Most proteins that reside within subcellular compartments have tags within their sequence that tell them where to go, much like a ZIP code would direct a package. Nuclear proteins synthesized in the cytoplasm have a “nuclear localization sequence” (NLS) that directs them to be transported through the nuclear pore, a large complex of approximately 30 proteins that regulates entry of large macromolecules into the nucleus. Similarly, a “nuclear export sequence” (NES) allows proteins to exit the nucleus through the same pore. Proteins with both an NLS and NES can shuttle in and out of the nucleus, and this localization is regulated by intracellular signals such as stress.

Such is the case with the protein TDP-43, normally localized to the nucleus in healthy cells, but mislocalized to the cytoplasm in more than 96 percent of ALS cases. This change in TDP-43 location—missing from the nucleus where it has an essential function and aggregation in the cytoplasm where it becomes toxic—is a leading theory for the underlying cause of most forms of ALS. In the rare cases of familial ALS caused by mutations in TDP-43, the mutant protein is thought to be more aggregate prone, and this may be the driving force for accumulation in the cytoplasm. However, in the vast majority of sporadic and inherited ALS, the cause of TDP-43 mislocalization has been unknown.

Three papers published in September 2015 suggest that disruption of the normal cellular machinery responsible for transporting RNA and proteins in and out of the nucleus is the most likely culprit in ALS caused by mutations in C9orf72 (called C9 for short). Using independent approaches and different model systems including yeast, fruit flies, and induced pluripotent stem cell (iPS) neurons, these three groups all converged upon the nuclear pore as the key cellular defect caused by the expanded GGGGCC repeats in the C9 gene. Although the exact cause of the defect is still unclear, the fact that three groups...
New Opportunities for Funding and Research
Discoveries Fuel the Field Forward

This year has seen some exciting new funding opportunities with the launch of the Assistive Technology Challenge for Communications together with Prize4Life and the TDP-43 PET Tracer Challenge in partnership with ALS Finding a Cure. Both initiatives will have significant impact on those living with the disease and our ability to better track disease progression.

Progress in therapy development was highlighted this January with the announcement from Biogen-Idec that the clinical trial for SOD1 antisense has enrolled the first participants. This is the second phase I clinical trial for SOD1; however, this trial will use a newer version of the antisense thought to have improved safety and bioavailability. While this trial begins, work continues to develop an antisense approach for the more recently discovered hexanucleotide repeat expansions in C9orf72, which accounts for at least 10 percent of ALS. This edition of Research ALS Today features new discoveries being made in our understanding of how these expansions cause disease and provide new avenues for intervening—a pathway already being tested, in partnership with Karyopharm Therapeutics Inc., with compounds that may reverse the abnormalities. It is encouraging to see the increased pace of translational discoveries with interesting findings in academic laboratories being rapidly embraced by industry partners to further their development into potential therapies.

The Association is pleased to partner with Prize4Life to expand the ALS Research Forum, a website and tool for the research community to identify resources and information in a central location. Exciting new features, including job opportunity postings, have been developed to better inform the investigators and encourage collaboration.

Finally, I would like to highlight three exciting awards and the deserving recipients. The ALS Association is proud to feature the Sheila Essey award, now in its 20th year, and the extraordinary clinician scientist that will be honored this year at the America Academy of Neurology meeting in Vancouver. In addition, through our programs, we encourage young clinician scientists to direct their research interests towards ALS. These clinician scientists play a critical role in ALS discoveries and with the guidance from their mentors will become the leaders in ALS in the future.

—Lucie Bruijn, Ph.D., M.B.A.

Professor Ammar Al-Chalabi Receives the Sheila Essey Award

Each year, The ALS Association and the American Academy of Neurology (AAN) proudly present the Sheila Essey Award for ALS Research to acknowledge and honor an individual who is making significant contributions in research for the cause, treatment, prevention or cure for ALS. This award is made possible through the generosity of the Essey Family Fund now in its 20th anniversary, in memory of Sheila Essey, who battled ALS for 10 years and died from the disease in 2004. Past recipients have used funds to continue ALS research or to support promising young scientists on their research teams.

This year, The Association and AAN are very pleased to award Professor Ammar Al-Chalabi, who has made major contributions to the understanding of ALS and the search for new therapies.

“I feel very honored to receive the Sheila Essey Award and I am grateful to my research team and colleagues who make our ALS research possible,” said Dr. Al-Chalabi.

Ammar Al-Chalabi, Ph.D., FRCP, DipStat is a Professor of Neurology and Complex Disease Genetics at King’s College, London, where he is also Director of a highly regarded and largest ALS clinic in London, called King’s MND Care and Research Center, which is at the forefront of ALS research.

Dr. Al-Chalabi has an illustrious career in ALS research and is a world-leader in the complex genetics of sporadic and familial ALS. He is a high achiever and obtained many prestigious awards, such as a Medical Research Council Clinical Training Fellowship, working with Nigel Leigh, M.D. on genetic risk factors in ALS. In that position, he set up one of the first DNA banks in ALS research, which is connected with a clinic database that carries clinical information extending more than 20 years. He went on to obtain his Ph.D. at the University of London and was the first recipient of an international competitive prize, the Charcot Young Investigator Award for Outstanding Research into ALS. He then won a Medical Research Council Clinician Scientist Fellowship to take his genetic research further,

Continued on page 3
Announcing the New Clinical Research Fellowships

The ALS Association and the American Academy of Neurology (AAN) are proud to announce this year’s recipients for the Clinical Research Training Fellowship and Clinician-Scientist Development Award in ALS Research. These awards are designed to recruit talented, promising young clinicians who propose innovative clinical ALS studies and to foster their development to make significant contributions to ALS clinical research. This year our recipients herald from Washington State University in St. Louis and Johns Hopkins University in Baltimore to creatively address the gaps in ALS clinical research.

Cindy Ly, M.D., Ph.D.,
under the mentorship of Timothy Miller, M.D., Ph.D., at Washington University in St. Louis, received the Clinical Research Training Fellowship in ALS Research. Their research project focuses on investigating innate immunity and autophagy (a type of degradation system in cells) in TANK-binding kinase 1 (TBK1)-associated ALS. TBK1 is a novel gene in sporadic and autosomal dominant ALS suggesting a risk-conferring and potentially causative role in disease. The mechanism by which TBK1 disruption leads to motor neuron loss in ALS is largely unknown. This study will investigate whether TBK1 mutations impair autophagy and disrupt inflammatory responses by examining the fidelity of key protein interactions and their impact on these cellular processes. They will also explore whether TBK1 is misregulated more broadly in sporadic ALS. The goal is to provide valuable insights into the role of TBK1 in ALS pathogenesis and clarify its potential as a therapeutic target.

“I am very honored to receive this award and grateful to The ALS Association, American Academy of Neurology, and American Brain Foundation for their support. This award will provide critical early funding for me as I begin my career as a physician-scientist, and allow me to pursue research that I hope will advance therapy development for ALS.”

Lindsey Hayes, M.D.,
under the mentorship of Jeffrey Rothstein, M.D., Ph.D., from John Hopkins University in Baltimore, received the Clinician Scientist Development Award in ALS Research. Their project focuses on the development of pharmacodynamic biomarkers to use in antisense oligonucleotide (ASO) therapy against C9orf72, the most common cause of ALS. ASO therapy has shown promising results in the laboratory and the goal is to translate this therapy to patients. To accomplish this, they propose to develop a cerebral spinal fluid (CSF) biomarker to verify that the ASO drug is reaching its target and having the desired effect. This study will generate the preclinical data necessary to move C9orf72 ASO therapy forward to the clinic.

“I am very honored to receive this award and grateful to The ALS Association, American Academy of Neurology, and American Brain Foundation for their support. This award will provide critical early funding for me as I begin my career as a physician-scientist, and allow me to pursue research that I hope will advance therapy development for ALS.”

Lindsey Hayes, M.D.
John Hopkins University
Baltimore, Maryland

Al-Chalabi Receives Essey Award

Continued from page 2

a year in the laboratory of Dr. Robert Brown at Massachusetts General Hospital (MGH).

He is widely recognized as one of the world’s top clinician scientists organizing large-scale genome wide scans in ALS. His prowess in complex genetics of neurodegeneration is exceptional. While at MGH, Dr. Al-Chalabi completed a genetic linkage study that demonstrated the possibility that ALS and frontotemporal dementia (FTD) could stand as a single disease. This study also found a signal on chromosome 9 that would later reveal itself as the C9orf72 gene mutation, the most common genetic cause of ALS. He subsequently led an international genome-wide association study that narrowed the list of possible ALS genes on chromosome 9 to just three, which directly led to the discovery of the C9orf72 expansion mutation.

Dr. Al-Chalabi has established long-standing collaborations with most of the world’s major ALS genetic research centers and succeeded in obtaining significant United States and European Union funding to lead two multinational ALS research consortia. It is a high accolade that Dr. Al-Chalabi has been invited to lead the Cold Spring Harbor course in complex genetics for several years. He contributed to a national MNDA and Wellcome Trust Fund-supported DNA Bank and Epidemiology project that led to a nationally funded ALS population register. In addition, The ALS Association has funded him for several studies including the ALSoD, an ALS online genetic database.

http://www.alsa.org/assets/pdfs/summary_alsod.pdf
He also serves as a judge for the Assistive Technology Challenge and as a reviewer for ALS Association research grant programs.

RESOURCES

ALS mutations database
http://alsod.iop.kcl.ac.uk/home.aspx

ALS iPSC cell lines
http://cedars-sinai.edu/Research/Research-Cores/Induced-Pluripotent-Stem-Cell-Core/ALS-iPSC-Lines.aspx

ALS Epidemiology
http://aces.stanford.edu/ForRes.html

SOD1 mutant rats, Taconic
http://www.taconic.com/rat-model/sod1-rat

Jackson Laboratories ALS Mouse Repository
https://www.jax.org/search?q=ALS+mouse+repository

ALS Mouse Model Summary
http://www.alsforum.org/research-models/als

ALS Untangled
http://www.wfnals.org/alsu.html

ALS Association Research Webinars
http://www.alsa.org/research/research-webinars.html

ALS Research Forum
http://www.alsresearchforum.org/
NUCLEAR TRANSPORT

Continued from page 1

independently came to the same conclusion strongly suggests that disruption of nuclear transport is a critical event in the pathogenesis of C9-mediated ALS.

Our group, comprised of three labs at Johns Hopkins, came to the conclusion that nuclear transport was important by testing previously identified GGGGCC RNA-binding proteins (RBPs) in a fruit fly model of C9-ALS. Using this approach, Ke Zhang, Ph.D., a Milton Safenowitz post-doctoral fellow in my lab, tested more than 400 fruit fly homologues of putative GGGGCC RBPs for proteins that suppress neurodegeneration in the fly eye. The strongest hit in this screen was a protein called RanGAP (Figure 1), thought to be a “master regulator” of nuclear transport.

Aaron Hauesler, Ph.D., a post-doctoral fellow in Jiou Wang’s lab at Johns Hopkins, confirmed that the GGGGCC RNA repeats do indeed directly bind to purified RanGAP protein.

RanGAP accelerates the conversion of Ran-GTP to Ran-GDP, and this switch allows NLS-containing proteins to enter the nucleus through the nuclear pore. Interestingly, multiple genetic manipulations in the fly that either stimulate nuclear import or block nuclear export also rescue degeneration in the fly eye, suggesting that degeneration is caused by an imbalance in nuclear transport. To determine if the GGGGCC repeats directly alter nuclear transport, Dr. Zhang expressed a GFP protein containing NLS and NES sequences and found that like TDP-43, NLS-NES-GFP is expressed in nuclear transport. To determine if the GGGGCC repeats disrupt nuclear transport were occurring in ALS patients, Chris Donnelly, Ph.D., a post-doctoral fellow in Jeff Rothstein’s laboratory at Johns Hopkins, analyzed localization of an NLS-NES-containing fluorescent reporter and TDP-43 in iPS neurons derived from C9-ALS patients.

Interestingly, he saw the same disruption in nuclear import in patient cells that was seen in fly cells (Figure 3). Furthermore, in human brain tissue, there were also clear signs of RanGAP abnormalities and disrupted nuclear transport in C9-ALS patients. These data confirm that the same nuclear transport defects occur in C9-ALS patients as occur in fruit fly and iPS cell models.

One of the critical outstanding questions in C9-ALS is whether toxicity arises directly from the GGGGCC repeat RNA or from dipeptide repeat proteins (DPRs) that can be translated from the RNA. Multiple studies in cells, flies, and mice have shown that arginine-containing DPRs are quite toxic themselves, even in the absence of the GGGGCC repeat RNA. Using different fly models and an unbiased screening approach, Paul Taylor’s lab at St. Jude’s Hospital and Fen-Biao Gao’s lab at University of Massachusetts independently identified genes that regulate nuclear transport and the nuclear pore complex as potent modifiers of neurodegeneration. Many of the genes identified in their screens regulate RNA export, and indeed, RNAs accumulate in the nucleus in these fly models and

...
NUCLEAR TRANSPORT

Continued from page 4

in iPS neurons from C9-ALS patients. Furthermore, expression of DPRs causes similar effects, suggesting that the nuclear transport abnormalities may be due to DPRs. Thus, their data suggest RNA export through the pore is blocked, and that this defect may be due to GGGGCC repeat RNA and/or the DPRs themselves.

In a separate study, Aaron Gitler’s lab at Stanford University found that arginine-containing DPRs are toxic in yeast, and he performed a screen for modifiers of this toxicity. This screen identified multiple nuclear transport genes as potent modifiers of DPR toxicity. One of the most potent suppressors of yeast cell death was overexpression of karyopherin genes, also called importins, known to bind NLS-containing proteins and import them into the nucleus. Nuclear transport disruption was also seen in human neurons directly converted from skin cells of C9-ALS patients (called iNeurons). As in our study, their results also suggest that a disruption in nuclear import is a key pathway disrupted in C9-ALS, although their findings suggest that this defect is due to the DPR proteins themselves.

Thus, these three studies all point to the nuclear pore complex (Figure 4), responsible for importing and exporting protein and RNA molecules in and out of the nucleus, as a key target for pathogenesis in C9-ALS. Future studies will be needed to determine if the toxic species is the GGGGCC RNA, the translated DPR proteins, or both. Another critical question is what are the downstream consequences of nuclear pore disruption? Our study suggests that TDP-43 cytoplasmic mislocalization may be a consequence of blocked nuclear import, but there are many other proteins with NLS and NES domains, including other proteins thought to be important for motor neuron health. Paul Taylor’s data suggests that mRNA export is also disrupted, and understanding how transport alterations correlate with protein synthesis abnormalities in C9-ALS neurons will be an important next step.

Another important question is how might aging make the nervous system of C9-ALS patients susceptible to the GGGGCC repeats. Interestingly, the nuclear pore complex is one of the largest and longest-lived protein complexes in the mammalian brain. This lack of turnover makes the pores likely to accumulate damage over time, and recent evidence suggests that nuclear pores do indeed become dysfunctional with aging. Thus, one possible scenario is that alterations in the nuclear pore with aging make them more susceptible to the toxic effects of the GGGGCC RNA and/or DPRs. Since a single nuclear pore complex is normally able to transport approximately 60,000 proteins per minute, even slight “clogging” of the pores may have profound consequences for motor neuron health over time.

Might similar nuclear transport defects also occur in other forms of ALS? Given the mislocalization of TDP-43 in almost all ALS cases, this seems like a strong possibility. We are currently testing iPS neurons and brain tissue from sporadic ALS and other familial forms of ALS for similar nuclear transport abnormalities.

We suspect that nuclear pore abnormalities may be a common cause of ALS.

Could the nuclear transport pathway be amenable to therapy in ALS? Since either genetically inhibiting nuclear export or stimulating nuclear import rescued neurodegeneration in our fly model, we searched the literature for small molecule compounds that might have similar effects. Indeed, selective inhibitors of nuclear export (SINE compounds) are in phase II clinical trials for different forms of cancer. SINE compounds inhibit exportin (XPO1), a protein required for nuclear export in all cells, and surprisingly these compounds are well tolerated in patients. We tested a SINE compound developed by Karyopharm pharmaceuticals called KPT-276 in our fly model and found that indeed, it did rescue neurodegeneration in the eye. Although KPT-276 is not in commercial development due to off-target effects, these findings suggest the exciting possibility that other XPO1 inhibitors may have therapeutic efficacy for C9-ALS. In collaboration with Karyopharm and the Rothstein lab, we are now testing this hypothesis in multiple ALS models.

**LEGEND**

Figure 1: Identification of RanGAP in a fly eye screen. Expression of GGGGCC repeats causes visible degeneration of the fly eye (left panels) and photoreceptor neurons (right panels, normally seven per cluster). Degeneration is suppressed with mutations that activate RanGAP. Images provided by Dr. Ke Zhang.

Figure 2: Nuclear import defect in fly model of C9-ALS. Both NLS-NES-GFP and TDP-43 are predominantly present in the nucleus (asterisk) in fly salivary gland cells (top panels). However, expression of GGGGCC repeats (bottom panels) inhibits import into the nucleus, and these NLS-NES-containing proteins accumulate in the cytoplasm. Similar findings were made in fly motor neurons and iPS neurons derived from C9-ALS patients. Images provided by Dr. Ke Zhang.

Figure 3: Nuclear transport defects in iPS neurons from C9-ALS patients. Ran (red) is normally localized to the nucleus (outlined with dotted line in left panels, labeled in blue in right panels). However, in C9-ALS patients, Ran becomes mislocalized to the cytoplasm (asterisk, labeled with marker for microtubules (MTs) in green). Images provided by Dr. Ke Zhang.

Figure 4: Model for nuclear pore defects in C9orf72-ALS. The GGGGCC repeat RNA is transcribed in the nucleus and is exported into the cytoplasm through the nuclear pore. The RNA may cause nuclear pore dysfunction either by interacting directly with RanGAP as it is being exported through the pore, or by unconventional translation into dipeptide repeat proteins (DPRs).

**IMAGES**

All images were provided by Dr. Ke Zhang.

**TIMELINE cont.**

<table>
<thead>
<tr>
<th>Event</th>
<th>Year</th>
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<tbody>
<tr>
<td>SOD1 gene mutation (chromosome 21) discovered in familial ALS</td>
<td>1993</td>
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<tr>
<td>Trials using glutamate blocker riluzole begin</td>
<td>1994</td>
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<td>Animal studies combining CXNT and BDNF demonstrate decreased motor neuron loss</td>
<td>1995</td>
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<td>GSNO rescues degenerating motor neurons during development in an in vitro experiment</td>
<td>1996</td>
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<td>FDA approves riluzole</td>
<td>1998</td>
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<td>Toxic properties of the SOD1 enzyme discovered and linked to familial ALS</td>
<td>2000</td>
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<td>RNAi discovered by Craig Mello and Andrew Fire</td>
<td>2001</td>
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<td>The ALS Association co-sponsors workshop on high-throughput drug screening with NINDS</td>
<td>2002</td>
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<tr>
<td>RNA discovered by Craig Mello and Andrew Fire</td>
<td>2002</td>
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<tr>
<td>The ALS Association/NINDS collaborative effort begins screening drugs</td>
<td>2002</td>
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The ALS Association holds scientific workshop on “Environmental Factors and Genetic Susceptibility”

Aggressive search for new ALS genes funded by The ALS Association

Scientists complete map of mouse genome

Agency of Toxic Substances and Disease Registry awards five grants focused on ALS

Department of Defense approves funding for ALS-specific research
The ALS Association and Prize4Life Announces the ALS Assistive Technology Challenge

“Even more important than the freedom of speech is the freedom to speak.”
—Stephen Hawking

Amyotrophic lateral sclerosis (ALS) robs people of the ability to eat, speak, move and eventually breathe. The disease is 100 percent fatal. As the disease progresses, patients will lose all abilities to communicate. Communication solutions are vital to keep people living with ALS connected to those around them.

The ALS Assistive Technology Challenge, through The ALS Association and Prize4Life, is offering a $400,000 prize for the development of flexible, accessible technology to help people with ALS communicate with ease.

The Challenge is open to academics, industry, young start-ups and anyone that believes that they can make a difference for people living with ALS.

Registration for the Prize Phase started on March 1, 2016, and runs through July 29, 2016, on a rolling basis.

Learn more and enter at: http://www.alsa.org/research/als-assistive-technology-challenge-2.html

The ALS Association Announces Grand Challenge to Develop a TDP-43 Biomarker

The ALS Association, in partnership with ALS Finding a Cure Foundation, is pleased to announce the Grand Challenge to generate a biomarker to track TDP-43 aggregation. The successful team(s) with the most developed plan will receive up to $1 million investment.

TDP-43 is the primary protein aggregate (i.e. clumps of protein) found in the brain and spinal cord of people with ALS and mutations in TDP-43 are also a genetic cause of ALS. It is crucial to understand how TDP-43 is linked to clinical disease and whether TDP-43 aggregates are toxic to cells.

The Grand Challenge will lay the groundwork to develop a positron emission tomography (PET) tracer specific for TDP-43 aggregates to use as an ALS biomarker. Once developed, its use would be widespread from tracking TDP-43 aggregation during the disease process to enhancing ALS clinical trials targeting TDP-43.

Letters of intent and abstracts were due March 22, 2016. The Challenge Committee will issue requests for full proposals to the groups with the most promising proposals on April 11, 2016. Full proposals are due May 20, 2016, with winners announced in July 2016.

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**TIMELINE cont.**

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<th>Year</th>
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<tbody>
<tr>
<td>2003</td>
<td>Study shows surrounding support cells play key role in ALS</td>
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<td>Study shows that human embryonic stem cells can be stimulated to produce motor neurons</td>
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<td>Gulf War study shows that vets deployed to Persian Gulf in 1991 developed ALS at twice the rate of those not deployed there</td>
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<td>KIF1 gene therapy study proves beneficial in mice with ALS</td>
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<td>VEGF gene abnormalities shown to be potential factor in ALS</td>
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<td>The ALS Association collaborates with U.S. Department of Veterans Affairs to enroll all vets with ALS in registry</td>
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<td>Early tests of ceftriaxone appear to increase survival in mice with ALS</td>
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<td></td>
<td>Combination of creatine and minocycline prove more effective together in mouse model than either drug alone</td>
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<td>Study implicates smoking as likely risk factor in sporadic ALS</td>
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<td>Study releases evidence that mitochondrial malfunction may play an important role in ALS</td>
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<td>Study funded by The ALS Association to find biomarkers in cerebrospinal fluid and blood</td>
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<td></td>
<td>Ceftriaxone increases levels of the glutamate transporter GLT1 in a mouse model of ALS</td>
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<td>First international workshop on frontotemporal dementia discusses link to ALS</td>
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<td>Stem cells engineered to make GDNF survive when transplanted into rats modeling ALS</td>
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<td>Early data suggests that mutant SOD1 may be secreted by and may activate microglia</td>
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<td>Launch of TREAT ALS initiative (Translational Research Advancing Therapies for ALS) to accelerate clinical trials in ALS</td>
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<td>VEGF increases survival in a rat model of ALS while improving motor function</td>
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<td>ALS patient samples collected to NINDS ALS Repository</td>
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<td>Repository samples allow genome analysis for sporadic ALS</td>
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<td>First TREAT ALS clinical trials funded</td>
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<td>First TREAT ALS clinical trials begun</td>
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<td>TDP-43 discovered as a common link in FTD, ALS Chromosome 9 region intense focus for FTD</td>
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<td>Stem cell study shows SOD1 mutant support cells can kill any motor neuron</td>
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<td>ALS U.S. registry efforts gaining ground in Congress</td>
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<td>Fish model of ALS: Progress reported</td>
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<td></td>
<td>SOD1 in altered form common to both sporadic and inherited ALS</td>
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<td></td>
<td>Engineered stem cells making GDNF help motor neurons survive in SOD1 mutant rats</td>
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<td></td>
<td>First genome screening data published based on NINDS ALS Repository</td>
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MHC Class I Molecules Decline on ALS Motor Neurons, Increasing Susceptibility to Astrocytes

It has been clear for several years that astrocytes are toxic to motor neurons in the ALS disease process, but how they exert toxicity has been unclear. In a new study, Song et al. show that loss of expression of major histocompatibility class I (MHC-I) molecules on motor neurons makes them susceptible to astrocyte-mediated toxicity, and that overexpression of an MCH-I molecule can protect them.

The authors became intrigued by evidence that in Cu/Zn superoxide dismutase (SOD1) mutant mice, a common disease model for ALS, higher levels of MHC-I expression on motor neurons is associated with slower disease progression, and that reduction in MHC-I presentation accelerates progression. To further understand the role of MHC-I in ALS, they first showed that in mutant SOD1 mice, MHC-I expression was reduced on motor neuron soma but increased on axons after disease onset, consistent with a transport away from the cell body. In human spinal cord of both familial and sporadic ALS, MHC-I was reduced compared to non-ALS spino-cordal cord. Co-culture of motor neurons with SOD1 mutant astrocytes led to reduction in MHC-I expression within 24 hours. No effect was seen in GABAergic neurons, which are spared in ALS. The effect on motor neu-rons MHC-I expression was not dependent on cell contact, suggesting a secreted factor, a finding that echoes previous work on astrocyte-mediated toxicity.

Overexpression of the mouse MHC-I molecule H2-Kb protected motor neurons, and early treatment of SOD1 mutant mice with adeno-associat-ed virus containing the H2-Kb gene improved motor function and increased survival by 21 days. Reduced expression of the human MCH-I molecule HLA-F was found in human ALS spinal cord, and increasing its expression in neurons in culture protected them from toxicity from astrocytes from both familial and sporadic ALS.

“Those findings suggest that viral vectors (such as AAV9) that have been developed to treat CNS disorders can be used to deliver HLA-F to motor neurons and hamper astrocyte toxicity in individuals with FALS or ALS,” the authors concluded.


SOD1 Misfolding and Propagation

Mutations in SOD1 have been known to cause ALS for over 35 years, but the mechanism remains elusive. Much research has focused on the potentially toxic effects of misfolding, and two new studies lend further support to the misfolding hypothesis. The normal quaternary structure of wild-type SOD1 is a dimer, but Proctor et al. showed, using high-speed atomic force microscopy to directly image SOD1 oligomers in solution, that trimeric SOD1 formed under some conditions. Mutations that sta-bilized the trimeric form promoted motor neuron death in culture, while those that destabilized it did not. A majority of disease-causing mutations are located in the likely interface regions that bind the trimer, suggesting they may promote disease by stabilizing an otherwise unusual quaternary structure. The mechanism of trimer toxicity is under investigation. Pokie-shkevsky et al. showed that ALS-causing mutations in either FUS or TDP-43 can induce misfolding in wild-type SOD1, and trigger its intercellular propaga-tion in a prion-like manner. Conditioned media from FUS or TDP-43 mutation-expressing cells, when exposed to primary spinal cord culture cells, caused WT SOD1 to misfold in motor neurons, an effect that could be blocked with antibodies against misfolded SOD1. The results suggest that mutations in either gene may contribute to a common disease propa-gation pathway, in which disease spreads through cell-to-cell transmission of misfolded wild-type SOD1, and support the development of anti-SOD1 immunotherapy in ALS.


Disease Mechanisms in FUS-related ALS

FUS (fused in sarcoma, also called TLS, or translocated in liposarcoma) is a rare cause of ALS. FUS is a known RNA-binding protein, but how muta-tions in FUS cause ALS is unknown. Two new studies shed some light on this question. Sharma et al. created multiple transgenic FUS mouse models, including one in which the FUS transgene could be conditionally deleted. Mutant protein was mislocalized to the cytoplasm, as in human FUS-mediated ALS, and the amount of cytoplasmic protein was greater in more aggressive mutants. Mutation was selectively toxic to motor neu-rons, and led to early abnormalities at the neuromuscular junction that preceded axon withdrawal. Complete loss of FUS expression in post-natal

Launched of the Expanded ALS Research Forum

The ALS Association, in partnership with Prize4Life, is pleased to announce the launch of a joint partnership to expand the ALS Research Forum, a web-based news and resource portal. The Forum is a gateway to a collection of some of the most important and valuable ALS-related resources targeted specifically towards both academic and industry-based ALS researchers. Resources include up-to-date research and drug news, review articles, avenues for sharing scientific breakthroughs, portals to ALS databases, announcements of funding opportunities, scientific meeting list-ings and much more. The content is provided in partnership with the Alzheimer Research Forum. This web-based collaborative effort brings together valuable ALS-related resources together in one place for use worldwide.

Learn more at: http://www.alsresearchforum.org

JOURNAL NEWS

GENES AND DISEASE MECHANISMS

February: Identification of CBAN translated peptide
March: The ALS Association hosts 2nd Drug Discovery Workshop for ALS
September: Researchers find genetic region influencing age at which people develop ALS

MARCH: Mutations in Matrin 3 identified linked to ALS
August: ALS Ice Bucket Challenge
September: Mutations in mitochondrial gene CHCHD10 linked to ALS
October: Mutations in microtubule associated gene TUB4A linked to ALS

July: New studies shed light on TDP-43 disease mechanism
August: C9orf72 repeat expansion mutations—disease mechanisms begin to unfold

January: Antisense clinical trial for SOD1 started

TIMELINE cont.

Stem cells generated from ALS patients
Identification of new gene linked to familial ALS
Fused in Sarcoma (FUS) on Chromosome 16
FDA approval of SOD1 antisense and stem cell trials in U.S.
First patients enrolled for antisense and stem cell trials in U.S.
Ubiquitin-2 discovery; C9orf72 discovery
March: The ALS Association hosts 2nd Drug Discovery Workshop for ALS
September: Researchers find genetic region influencing age at which people develop ALS

February: Identification of CBAN translated peptide
March: First human antisense trial published—approach safe in people with ALS
November: Progress in understanding effects of C9orf72 gene in ALS

March: Mutations in Matrin 3 identified linked to ALS
August: ALS Ice Bucket Challenge
September: Mutations in mitochondrial gene CHCHD10 linked to ALS
October: Mutations in microtubule associated gene TUB4A linked to ALS

July: New studies shed light on TDP-43 disease mechanism
August: C9orf72 repeat expansion mutations—disease mechanisms begin to unfold

January: Antisense clinical trial for SOD1 started

2008 Identification of new gene linked to familial ALS
2009 Fused in Sarcoma (FUS) on Chromosome 16
2010 FDA approval of SOD1 antisense and stem cell trials in U.S.
2011 First patients enrolled for antisense and stem cell trials in U.S.
2012 Ubiquitin-2 discovery; C9orf72 discovery
2013 March: The ALS Association hosts 2nd Drug Discovery Workshop for ALS
2014 September: Researchers find genetic region influencing age at which people develop ALS
2015 February: Identification of CBAN translated peptide
2016 March: Mutations in Matrin 3 identified linked to ALS

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not lead to motor neuron loss, arguing against a loss of function as the primary pathogenic event in FUS mutation. Sugiura et al. show that wild-type FUS plays a role in controlling the subcellular localization of Mena, an actin regulatory protein. Loss of FUS prevented Mena from suppressing cell motility and promoting neurite outgrowth, suggesting that loss-of-function mutations in FUS may partially exert their effects through loss of regulation of actin. The likelihood that both loss- and gain-of-function effects are at work in FUS-related ALS was explored by Scekic-Zahirovic et al., who created both a knock-out mouse, expressing no FUS, and a knock-in mouse, in which expression was restricted to the cytoplasm (most FUS is normally found in the nucleus). They found that both mice displayed respiratory insufficiency, reduced weight and perinatal lethality, and gene expression changes were similar between them. However, the knock-in mouse also had a reduction in motor neuron number at birth, arguing for a crucial role for a toxic gain of function as a result of mislocalization of FUS to the cytoplasm.


Liu et al. showed the feasibility of direct transformation of fibroblasts into motor neurons, skipping the conversion into induced pluripotent stem cells (iPS cells) that are more commonly used to create motor neurons for studies of ALS. While the direct transformation means comparatively few cells can be derived from a given patient sample, the resulting cells maintain epigenetic markers of aging, unlike iPS cells, which may be important for understanding age-related neurodegenerative processes. Sances et al., who include several of the most prominent leaders in the field of modeling ALS with iPS cells, offer a review of the current state of the art of converting iPS cells into motor neurons, including the developmental principles underlying methods in use and suggestions to improve the maturation and characterization of such motor neurons. “To capitalize on [the development of large iPSC banks, a single, unified method for studying human ALS and its cellular and molecular mechanisms] will be a major advance in the field of ALS research,” the authors suggest.


The C9orf72 repeat expansion is the most common genetic cause of ALS and frontotemporal dementia. Bauer showed that methylation of the C9orf72 repeat expansion in cell culture led to a reduction in the production of RFI1, one of the pathologic hallmarks of the expansion, as well as reduction in the production of dipeptide repeat proteins, which are produced by translation of the repeat and which may be toxic. Previous work has shown that methylation of histones that bind the gene correlate with reduced gene expression, a potentially protective effect; Bauer extends that idea by showing that methylation of the repeat itself may be protective.


Motor Neurons from Patient Cells: Direct Conversion and State-of-the-art Review

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THERAPY DEVELOPMENT

AAV9 Delivers Anti-SOD microRNA to Motor Neurons

Multiple experiments have shown the therapeutic potential of reducing expression of mutant SOD1 in mouse models of the disease, leading to initial clinical trials of antisense oligonucleotides for SOD1 reduction in people with ALS. Multiple means have been explored for delivery of various therapeutic molecules, including direct injection, gene therapy and others. In a new study, Stoica et al. showed that adenovirus-associated virus (AAV9), a neurotropic form of the virus, has the potential to deliver an artificial microRNA that triggers degradation of mutant SOD1 by the cellular machinery by a pathway distinct from antisense-mediated degradation. Transgenic mice expressing mutant SOD1 received the microRNA by injection into the cerebral ventricles shortly after birth. Treatment extended survival by 50 percent, and improved multiple measures of disease progression, including motor neuron loss and neuroinflammation.


Can Increasing Copper in the CNS Be Therapeutic?

SOD1 is a copper-containing enzyme, which receives its copper from a chaperone protein called copper chaperone for SOD (CCS). Overexpression of CCS in mutant SOD1 mice causes early lethality, a paradoxical phenomenon that Williams et al. hypothesized was due to induced copper deficiency in the spinal cord. Treatment with the PET imaging agent CuASTM, which delivers Cu to the central nervous system, rescued the phenotype, and greatly prolonged survival in mutant mice, suggesting that CuASTM (but not oral copper supplements, which do not reach the central nervous system) might be therapeutic in ALS.


Dietary BMAA Causes Neuropathology in Monkeys: A Clue to Guamanian ALS?

The discovery of ALS-parkinson-dementia complex (ALS/PDC) in Guam after World War II led to the hypothesis that ingestion of the neurotoxic non-standard amino acid BMAA, bioconcentrated in the indigenous diet, might contribute to neurodegeneration in the disease. Cox et al. have now shown that dietary intake of BMAA at levels comparable to those eaten in Guam can cause a neurodegenerative phenotype in vervet monkeys. Monkeys fed BMAA for 140 days developed tau-positive neurofibrillary tangles and sparse amyloid plaque-like deposits in multiple brain regions. Co-administration of serine reduced the development of deposits, possibly by competing with BMAA for incorporation into proteins. BMAA is produced by cyanobacteria throughout the world, and epidemiologic efforts are ongoing to determine whether and how it may contribute to ALS or other neurodegenerative phenotypes outside of the South Pacific.