

Neurodegeneration: From Cellular Concepts to Clinical Applications

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Abstract

Developing therapies for neurodegenerative diseases will require new scientific approaches that take into account the complex multicellular interactions of the nervous system.

Introduction

The growing incidence of neurodegenerative diseases is a ticking time bomb. In addition to the toll these conditions take on patients and caregivers, their drain on the global economy is huge. Alzheimer's disease, the most common neurodegenerative disorder, affects one in nine people over the age of 65 and accounts for more than 60% of dementia worldwide. The global cost of Alzheimer's disease and dementia is projected to hit \$1 trillion by 2018 (<http://www.alz.co.uk/research/WorldAlzheimerReport2015.pdf>). In the United States, currently Alzheimer's disease affects 5.4 million people and this is expected to triple by 2050. Other neurodegenerative diseases, although not as common, are no less debilitating.

In the face of great clinical need, however, there has been little success in developing effective therapies for neurodegenerative diseases. Between 2002 and 2014, 413 clinical trials assessing 214 compounds to treat Alzheimer's disease yielded just one new approval by the US Food and Drug Administration (FDA) — a success rate of 0.4% (2). No new treatments have been approved since 2003. Other neurodegenerative diseases have fared no better. Even for those with a defined monogenic etiology, such as Huntington's disease, no treatment exists that meaningfully modifies disease progression.

As researchers develop an in-depth understanding of the basic mechanisms underlying neurodegeneration, the interrelation of a number of these disorders is becoming clear. Breaking the therapeutic stalemate will require multidisciplinary and combinatorial approaches, as well as the long-term engagement of all players involved. The high costs of developing new

medications necessitate careful consideration of how to best use the available resources, as well as how to build more productive collaborations between industry, academia and public organizations. Equally important will be the social dimensions surrounding neurodegenerative disease research and policy, such as public awareness, breaking the taboo around dementia, public education, implementation of preventative measures, and getting sufficient support for research.

In April this year, more than 400 people assembled at Baylor College of Medicine to address these challenges at the third biennial symposium of the Jan and Dan Dunan Neurological Research Institute. Attendees and speakers consisted of academic researchers conducting basic mechanistic studies on neurodegeneration, clinicians at the front line of patient care, pharmaceutical company scientists working on translating basic research discoveries into effective therapies, foundations and government agencies that support research, as well as patients and advocates. This diverse group identified research priorities and stressed the importance of embracing data sharing and more inclusive publishing models.

Snapshots of Neurodegenerative Disease

The proteinopathies: Accumulation, seeding and aggregation.

Several neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, Huntington's disease, frontotemporal dementia, and Spinocerebellar Ataxia type 1 are so-called proteinopathies, characterized by misfolding, accumulation, and aggregation of a specific disease-driving protein. The appearance of these aggregates correlates to different extents with disease pathogenesis. A clear understanding of the mechanisms underlying the potential pathogenicity of protein aggregates is still lacking, however.

A common theme throughout many neurodegenerative disorders is the appearance and spreading of abnormal protein aggregates throughout the brain. One hypothesis postulates that initial protein aggregates that are formed because proteins such as alpha-synuclein, Tau, amyloid- β can adopt an abnormal conformation. Such abnormal conformers can, once generated, act as "seeds" that somehow convert normal protein into a pathological state. Many researchers are exploring how such seeding occurs. For example, recent work has shown that the tau protein that cause distinct tauopathies, folds in different pathological conformations, which trigger different aggregation patterns. At the moment only biological assays are available that can follow this phenomenon, i.e. it can be demonstrated that these

different Tau conformers, when seeded in cultured cells expressing tau, induce the propagation of different “strains” of abnormal Tau aggregates (3). Furthermore, when these different tau species were re-inoculated into the brains of transgenic mice expressing mutant human tau protein, they induced different tau pathologies that resembled the aggregate patterns seen in patients with tauopathies (4, 5). This suggests that individual tauopathies are associated with unique “strains” of the tau protein and provides an avenue for investigating how a single protein pathology can yield multiple disease phenotypes. In other work, neurons differentiated from patient-derived induced pluripotent stem cells carrying tauopathy-associated mutations showed earlier electrophysiological maturation, altered mitochondrial transport, and early alterations in Tau splicing or Tau distribution before tau began to aggregate, pointing to an aggregation-independent aspect of the pathogenesis (6). Questions such as which tau assemblies seed further propagation, whether they exert cell-specific toxicity and what makes these assemblies toxic are under investigation.

In detailed molecular studies of another neurodegenerative proteinopathy, the autosomal genetic disease spinocerebellar ataxia type 1 (SCA1), several complementary strategies are being explored to lower the mutant disease-causing protein, Ataxin-1. In one approach, unbiased genetic screens were used to identify genes that modulate expression of mutant Ataxin-1 (7). Running parallel screens in human cells and flies unveiled components of the RAS/MAPK/MSK1 signaling pathway that, when downregulated, reduced expression of mutant Ataxin-1 and partially ameliorated neurodegeneration. The therapeutic potential of these targets is currently being explored in preclinical studies using small molecule inhibitors against key mediators of this pathway. An alternative approach is to use antisense oligonucleotides (ASOs) to target the disease-driving protein. ASOs directly targeting mutant and wildtype alleles of *Ataxin-1* transcripts in a mouse model of SCA1, for example, increased survival and ameliorated SCA1 pathology. This strategy is also being explored in monogenic disorders such as amyotrophic lateral sclerosis (ALS) and Huntington’s disease, for which ASOs targeting the disease-driving protein are already in clinical trials. The development of ASO and related therapies allows to test very directly therapeutic strategies that are based on genetic insights. It is likely that such approaches will become very important next to more classical pharmacological strategies for the treatment of diverse neurodegenerative disorders (8,9).

Altered RNA homeostasis

Most genetic studies of neurodegenerative diseases have focused on identifying protein coding variants that lead to changes in protein function. Although many causes of Mendelian disorders have been identified with this strategy, genetic risk factors for more complex common diseases have been a greater challenge to define comprehensively, and even with large amounts of

genomic sequencing of exonic variants, much missing heritability remains. A complementary way forward is to look for variation beyond the coding sequence, including assessment of variants that alter gene expression at the level of RNA regulation. Post-transcriptional processes such as alternative splicing, mRNA localization and translational regulation within different cellular compartments are key regulators of brain function and disturbed RNA homeostasis has been implicated in a number of neurodegenerative disease pathologies.

Mammalian neurons have unique systems of regulating RNA metabolism, mediated by several RNA binding proteins (RBPs) that are not expressed in any other cell type (10). These proteins regulate RNA at multiple levels, most clearly defined through regulation of splicing of transcripts that encode multiple functional properties of neurons. For example, differential splicing via one group of neuron-specific RBPs, neuron Elav-like (nElavl), maintains glutamate production and thereby contributes to the control of excitation and inhibition in the brain (11). Using a genome-wide *in situ* crosslinking assay (CLIP) to map specific points of nELAVL regulation, Scheckel et al (12) found that nELAVL bound transcripts of multiple genes implicated in neurodegenerative diseases. In postmortem brain tissue from Alzheimer's disease patients, nELAVL showed increased binding to the Y non-coding RNA subset (hY3 RNAs), which have been linked to the stress response. The sequestration of nELAVL by this intronic transcript may represent a mechanism by which genetic variation in noncoding regions of the genome may be traced back to changes in splicing and ultimately to phenotypic changes in neurodegenerative disease.

Several neurodegenerative diseases including ALS, frontotemporal dementia, and inclusion body myopathy show clinical, pathological, and genetic overlap. Underlying mechanisms are related to ubiquitin-dependent autophagy and RNA biology, suggesting an avenue for exploring therapeutic targets in pathways shared by multiple diseases. One such shared pathway is the functional impairment of membrane-less organelles, including nucleoli, stress granules and RNA transport granules. In the last year, studies from multiple labs have found that membrane-less organelles are assembled by liquid-liquid phase transitions of RNA-binding proteins that harbor low complexity sequence domains (13). Whereas membrane-less organelles are biologically advantageous, persistent assembly of low complexity domains in a highly concentrated liquid state risks further phase transition to pathological fibrils. Studies of the RNA-binding proteins hnRNPA2B1 and hnRNPA1, for example, suggest that mutations in low complexity sequence domains of these proteins alter the balance of this phase transition, leading to the fibrillar protein pathology characteristic of ALS (14). Given that phase transitions are reversible, these insights suggest a therapeutic strategy based on tipping the balance in favor of disassembly.

The role of glia and inflammation

Tau, amyloid precursor protein (the cleavage of which generates amyloid- β peptide), and other aggregate-prone proteins such as α -synuclein are expressed in neurons, but research is now showing that non-neuronal cells such as glia are also involved in neuronal injury and cell death. Increasingly, neurodegenerative diseases are being treated less like neuro-centric diseases and more like multi-cellular diseases.

Until recently, just one major genetic risk factor for sporadic AD was known: the *APOE* gene where the $\epsilon 4$ allele increases risk for AD and the $\epsilon 2$ allele decreases risk. ApoE's effect on AD is thought to occur partly through modulating A β aggregation and clearance. Interestingly, APOE is mainly expressed in glia cells in the brain, calling attention to non-neuronal cell involvement in AD. Three years ago, researchers reported that rare mutations in the *TREM2* gene, which is also mainly expressed in non-neuronal cells, i.e. the brain microglial cells, are also associated with a 2 to 4-fold increase in AD risk (15). Research investigating the biology of TREM2 points to a possible role for this receptor in modulating the brain's response to protein aggregation by enabling microglial cells to contain neuritic damage (16-18). APOE and TREM2 may thus represent a link between A β aggregation, toxicity, and the clinical presentation of AD.

In another example of the importance of non-neuronal cells, astrocytes derived from people with ALS causing mutations in *SOD1* trigger motor neuron death, presumably through the release of a toxic factor (19). Genome-wide expression and proteomic analyses were used to identify the astrocyte-mediated toxic signal (20).

These three examples highlight the potential role of non-neuronal cells in neurodegeneration, and reveals likely a prospective source of therapies and new biomarkers (see for instance 21).

Another intriguing dimension in neurodegenerative disease pathology is the role of neuroinflammation. New evidence has emerged for involvement of the innate immune system in Parkinson's disease. The two classes of major histocompatibility complex (MHC) molecules allow the immune system to recognize self and foreign peptides. MHC class I molecules, which tag antigens for identification by cytotoxic T cells, are expressed by most cell types, but they have generally not been thought to be expressed in the mammalian central nervous system. However, recent research has identified MHC class I expression in rodent and human brain tissue, raising the possibility that neurons expressing MHC class I could be selectively targeted for immune system destruction.

In 2014, Cebrián et al (22) reported that MHC class I molecules are expressed in catecholamine-expressing neurons in the substantia nigra, and that this expression can be induced by microglial activity. One could speculate that microglial activation spurred by the death of

dopaminergic neurons in Parkinson's disease may upregulate neuronal MHC class I expression and display of antigens. If some sort of peripheral insult simultaneously leads to T lymphocytes breaching the blood brain barrier, those T cells could target and kill the antigen-expressing neurons. If this scenario is borne out, immunosuppressive therapeutic strategies used for treating autoimmune disorders may be applicable to Parkinson's disease, and perhaps other neurodegenerative diseases.

Cellular organelles and neurodegeneration

Much evidence points to a functional impairment of lysosomes – the cytoplasmic organelles responsible for clearing cellular debris – in the pathogenesis of multiple neurodegenerative diseases. Lysosomes are primarily known for their role as cellular incinerators, to which extracellular materials are transported through endocytosis and intracellular materials are delivered through autophagy. Genetic disruptions in lysosomal function are known to cause more than 50 rare, debilitating multisystem disorders collectively called lysosomal storage diseases. Many of these diseases have a neurodegenerative component, and many disease-causing genes in lysosomal storage diseases have also been linked to neurodegenerative disease. For example, whereas homozygous mutations in the gene encoding the enzyme glucocerebrosidase cause Gaucher's disease, heterozygous mutations for the same gene are one of the strongest genetic risk factors for Parkinson's disease (23).

Indeed, recent work suggests that lysosomal and autophagy dysfunction are key mechanisms underlying the defective cellular clearance and accumulation of neurotoxic proteins characteristic of many neurodegenerative diseases such as AD, Parkinson's disease, and Huntington's disease. Traditionally, the lysosome has been viewed as a static organelle that is not subject to regulation. However, work published in 2009 identified a lysosomal gene network and its master regulator gene, TFEB, that controls lysosomal biogenesis and function in response to environmental cues (24). Further work showed that TFEB also regulates autophagy, mechanistically linking the delivery of materials to the lysosome through that process with their degradation (25). Activating TFEB in neurodegenerative disease mouse models rescued the disease phenotype, suggesting that targeting this network may represent a new therapeutic strategy for treating these conditions (26).

Dysfunction in another organelle, the mitochondrion, has long been implicated in the pathogenesis of Parkinson's disease. Dopaminergic neurons, which are vulnerable in Parkinson's disease, are especially sensitive to mitochondrial dysfunction. For instance, neurotoxins causing Parkinson's disease-like symptoms are thought to block mitochondrial respiratory chain activity. Moreover, several genes in which mutations are causative for the disease are associated with mitochondrial function both in genetic and sporadic cases of the

disease. For example, *PINK1* mutations, associated with early onset Parkinson's disease, dysregulate mitochondrial function by interfering with the electron transport chain and mitochondrial membrane potential (27). Several molecules including Vitamin K2 rescue this deficit (28) and are being investigated in preclinical studies. Other research shows that Pink1's normal function is to recruit autophagy markers. In contrast, Parkin — encoded by a gene that also causes early onset Parkinson's disease when mutated—amplifies the Pink1 mitophagy signal, thereby protecting endogenous neurons from mitochondrial dysfunction (29, 30). A better understanding of the role played by mitochondria in Parkinson's disease might also shed some light on pathological processes of AD and ALS, in which mitochondrial dysfunction is often present.

Recommendations for the field

_Multi-disciplinarity, integration, and data sharing

Available animal models fall short in mimicking neurodegenerative processes in humans. Moreover, too many investigators work blindly and competitively alongside each other rather than collaborating. There needs to be integration of data from many different animal models, and integration of animal data with patient-based research. Data sharing of both positive and negative data between different research groups across industry and academia is also crucial. These efforts are especially important for pre-clinical research, where too much information currently remains buried in individual laboratories.

One important way forward is to promote transparency about inconsistent results or problems with data reproducibility. Scientists in academia and industry should publish data when they find an inability to reproduce published results. Industry scientists should also share more readily insights about flawed studies with academic investigators. Moreover, granting agencies should encourage and support research groups to reproduce hallmark findings. Lastly, journals need to be willing to dedicate a section of their journal for publishing such data.

Another important bottleneck impeding progress is the slow dissemination of results. Two robust solutions were proposed: first the creation of rapid publication journals that make information available faster in an open access format, and that would also publish genetic screens, large data sets, and, crucially, negative data. The second suggestion was to provide alternative ways to give authorship credit to investigators and their groups when they generate and make available a large biological dataset, therefore eliminating the need to wait for publication before making the data available.

Alongside the great need for data sharing and integration, it is important to acknowledge the distinct roles played by academic research and industry. Whereas academics are the driving force behind mechanistic characterization and identification of potential therapeutic avenues, the pharmaceutical industry is better resourced and positioned to conduct high powered studies and to identify chemical agents that could be developed into therapies. Government agencies should prioritize funding biology rather than chemical screens and clinical trials; meanwhile, industry should establish partnerships with academic labs to gain access to new scientific approaches that could then be developed into therapies for testing in clinical trials.

New approaches to understand neurodegenerative disease

A full understanding of neurodegenerative disease biology will require broadening our strictly neurocentric viewpoint. Cutting edge techniques that probe the role of non-neuronal cell types— for example, using genetic approaches to study the contribution of glial cells to neuronal networks -- should be more widely implemented to address questions such as how the diverse array of cellular interactions in the brain goes astray in different diseases. Single cell approaches are needed to better understand the contribution of individual cells to molecular and functional neuronal networks.

It will also be important to expand the research focus beyond disease conditions and to develop a better understanding of the mechanisms underlying healthy aging. Such an approach could open the door to therapeutic strategies that build on molecular processes that bolster the brain's resilience to damage accrued during aging. A focus on identifying genetic modifiers present in healthy long-lived individuals and populations could be an initial step in this direction.

Hypothesis-driven approaches have limitations but are the norm in the way public bodies prioritize their funding. The US National Institutes of Health (NIH) and other funding agencies should acknowledge the importance of screening methods and unbiased, not necessarily hypothesis driven approaches to explore new areas of biology relevant to neurodegenerative disease.

Learning from the cancer field

Therapeutic development in the cancer field has shown that there is no such thing as a magic bullet cure and that multi-level approaches are needed. Very likely, the treatment of neurodegenerative disorders will consist of combinatorial therapies and also may be based on

personalized genomics. A major issue complicating therapeutic development is the need for chronic treatment in neurodegenerative conditions: the issues of cost and of potential side effects are much more important for the neurodegenerative field than for most other fields of medicine that warrant in many cases shorter courses of therapy.

Chances for young investigators

The importance of training and mentoring young scientists and helping them to establish their careers is paramount to continued development of neurodegenerative disease research. Seasoned principal investigators should open the door (and also specifically their own doors) for their younger colleagues. The funding systems should more actively incentivize and reward young investigators who participate in interdisciplinary partnerships that address major questions or solve major problems. Arguably, the need to promote early-career scientists is even more pressing for female researchers, who continue to be vastly underrepresented at higher levels of the academic ladder and on research governing boards. Directed efforts at leveling the playing field and ensuring a fair representation are an immediate priority.

Optimism for the future

Despite the harsh assessment of therapeutic progress to date, prospects for developing therapies to treat neurodegenerative diseases are improving. New molecular and imaging techniques are providing researchers with unprecedented tools to study disease mechanisms and assess the efficacy of experimental compounds. Public perception has generally held dementia to be an inevitable consequence of aging, but the accumulation of research demonstrating that it is a pathological state — and in theory, at least, a treatable one — is turning the tide of negative and fatalistic thinking with regard to these diseases in society.

Funding for neurodegenerative disease research is at an all-time high. Five years ago, the United States launched an ambitious AD initiative and this year research on AD and related dementias received a historic \$320 million increase in federal funding. Several large-scale brain research projects have been launched in the past few years, most notably the three-year-old BRAIN Initiative, a US public-private partnership with some \$500 million in funding this year that aims to develop the tools to decode the mysteries of the brain in health and disease. Meanwhile, pharmaceutical companies, which have shied away from neurological indications for the past decade, are cautiously returning to the field. These optimistic developments signal an ideal moment for neurodegenerative disease researchers to take stock of the field, identify the gaps in knowledge and challenges in addressing them, and map out how to most efficiently

enable discoveries that can potentiate the development of new therapies for treating these devastating conditions.

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Figure Legend

The major cellular and molecular processes contributing to neurodegeneration. There are multiple processes that drive neurodegeneration as a result of specific genetic vulnerabilities or aging. Such processes include abnormally altered expression of some disease driving RNAs and proteins, dysfunction of specific cellular organelles such as mitochondria or lysosomes, neuroinflammation and altered responses of glia in the brain. Lysosomes and mitochondria are shown in purple and red, respectively. Abnormal protein accumulation and altered RNA-protein interactions are depicted as black spots.

