Austin Neurology

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Editorial

Widespread RNA Dysregulation in Neurodegeneration: Challenges and Opportunities

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Received: June 10, 2016; **Accepted:** June 13, 2016; **Published:** June 15, 2016

Abbreviations

AD: Alzheimer's Disease; ALS: Amyotrophic Lateral Sclerosis; C9ORF72: Chromosome 9 Open Reading Frame 72; Cas9: CRISPR associated protein 9; CRISPR: Clustered Regularly Interspaced Short Palindromic Repeat; DM1: Myotonic Dystrophy type 1; FTLD: Frontotemporal Lobar Degeneration; FUS: Fused in Sarcoma; FXTAS: Fragile X-associated Tremor and Ataxia Syndrome; HD: Huntington's Disease; PD: Parkinson's Disease; RAN: Repeat-Associated Non-ATG; SCAs: Spinocerebellar Ataxias; SILAC: Stable Isotope Labeling with Amino acids in Cell culture; SMA: Spinal Muscular Atrophy; SMN: Survival of Motor Neurons; TDP-43: Transactive response DNA binding Protein of 43 Kda.

Editorial

The brain accounts for 2% of the body weight but consumes approximately 20% of the oxygen in the air breathed in as neurons heavily rely on the energy produced by oxidative phosphorylation. Increased reactive oxygen species and mitochondrial alterations are common features attributed to neurodegenerative diseases. The molecular mechanisms involved in the progressive death of neurons are however far more complex involving the additional dysregulation of multiple biological processes. These include the deposition of insoluble intracellular protein aggregates, the dysfunction of protein degradation and recycling pathways (ubiquitin/proteasome, autophagy/lysosomes), the swelling of axons and the induction of programmed cell death. Increasing evidence has also linked neurodegeneration to alterations of the RNA metabolism. In particular, pre-mRNA splicing defects were found to play key pathological roles due to reduced levels of SMN (Survival of Motor Neurons) in Spinal Muscular Atrophy (SMA) [1,2] and cytoplasmic mislocalisation/aggregation of TDP-43 (Transactive response DNA binding Protein of 43 kDa) in Frontotemporal Lobar Degeneration (FTLD) and in most cases of Amyotrophic Lateral Sclerosis (ALS) [3-6]. TDP-43 has since been involved in the regulation of multiple steps of gene expression which are all affected in neurodegeneration. These include miRNA biogenesis, transcriptional regulation, alternative splicing of conserved and non-conserved cryptic exons, axonal transport and stress-related translational control of mRNA. Widespread RNA dysregulation, with downstream alterations of coding and non-coding transcripts, has now been recognised to play a prominent role in causing five neurodegenerative diseases, SMA, Huntington's Disease (HD), Spinocerebellar Ataxias (SCAs), Fragile X–associated Tremor and Ataxia Syndrome (FXTAS) and some of the major subtypes of ALS (C9ORF72, TDP-43, FUS).

Extensive analysis of transcriptomes revealed that the expression levels and processing of hundreds to thousands of coding and non-coding RNA molecules are affected during the course of RNA-mediated neurodegeneration (reviewed in [7]). Given the large number of affected transcripts and their involvement in multiple cellular pathways, it is not feasible to investigate all of the gene expression changes and distinguish between those causing death of neurons and those arising from consequences of initial perturbations. Identifying the molecular mechanisms of neurodegeneration triggered by altered expression of genes remains in fact the main challenge in the RNA field of research. It considerably restricts the development of novel and effective therapeutic strategies of neuroprotection. The nature of biological samples further complicate the analysis of widespread RNA dysregulation as cell or animal models of neurodegenerative disorders and post-mortem tissue from patients might provide different results and directionality of changes depending on the disease stage, the fold expression of the transgene or the model organism. A typical conundrum for interpreting data from post-mortem tissue is to define whether gene expression changes are attributable to neurons that survived neurodegeneration or to neurons that were in the process of dying.

In general, new cell and animal models that better mimic the human neurological diseases and do not over express the mutated alleles are required. Technological advances derived from CRISPR-Cas9 genome-editing approaches will allow the generation of novel cell and animal models based on chromosomal insertion of disease-causing mutations (reviewed in [8]). This technology is also useful for the creation of isogenic controls by removing the disease-causing mutations in patient-derived cells, taking thus away genetic variations in standard analysis that use healthy control individual and disease-affected patient-derived cells for the normalization of gene expression changes. Moreover, the expanding repertoire of protocols for the reprogramming of patient skin fibroblasts into various types of neurons, astrocytes or oligodendrocytes provide great opportunities to generate large amounts of neuronal materials, which are relevant to diseases and retain the aging-associated characteristics, for the biochemical investigation and genome-wide analysis of altered RNA molecules and proteins. On the other hand, the emerging next generation sequencing of RNA molecules isolated from single reprogrammed patient-derived cells appears to constitute a promising method with increased signal to noise ratios which allow for greater sensitivity in the identification of specific gene expression changes [9]. A key consideration remains to be taken into account. Most neurodegenerative diseases involve the progressive death of neurons with rapidly escalating motor and/or cognitive deficits

Citation: Hautbergue GM. Widespread RNA Dysregulation in Neurodegeneration: Challenges and Opportunities. Austin Neurol. 2016; 1(1): 1002.

developing in late adulthood. Recent advances in the engineering of stable inducible cell and animal models might help address the progressive yet sudden onset nature of neurodegeneration. In these models, the transgene causing neuron injury is stably integrated at a defined chromosomal locus under the control of a tetracycline-inducible promoter. Expression of the transgene and induction of disease is conveniently turned on at any time by the simple addition of tetracycline or derivatives into the tissue culture medium or the drinking water. These systems, which also allow building isogenic controls, provide powerful tools for identifying early events of dysregulation more likely to cause neuron injury and for monitoring the widespread consequences of initial transcript alterations over time.

The advent of transcriptomics and next generation sequencing technologies over the past 20 years has greatly improved our knowledge of the multifactorial processes that are involved in neuron injury. However, the mechanisms governing progressive death and age-related onset remain very poorly understood. Much more effort in a genomic medicine era is needed for the curation, the management, the integration and the optimal use of published data. This is increasingly relying on the development of interactable repositories for the sharing of expanding omics datasets and on user-friendly publically-available interfaces for personalized web-searches and maximal exploitation of resources within the research community [10]. The biological interpretation of neurodegenerative transcriptomes has moreover been limited by the complexity of data and by the overwhelming number of differentially expressed transcripts with low fold changes. Is a 1.5-fold change pathologically more important than a 3-fold variation and when does statistical significance reach pathophysiological relevance are critical questions that usually remain unanswered. Another caveat concerns the directionality of gene expression changes. Up-regulation of mRNA levels does not necessarily correlate with increased levels of the corresponding proteins but often down regulation as cells compensate low levels of proteins by activating transcription. If thousands of altered transcripts have been characterized in neurodegeneration, the functional consequences of widespread RNA dysregulation remain largely unknown at the protein level. The proportion and identity of abnormally processed transcripts that are translated into non-physiological potentially neurotoxic proteins has yet to be investigated. Novel methodologies are emerging for the identification of translating RNA molecules (translatome, reviewed in [11,12]) and for measuring the synthesis rate of proteins (pulsed Stable Isotope Labeling with Amino acids in Cell culture (SILAC) proteome [13,14]). This is a burgeoning and exciting field of research however these technologies remain practically complex and challenging as indicated by the very low number of publications. Optimization of the current methods and novel developments are required. Pulsed SILAC allows for partial investigation of the proteome (approximately 4,000 target proteins) and current translatome technologies do not necessarily discriminate between RNA molecules that are actively translated into proteins and RNA molecules indirectly associated to ribosomes *via.* other RNA-binding proteins or stress granules.

The mechanisms involved in RNA-mediated neurodegeneration also include non-conventional cellular pathways such as the unexpected discovery of Repeat-Associated Non-ATG (RAN) translation in SCA type 8 and Myotonic Dystrophy type 1 (DM1) [15]. Since, both non-coding and coding microsatellite repeat expansions in FXTAS [16], C9ORF72-related ALS [17] and HD [18] have been found to be translated in all reading frames leading to the production of polymeric repeat proteins with aggregating properties. The neurotoxicity-conferred by repeat proteins is complex involving nucleolar stress and the disruption of several cellular processes which include the altered nucleocytoplasmic transport of proteins and RNA as well as widespread defects in the nuclear processing of RNA. Repeat proteins complicate therefore the repertoire of widespread RNA dysregulation in neurodegeneration. On the other hand, they provide novel important therapeutic targets. The mechanisms of RAN translation are however yet poorly understood sparking enormous interest and focus in the field of microsatellite expansion disorders.

At the forefront of the discussed challenges and opportunities, the pace of RNA research in neurodegeneration and its translational applications are increasing with an explosion in the establishment of new research groups and publications over the past few years. This is a tremendously exciting area of research. Challenges are facing researchers each steps of their way from the understanding of the dysregulated mechanisms which cause progressive death of neurons, the engineering of new models of diseases, the development of novel technologies to the design and testing of new therapeutic strategies of neuroprotection. Investing in neurodegenerative research represents not only finding treatments to slow down or cure the relentless progression of neurological disorders - it is also about the huge impact on families, carers and society as well as on the potential discovery of other unknown cellular mechanisms that improve our general scientific understanding of life and disease.

Acknowledgement

Hautbergue GM acknowledges support from the Medical Research Council MR/M010864/1 and The Royal Society RG140690 research grants.

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