, binding domain; BDNF, brain-derived neurotrophic factor; Cas9, CRISPR-associated protein 9; CCPG1, cell cycle progression 1; CCR4, chemokine (C-C motif) receptor 4; COX2, cyclooxygenase 2; CRISPR, clustered regularly interspaced short palindromic repeats; CSDC2, cold shock domain containing C2, RNA binding; CSF, cerebrospinal fluid; DHX9, DEAH (Asp-Glu-Ala-His) box helicase 9; ED, effector domain; Emx2, empty spiracles homeobox 2; eRNA, enhancer RNA; Foxg1, forkhead box G1; FRDA, friedreich's ataxia; FTD, frontotemporal dementia; FXN, Frataxin; GFP, green fluorescent protein; GOI, gene-of-interest; HD, Huntington's disease; HTT, huntingtin gene; IGF2, insulin-like growth factor 2; IL24, interleukin24; IL32, interleukin32; iPSC, induced pluripotent stem cells; LDLR, low density lipoprotein receptor; lincRNAs, long intergenic non-coding RNAs; LINE, long interspersed nuclear element; lncRNAs, long non-coding RNAs; miRNAs, microRNAs; NAT, natural antisense transcript; NMHVs, nuclear localization signal-MS2 coat protein RNA interacting domain-; HA epitope, (3x)VP16 transactivating domain; PABP, poly (A)-binding protein; PD, Parkinson's disease; piRNAs, piwi-interacting RNAs; bPLA2G4A, phospholipase A2; polyA, polyadenylated; PR, progesterone receptor; PSEN1, presenilin 1; RAN, repeat-associated non-ATG; RAPGEF3, rap guanine nucleotide exchange factor (GEF) 3; RIP, RNA immunoprecipitation; RISCs, RNA-instructed silencing complexes; RNAa, RNA activation; RNAi, RNA interference; S/AS, sense/antisense pairs; saRNA, small activator RNA; SHANK2, SH3 and multiple ankyrin repeat domains 2; shRNA, small hairpin RNA; SINE, short interspersed nuclear element; SINEUP, SINEB2 sequence to UP-regulate translation; siRNAs, small interfering RNAs; SLC39A, solute carrier family 39; SMA, spinal muscular atrophy; SMN, survival of motor neuron; SOD1, superoxide dismutase 1; SYNGAP1, synaptic ras GTPase activating protein 1; TEs, transposable elements; TFs, transcription factors; TGS, transcriptional gene silencing; TSS, transcription start site; UBE3A, ubiquitin protein ligase E3A; Uchl1, ubiquitin carboxyl-terminal esterase L1; UTR, untranslated region; VEGF, vascular endothelial growth factor; wt, wild type.

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1. The post-genomic era of molecular therapy

In the last years, our understanding of the functional output of the mammalian genome has enormously increased. This has led to profound changes in the current view of how a cell works and how evolution has shaped biological complexity. These discoveries provide new opportunities for translational research with a potentially great impact on molecular medicine.

The classic approach to drug discovery has stemmed from the concept that genomes contain the information to encode for proteins and these are the building blocks of organisms. Proteome complexity is the way to structure cells and tissues with different shapes and functions. Drugs are therefore modifiers of protein activities inhibiting or activating specific signaling pathways.

Despite a long list of success stories that have positively affected the well being of patients, this approach has posed tremendous challenges to drug discovery, resulting in staggering costs, poor specificities and difficulties in addressing major complex diseases. Importantly, it has negatively impacted the ability to address rare diseases that present highly heterogeneous molecular profiles in spite of the small number of patients. Many proteins remain difficult to be targeted, limiting the repertory of therapeutics.

The sequence of the human genome has confirmed that less than 5% is involved in encoding proteins, leaving the remaining sequences, the so-called Junk DNA, with no function and therefore no role in pharmacology.

A pioneering work previously showed that Junk DNA might indeed be transcribed unveiling the existence of small RNAs and the expression of repetitive elements (Klein et al., 1974). However, the first evidence that these unconventional transcripts may have an important functional role comes from the discovery of RNA interference in the late 1990s (Fire et al., 1998). Immediately, it was evident that these RNAs may provide a new class of nucleic acid-based drugs to inhibit gene expression *in vivo*. As for the discovery of recombinant molecular antibodies, the initial excitement and the consequent large investments crashed against technical difficulties that seemed unsolvable. Two decades later, we are now witnessing a renaissance of interest in small inhibitory RNAs due to the refinement of RNA targeting and the optimization of *in vivo* delivery. More than 50 RNA molecules and their derivatives are currently formulated as drugs and being tested in clinical trials for a number of disorders across different organs. Overcoming initial failures, we have now entered a second phase of RNA therapeutics, where we can optimistically expect to witness the first real impact of RNA medicine on patients and society.

Meantime, large genomic projects as those from the ENCODE (Derrien et al., 2012) and FANTOM Consortia (Forrest et al., 2014) have increased enormously the description of the molecular constituents of cells and have contributed to hamper the classical view of gene expression regulation. In addition to a previously underestimated number of alternative variants for protein-coding genes, pervasive transcription of the mammalian genome gives rise to a large repertory of non-coding transcripts, whose expression is tightly regulated in space and time. These include long non-coding RNAs (lncRNAs), small non-coding RNAs and transcripts derived from Transposable Elements (TEs), such as SINE (short interspersed nuclear element) and LINE (long interspersed nuclear element) (Faulkner et al., 2009; Fort et al., 2014; Kapranov et al., 2010; Katayama et al., 2005). Interestingly, the vast majority of genomic *loci* are extensively transcribed from both strands where two genes are located in opposite orientation, giving rise to Sense/Antisense pairs (S/AS) (Derrien et al., 2012; Katayama et al., 2005).

In this context, it is becoming evident that the major transcriptional output of mammalian cells is represented by lncRNAs. While functional annotation of the genome has previously revealed almost 15,000 independent lncRNA genes (Derrien et al., 2012), the most recent version of LNCpedia database contains more than 90,000 annotated human lncRNAs (Volders et al., 2015). By definition, lncRNAs are transcripts longer than 250 nucleotides, with features similar to those of protein-coding genes but without a functional open reading frame (ORF). lncRNAs