Science AMA Series: Hi, I'm Jonathan Ling, a researcher that's here to share our new breakthrough discovery for ALS (amyotrophic lateral sclerosis)

ALS-researcher [1] and Jonathan Ling[1]

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Abstract

Hey reddit, Today, in the journal Science, you can find our paper which describes the function of TDP-43, an important protein in ALS (the disease that the ice bucket challenge raised money for) tl;dr: TDP-43 doesn't do its job in 97% of all ALS cases. Scientists didn't really know its function—now we do. We also show that it's something that can be fixed! ELI5 Cells in your body are constantly reading your DNA to make proteins. DNA is located in the nucleus of a cell. You can think of a nucleus as a library except that instead of having books neatly lined up on shelves, the books in a nucleus have all of their pages ripped out and thrown around randomly. To sort through this mess, the cell has great librarians that go around collecting all these pages, collating them and neatly binding them together as books. These librarians then ship these "books" out of the nucleus so that other workers in the cell can do their jobs. Think of these books as instruction manuals. TDP-43 is a very special type of librarian. TDP-43's job is to ensure that nucleus librarians don't accidentally make a mistake and put a random nonsense page (usually filled with gibberish) into the books that they ship out. If one of these nonsense pages makes it into an "instruction manual", the workers in the cell get really confused and mess things up. For terminology, we call these nonsense pages "cryptic exons". Here's an image to help illustrate my analogy. In the brains of ALS patients, some cells begin to get sick because TDP-43 becomes really sticky and clumps together outside the nucleus, where it can't do its job. See this image here. We've known about TDP-43 for nearly a decade but never really understood what it did. Today, in our Science paper, we actually show evidence of cryptic exons in the brain autopsies of ALS cases, suggesting that some of our theories were right all along: TDP-43 isn't doing its job correctly in ALS. So, what does this mean for potential therapies? Well, we took mouse stem cells and completely deleted TDP-43 to show that without TDP-43, a cell can't survive more than 2-3 days. However, when we genetically inserted a special protein designed to mimic TDP-43's "librarian" function (i.e. prevent random nonsense pages from entering the instruction books of the cell), these cells came back to life and looked completely normal. In other words, these cells had absolutely no TDP-43 inside them but were almost completely healthy. Here's an image of those cells. If we are able to mimic TDP-43's function in the human neurons of ALS patients, there's a good chance that we could slow down progression of the disease! And that's what we're putting all our efforts into right now. Quick note for readers who are well versed in biology TDP-43's splicing repression mechanism is actually quite interesting and hints at a model for the evolution of exon-intron definition. I think biologists have long wondered how the cell can recognize short 50-200bp exons that are separated by gigantic 100kb introns. How is it that random exons don't just pop up in the intron region by chance? Well, it seems like the cell recruits microsatellite targeting RNA-binding proteins that act as general splicing repressors. This is further supported by the observation that the mechanism of cryptic exon repression is highly conserved across species but the targets are actually 100% different. Furthermore, expansions or contractions of these microsatellite "intronic splicing suppressor" elements could represent loci for disease risk. I think it's an exciting time for this discovery, especially with the advent of whole genome sequencing. Anyways I mainly wanted to do this AMA because I remember reading a lot of stories about people complaining that the ice bucket challenge was a waste and that scientists weren't using the money to do research, etc. I assure you that this is absolutely false. All of your donations have been amazingly helpful and we have been working tirelessly to find a cure. With the amount of money that the ice bucket challenge raised, I feel that there's a lot of hope and optimism now for real, meaningful

therapies. After all, the best medicines come from a full understanding of a disease and without the financial stability to do high risk, high reward research, none of this would be possible! Of course, there is always more to be done so please consider donating to the ALS Association or the Packard Center for ALS here at Johns Hopkins. If you're interested in supporting the work of our lab directly, you can also do so here. Here is a gallery of images as well That's it. I'll be back at 1 pm ET to answer your questions, Ask Me Anything! EDIT: Thank you everyone for all the questions! Sorry if I didn't get to you, I will check back on the AMA later and try to respond. -Jon

the WINNOWER

REDDIT

Science AMA Series: Hi, I'm Jonathan Ling, a researcher that's here to share our new breakthrough discovery for ALS (amyotrophic lateral sclerosis)

JONATHAN LING ALS-RESEARCHER

ABSTRACT

Hey reddit,
Today, in the journal *Science*, you can find our paper which describes the function of TDP-43, an important protein in ALS (the disease that the ice bucket challenge raised money for)
tl;dr: TDP-43 doesn't do its job in 97% of all ALS cases. Scientists didn't really know its function—now we do. We also show that it's something that can be fixed!
ELI5
Cells in your body are constantly reading your DNA to make proteins.
DNA is located in the **nucleus** of a cell. You can think of a nucleus as a library except that instead of having books neatly lined up on shelves, the books in a nucleus have all of their pages ripped out and thrown around randomly.
To sort through this mess, the cell has great librarians that go around collecting all these pages, collating them and neatly binding them together as books. These librarians then ship these "books" out of the nucleus so that other workers in the cell can do their jobs. Think of these books as instruction manuals.
TDP-43 is a very special type of librarian. TDP-43's job is to ensure that nucleus librarians don't accidentally make a mistake and put a random nonsense page (usually filled with gibberish) into the books that they ship out. If one of these nonsense pages makes it into an "instruction manual", the workers in the cell get really confused and mess things up. For terminology, we call these nonsense pages "**cryptic exons**".
Here's an image to help illustrate my analogy.
In the brains of ALS patients, some cells begin to get sick because TDP-43 becomes really sticky and clumps together outside the nucleus, where it can't do its job. See this image here. We've known about TDP-43 for nearly a decade but never really understood what it did. Today, in our *Science* paper, we actually show evidence of cryptic exons in the brain autopsies of ALS cases, suggesting that some of our theories were right all along: TDP-43 isn't doing its job correctly in ALS.
So, what does this mean for potential therapies?
Well, we took mouse stem cells and completely deleted TDP-43 to show that without TDP-43, a cell can't survive more than 2-3 days. However, when we genetically inserted a special protein designed to mimic TDP-43's "librarian" function (i.e. prevent random nonsense pages from entering the instruction books of the cell), these cells came back to life and looked completely normal. In other words, these cells had absolutely no TDP-43 inside them but were almost completely healthy.
Here's an image of those cells.
If we are able to mimic TDP-43's function in the human neurons of ALS patients, there's a good chance that we could slow down progression of the disease! And that's what we're putting all our efforts into right now.
Quick note for readers who are well versed in biology
TDP-43's splicing repression mechanism is actually quite interesting and hints at a model for the evolution of exon-intron definition. I think biologists have long wondered how the cell can recognize short 50-200bp exons that are separated by gigantic 100kb introns. How is it that random exons don't just pop up in the intron region by chance? Well, it seems like the cell recruits microsatellite targeting RNA-binding proteins that act as general splicing repressors. This is further supported by the observation that the mechanism of cryptic exon repression is highly conserved across species but the targets are actually 100% different. Furthermore,

expansions or contractions of these microsatellite "intronic splicing suppressor" elements could represent loci for disease risk. I think it's an exciting time for this discovery, especially with the advent of whole genome sequencing.
Anyways I mainly wanted to do this AMA because I remember reading a lot of stories about people complaining that the ice bucket challenge was a waste and that scientists weren't using the money to do research, etc. I assure you that this is absolutely false. All of your donations have been amazingly helpful and we have been working tirelessly to find a cure. With the amount of money that the ice bucket challenge raised, I feel that there's a lot of hope and optimism now for real, meaningful therapies. After all, the best medicines come from a full understanding of a disease and without the financial stability to do high risk, high reward research, none of this would be possible!
Of course, there is always more to be done so please consider donating to the ALS Association or the Packard Center for ALS here at Johns Hopkins. If you're interested in supporting the work of our lab directly, you can also do so here.
HERE IS A GALLERY OF IMAGES AS WELL

That's it. I'll be back at 1 pm ET to answer your questions, **Ask Me Anything!**
EDIT: Thank you everyone for all the questions! Sorry if I didn't get to you, I will check back on the AMA later and try to respond.
-Jon

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You say that it's happening in 97% of the cases. Is the other 3% different somehow, or are they just cases where you're not sure?

Staticblast
When you look at ALS from a genetics perspective, about 10% of the cases are called 'familial', i.e., lots of people in the family have ALS and it seems to be passed down. The other 90% of ALS appears to occur completely by unfortunate chance and we call that 'sporadic'.

As researchers, we look to the genetics for clues to study the disease. One of the first family-linked genes discovered was a gene called SOD1 that is found in about 30% of familial cases. But it's starting to seem like SOD1 is an outlier because TDP-43 doesn't seem to be messed up. Instead, SOD1 seems to clump together due to the mutation. We get the 3% because 30% of 10% familial is 3%.

In pretty much every other case you can see TDP-43 pathology, although there are some rare familial exceptions where another mutated protein seems to become unnaturally sticky. This is a common theme in brain disease, where there's usually some kind of sticky protein messing up the cells, like getting gum out of your hair.

Thanks for all of your hard work! A guy I worked with came down with ALS a couple of years ago and he passed away VERY quickly - I think it was about 6 months from the initial diagnosis to his passing. It's a terrible disease.

I know that breakthroughs like this can be very important in finding treatment or a cure, but for a non-medical guy like me, what's the real world impact for something like this? Meaning, how long is it from the 'aha' moment until there's a new treatment available for people affected?

Jeffbx
Sorry to hear that, it really is a terrible disease. Some other forms of neuromuscular diseases can last for decades with manageable symptoms but because ALS is so rapid and lethal, the patient and family members don't even have time to come to peace with the diagnosis. Even 6 months is on the short side for ALS, which is typically 2-5 years, so that's even more painful.

My best guess would be around 2-3 years to start clinical trials, provided all the experiments work correctly. The nice part is that we can piggyback on technologies that are already in use in the clinic. The reason it would take a few years is because I want to make sure that whatever final protein design we use is the absolute best version. We basically need time to test some variations.

That being said, things could go really well and a year from now we might have completed all the pre-clinical work, we just can't predict the future.

Hi Dr. Ling, thanks for doing this AMA.

Is restoring expression of a WT allele of TDP-43 in patient derived ALS cells in vitro*sufficient* to reverse ALS associated pathologies?

Is the TDP-43 protein amenable to pharmacological modulation, or would this strictly be a gene therapy therapeutic approach? Alternatively, are there drugs which can mimic TDP-43's functions (HAT inhibitors perhaps)?

SirT6
Is restoring expression of a WT allele of TDP-43 in patient derived ALS cells in vitro sufficient to reverse ALS associated pathologies?

ALS derived cells don't develop TDP-43 pathology and we've tried screening some ALS derived neurons for cryptic exons but with no luck. There probably needs to be other factors (decades of time and environment) to cause TDP-43 pathology in humans. As for restoring expression of a WT allele, what pathologies in the ALS cell lines would you be looking to reverse?

Is the TDP-43 protein amenable to pharmacological modulation, or would this strictly be a gene therapy therapeutic approach?

Yes, I think so! If we could find a compound that could break up TDP-43 inclusions and restore its function, that would be amazing and much easier than gene therapy. Our lab isn't working on that but I know many other labs are trying to find such compounds (I'm sure industry is as well).

Alternatively, are there drugs which can mimic TDP-43's functions (HDAC inhibitors perhaps)?

Sadly, I doubt we could find a drug that could mimic TDP-43's direction function. The mechanism is very precise and complex and to be honest, we don't even really understand how it represses cryptic exons, only that it does. That being said, science always surprises us so who knows!

Did your team receive any extra funding through the drive, if so how much did it contribute to this discovery?

Waimang_NINJA
Yes, partially, although much of the funding is only now being given out. There's a lag between receiving money and distributing it because smart people need to make sure that they choose the best research proposals to fund, and that takes time.

But regardless, the ALS association (ice bucket challenge organization) has funded and is continuing to fund our research.

Hi, thanks for doing this AMA and sharing your work.I had a few questions.

1. **Is anything known about why only certain subclasses of neurons are targeted in such neurodegenerative disorders? For e.g., is TDP-43 proteinopathy restricted to motor neurons in ALS? Or do other cell types have different mechansims for suppressing cryptic exons?**
2. **Going to therapeutic interventions, are there any plans underway to use this information in preclinical models or in clinical studies? If yes, would it involve providing functional/recombinant TDP-43, or do you plan to target the cryptic exons themselves?**

3. **Can TDP-43 function act as an early marker system for diagnosis of ALS? Is there any sense of when the proteinopathy arises during disease progression, and is there any non-invasive way to check TDP-43 states in the brains of living patients?**

amihappyornot
Is anything known about why only certain subclasses of neurons are targeted in such neurodegenerative disorders? For e.g., is TDP-43 proteinopathy restricted to motor neurons in ALS? Or do other cell types have different mechansims for suppressing cryptic exons?

No, it's not exactly clear. It could be random chance or it could be selective vulnerability. Another intriguing possibility is the idea that TDP-43 inclusions could spread like slow moving prions and maybe they spread through neuron connections rather than physical brain location, but that's speculative right now and evidence is hard to get. For the cryptic exons that are specific to TDP-43 (i.e. the ones that are next to stretches of 'UG' microsatellites) there doesn't appear to be another way to repress these guys.

Going to therapeutic interventions, are there any plans underway to use this information in preclinical models or in clinical studies? If yes, would it involve providing functional/recombinant TDP-43, or do you plan to target the cryptic exons themselves?

Yes, we're in the process of pre-clinical work right now. We can't put back normal TDP-43 because it would likely just clump with the aggregates already there and still never get into the nucleus. In our study, we designed a protein that took TDP-43's RNA binding side and cut off its protein clumping side. We replaced it with a known and well-characterized exon repressor. So now you have this protein that can bind to all the places that TDP-43 binds to in the nucleus and suppress cryptic exons, but doesn't clump together like TDP-43. We're trying to use variations of this idea in our mouse models and human cell culture models.

Can TDP-43 function act as an early marker system for diagnosis of ALS? Is there any sense of when the proteinopathy arises during disease progression, and is there any non-invasive way to check TDP-43 states in the brains of living patients?

YES! This is one part that I'm really excited about because we might be able to finish this in the coming months. Right now we're trying to get antibodies against cryptic exons because in **very rare** cases, some cryptic exons actually don't cause any problems in the gene. Instead, they just insert a new peptide sequence (which could be antigenic). So, we might be able to screen blood or CSF for cryptic exons pretty soon. Biomarkers are really important in the clinic because it makes the doctor's job a lot easier. They're also important for clinical trials to use as a benchmark for therapy efficacy.

Eventually, we might be able to find compounds that light up TDP-43 in brain scans like the Pittsburgh compounds do for Amyloid Beta in Alzheimer's but no, we don't have any of those things yet.

I'm also an ALS researcher.

Most of my work has been focused on C9orf72, but I did work on some TDP-43 projects.

The way I see it, it's not the lack of TDP's function causing the disease, but the aggregation of TDP-43 and other proteins (see the many other mutations causing ALS, including TDP-43, C9orf72, FUS...)

Wouldn't a better approach be stopping the prion-like aggregation of TDP-43?

botnut
C9orf72 also shows TDP-43 inclusions as do many other genes linked with ALS-FTD (VCP, GRN, OPTN, ATXN2, SQSTM1, UBQLN2, PFN1, TBK1, etc). Even the FUS inclusions show TDP-43 co-staining. TDP-43 is the common pathological phenotype across ALS and FTD except for things like

p62 staining which is more indicative of failures in autophagy.

In this study, we've identified the downstream effects of TDP-43. Because TDP-43 is shown to be important not only for neuron survival but also organism viability, it seems reasonably that repairing TDP-43's function could at least help in the disease.

But you're absolutely right, it would be fantastic if we could stop it from aggregating in the first place. We should still be trying to identify how to stop prion-like aggregation, not only in ALS but also all neurodegenerative diseases. It would be a far simpler strategy if we could get it to work. As I mentioned in another comment, I think pharmacological action to block TDP-43 aggregation is a promising route.

Goodmorning! I realize that you're not answering questions until one, so actually good afternoon! My question is that in your explanation you say complete removal of the TDP-43 causes cell death, and that when you use a protein synthesized to have the same function, that the cells come to life, was this a gross over simplification? Or could there be hope for those currently plagued by ALS for some recovery? Thank you so much for your hard work and time!

byrneboy
The mods said I could answer questions earlier so I will be answering them throughout the day.

No it's not a gross over simplification, I was actually completely surprised by how normal the cells look! The protein synthesized basically took only the "targeting" part of TDP-43 (cutting everything else off) and connected it to a well studied exon repressor. In this way, we could really test our hypothesis because this protein binds to all the right places and repress cryptic exons without being sticky like TDP-43 is. See the other comments for our therapeutic strategies.

So you said you genetically induced a protein to mimic TDP-43. How would therapy for an ALS patient utilize the information you've found? Would it be as simple as a pill, or are we talking about gene therapy?

Edit: spelling

iammandalore
This would have to be gene therapy, we would need to get a mimic protein into the neurons of ALS to either rescue the neurons with TDP-43 inclusions or "vaccinate" the remaining neurons so that they're resistant to TDP-43 loss of function.

However, gene therapy isn't as big of an issue anymore and we're even using it to cure cancer. It really does seem like medicine has broken quite a few barriers in recent years and I'm excited for the future!

Hello and thanks to you and your team for your amazing work.

Can you give us a rough ballpark estimate of how long it will take for human trials and subsequent mass distribution given a best-case scenario?

Deckard__
Best-case scenario is entering clinical trials 2-3 years from now (and obviously having success!).

So many people have mentioned to me that they know some who had ALS or had a family member suffer the disease and every time it reminds me of the impact that research has for human lives. We're trying our hardest right now to accelerate the pre-clinical work.

Is there any evidence or promising theories of what is causing the onset of this TDP-43 "stickiness and clumping"? An effective therapy would, of course, be awesome but the ability to prevent ALS by removing the etiological agent from the environment or creating a "vaccine" type therapy for susceptible people would really be the gold standard.

fishguy2001
To the best of my knowledge, it seems like the genetics points to the "trash clearing" mechanisms of the cell. Most of the mutations associated with ALS are involved in two processes called autophagy and the ubiquitin-proteosome system. But those are fancy terms for basically cellular trash disposal.

The idea is this: when something clumps together in a cell, the cell needs to get rid of it and activates the trash disposal system. 99.9% of the time it works perfectly fine. But that 0.1% of the time, things get out of hand and the cell dies. In organs like the liver or skin, that's totally fine because one of the neighboring cells just divide and fill the space. But in the brain, neurons can't be easily replaced. Neurons **really** depend on the trash disposal system. The way the brain compensates is by having lots of "plasticity" and rewiring capability in order to withstand losing a few neurons. Over time though, with age and environmental stress, the brain can only compensate for so much and that's when you start seeing disease symptoms.

Proteins sticking together is a very, very common theme across most brain disorders.

I lost my mother to ALS just over a year ago. We cared for her the best we could for 3 years. Watching her gradually slip away from us was the hardest thing I think I'll ever have to do. I just want to thank you and the others in your field for the work you are doing. I hope that one day your research will save the victims of this terrible disease and their loved ones the pain my family has experienced. Once again, thank you.

rmt5020
I'm sorry for your loss, and thank you for the well wishes. Yes, wouldn't it be wonderful to get rid of this horrible disease!

I never thought I'd get so emotional reading about this. My dad died from ALS.

On one hand I'm all "you guys are too late". On the other hand I'm ecstatic that there is a potential cure out there. This is amazing.

Not a question obviously. But this makes me really emotional so i had to comment.

Tl;dr thank you:')

mpmmuirhead
Thank you :)

Warm wishes from people like you are the fuel that drives our research—and research across the world!. It really does mean a lot to hear and think about the impact our work might have for humanity.

It sounds like your main goal is to treat the disfunction of this protein. However, has there been much more insight into what causes this protein to malfunction in this way? And, natural progression, if so, has there been much more insight into prevention?

tclerguy
Yes, there has been some headway into understanding how TDP-43 aggregates in the first place, but as you can imagine, it's hard to really prove anything because it takes decades to develop these inclusions.

But, what we do know from genetics is that the cellular "trash cleaning" pathways are probably very important. If there's a failure to clean up the trash of the cell, TDP-43 might gradually build up to a point of no return. But again, this is all speculation.

As for prevention, as a field we're still trying to identify ways to "activate" the trash removal systems. But it's not easy because there are lots of side effects (you don't want your trash disposal team throwing away important things). My best recommendation is to avoid head trauma, it's by and far the clearest environmental factor for the development for brain disease.

And of course, eat healthy and exercise :)

I'm curious about the (theorized or known?) full path from cellular disorder to ALS symptoms. If the root cause is -- if I'm understanding correctly -- cell death caused by the mechanism you describe here, does that mean that the progressive loss of muscular control that ALS brings comes about because neurons (in the brain or elsewhere as well?) need to regenerate more frequently than other types of cells? To my layperson thinking, it seems like the cellular malfunction you're talking about would cause more catastrophic and general cellular decay, whereas ALS is very much a neural degradation.

As someone who had to watch a close family member be inexorably diminished and taken away by this disease, thank you so much for your exciting research, which seems extremely promising to help others.

sherkaner
I'm sorry to hear about your relative. It is a terrible disease.

As for your questions, neurons aren't really capable of regenerating except in very unique circumstances that wouldn't apply here. Instead, the brain compensates for neuron loss by using other neurons to fill the missing functions. You may have heard of this concept called "plasticity".

In ALS, the disease probably occurs a few years before symptoms because the brain is capable of making new connections each time a neuron drops out. But eventually, too many neurons get sick and there aren't enough neurons left to do the job. This is when you start seeing symptoms. We still aren't sure why TDP-43 begins to stick together, but it's likely because neurons can't regenerate that these protein clumps last so long in the cell.

I hope that makes sense.

I skimmed the manuscript on STM's website. You reached the conclusion that TDP43 is altered in ALS using a study where you examined a grand total of two diagnosed ALS patients?

I also looked at biogps and noticed that tdp43 has low brain expression. Why do you think a CNS disease would be the main pathophysiological manifestation of perturbating this protein's function?

Why does ALS take 40ish years to appear if this protein doesn't work well? Why did you test its function in an ovarian cancer cell line? Why not use patient-derived iPS neurons? Is altering tdp43 via simple knockout embryonic lethal (as you seem to indicate it is in cell culture)? Have you consistently observed perinuclear tdp43 accumulation in patient autopsy samples? Does this account for a subset of cases, or is it a ubiquitous factor?

nippycrisp
You reached the conclusion that TDP43 is altered in ALS using a study where you examined a grand total of two diagnosed ALS patients?

The diagnosis is based on initial clinical presentation but we've begun to understand that ALS and FTD (a type of dementia) are on the same spectrum. You have mutations in genes like TBK1 or C9orf72 where two siblings (both with disease causing mutations) initially present with completely different symptoms (one with ALS, the other with FTD).

Quite frequently, FTD patients will present with motor neuron symptoms and ALS patients will present with cognitive symptoms, but as the conditions worsen, clinicians aren't looking to make new diagnoses and instead focus on palliative care and quality of life decisions.

Nevertheless, you're right, we've begun categorizing cryptic exons in different regions of ALS and FTD patients to answer if there are different reasons for regional vulnerability. Likewise, many of our collaborators are beginning to do the same. Our study is just the beginning, it's not meant to be an extensive pathological study.

I also looked at biogps and noticed that tdp43 has low brain expression. Why do you think a CNS disease would be the main pathophysiological manifestation of perturbating this protein's function?

Actually, if you look at Dr. Barre's transcriptome database, you can see that Tdp-43 (gene name Tardbp) is actually very abundant in neurons. Otherwise, how could we see TDP-43 pathology in the brain autopsies of patients?

Why does ALS take 40ish years to appear if this protein doesn't work well?

It likely takes decades to develop TDP-43 inclusions because neurons normally clear misfolded TDP-43 (and other misfolded proteins) very effectively. It's important to note that there are no observed humans that have complete deletions of TDP-43. All the mutations in ALS are point mutations that slightly alter the protein at a single amino acid (probably pushing it towards a prion-like state). And if you delete TDP-43 from flies, zebrafish and mice (all the common scientific model organisms), they end up dying.

Why did you test its function in an ovarian cancer cell line?

We needed a proof of concept that TDP-43 is doing the same function in humans. We then went directly to neuron brain samples from patients to confirm the cryptic exons. Scientists use HeLa cell lines because they are easy to manipulate. The cell does not want to lose TDP-43 so there is a strong autoregulatory response when you try to reduce the protein. In other words, HeLa cells (and cell lines like this) are the only ones where we can study TDP-43 depletion.

Why not use patient-derived iPS neurons?

We screened patient-derived iPS neurons but they don't have cryptic exons and don't show TDP-43 pathology. Again, like you said, it takes decades to develop this pathology so it's unlikely that you would see results in iPS neurons that are only a few weeks old. There is also no clear evidence as to how many of these mutations cause ALS, so it would be difficult to study them (at least in this context) until we can explain their mechanism of disease.

Is altering tdp43 via simple knockout embryonic lethal (as you seem to indicate it is in cell culture)?

Yes, it's embryonic lethal in flies, zebrafish, mice and probably humans. It's an exceptionally well conserved protein and is highly autoregulated to maintain its levels in the cell.

Have you consistently observed perinuclear tdp43 accumulation in patient autopsy samples? Does this account for a subset of cases, or is it a ubiquitous factor?

Yes, it is extremely ubiquitous in ALS patients (97% show pathology) although not every neuron has it. In rare cases, you can even see TDP-43 up to 30-50% of all neurons but usually it's less than that. My guess is that if patients survived longer, you would see more inclusions, but the disease is very rapid in

terms of progression.

What does this mean for patients with advanced symptoms, Professor Hawking for example?

500yds
Hey, I answered your question below.

You state that "it's something that can be fixed", which is awesome and I fully expect that you clever science folks will crack the case. This is seriously awesome, and congrats to the people who worked on/are working on it!

I have two semi-hemi-demi related questions,

1. **What other information is missing about ALS which could prove necessary in developing a solution to it?**
2. **Knowing that something *can* be fixed seems like it's only half of the puzzle - What sorts of techniques and therapies could you imagine having a positive effect in humans in the real world?**

Thanks for doing this AMA!

OracularLettuce
What other information is missing about ALS which could prove necessary in developing a solution to it?

For our therapeutic strategy, there's not much more information that we need. The concept is pretty much all there. In terms of understand ALS, however, there's plenty left to figure out. Why does TDP-43 clump together in the first place? Why are motor neurons affected in some patients while other patients also develop dementia on top of the motor neuron disease? Are there any nutritional recommendations that we can make for ALS patients now that we have a better understanding of the disease?

Knowing that something can be fixed seems like it's only half of the puzzle - What sorts of techniques and therapies could you imagine having a positive effect in humans in the real world?

Well, one of the promising strategies that we're looking into is something called adenovirus delivery. Basically, we take the genes required for therapy, and wrap them in the skin of a virus. There's never any risk of virus replication because none of the virus genes are there (we replaced them with our therapy ones), but the virus can now deliver its payload to human cells.

The reason why adenovirus is so attractive is because there are different versions. Some versions infect skin or the lungs, some can infect neurons. If we use the skins of viruses that infect neurons, we can deliver our therapy more directly with fewer side effects. People are already trying this in the clinic, among other variations of this kind of gene therapy.

My dad is in the final stage of ALS - he is completely paralyzed & on a ventilator. Do you think there will ever be a treatment for someone in the advanced stages of ALS to improve? Everything I've seen so far is about delaying the symptoms.

reesuh
I'm sorry to hear that about your father. I know it's really tough to go through right now, but I hope you and your family have had a chance to make peace.

My hope is that eventually people won't have to reach the advanced stages of ALS. As with most

diseases, the more advanced stage a patient is, the more complications exist that are beyond the scope of modern medicine. If we can diagnose and treat earlier, there's a better chance for long-term survival.

Is it just ALS that is caused by these proteins malfunctioning? There are a variety of neuromuscular disorders out there that closely resemble ALS in terms of symptoms. If not THIS protein, could MS, MD, etc have similar root causes?

MrCobraKai
There are tons of examples of brain diseases with proteins sticking together: amyloid beta in Alzheimer's, alpha-synuclein in Parkinson's, tau in Pick's disease, the prion protein in prion disease, etc.

There are basically two things to do for each of these diseases:

1. Figure out how to stop the proteins from clumping together OR
2. Figure out what the clumping does to the cell and fix that.

What we've done here in this study is figure out what TDP-43 clumping does to the cell in ALS. And we've identified a way to fix it.

It looks like TDP-43 functions to suppress non conserved cryptic exons.

Is TDP-43 expressed in all cells in the body? Are these exons de repressed in all cells lacking TDP-43? If so, why is derepression of these exons specifically toxic to the cells of the neuromuscular system?

SirT6
Yes, it is expressed in every single cell that has been studied so far. We're in the process of publishing another paper that answers your second question in more depth but basically, we've found that TDP-43 represses different cryptic exons in different cell types. While some cryptic exons overlap between cell types, many cryptic exons appear to be tissue specific. This is primarily due to transcriptional differences (i.e. if a gene isn't transcribed in the first place, there's won't be a cryptic exon in that cell). But, there's also the dimension to consider of splicing factor differences between cells. Human motor neurons may have unique cryptic exons that cause predispose them for disease, but more research needs to be done. In my honest opinion though, motors neurons might just be a vulnerable population of cells (think weakest link in a chain) because their axons are so long and because they use so much energy.

Science AMAs are posted early to give readers a chance to ask questions and vote on the questions of others before the AMA starts.

Guests of /r/science have volunteered to answer questions; please treat them with due respect. Comment rules will be strictly enforced, and uncivil or rude behavior will result in a loss of privileges in /r/science.

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Doomhammer458
👍

What's the next step after this discovery? Further testing in humans?

iM0t0r

We've brought cells without TDP-43 completely back to life. Now we're going to rescue mice that have neurons without TDP-43 (every other cell has TDP-43) in order to model the human disease. Then we test in humans.

This is very fascinating to me, and I am very excited to hear of the progress being made. My mother passed away from ALS in 2011, about one year from diagnosis. It was the hardest thing I personally ever had to go through, or witness another person suffer. A cure can't come soon enough, and it really is one of the most awful diseases.

I have two questions:
- Does this mean we'll have an actual *test* for ALS soon?
- There is mixed information about whether or not ALS is hereditary or environmental. Does this discovery shed any new light on this?

Thank you so much for the work you are doing! I'm excited to see what else you'll discover soon.

Edit: I saw you actually answered the question about familial versus sporadic, so you may disregard unless you have more information to share on it. :)

AsthmaticHummingbird

I'm sorry to hear that, I hope you found a good support group to talk about your loss. There are many others like you and I know it's especially hard for those families where time from diagnosis to passing is very short.

Does this mean we'll have an actual test for ALS soon?

Hopefully so. We're in the process of making antibodies that will be able to identify cryptic exons in blood or CSF. At the very least, we should be able to detect if a person has cells where TDP-43 isn't working. Coupled with a clinical diagnosis of neuromuscular disease, this would be a powerful tool for ALS diagnosis.

There is mixed information about whether or not ALS is hereditary or environmental. Does this discovery shed any new light on this?

Right now, we can only identify hereditary ALS in about 10% of ALS cases. There is likely a major environmental component and the biggest factor, in my opinion, is head trauma. Apart from that, variables like neuron stress and nutrition may be other factors but there is no clear answer. If you're worried about your own risk, I would suggest talking with a genetics counselor / neurologist. But if you don't have anyone else in your family with motor neuron disease or dementia, there's a good chance that it's not familial.

Your work seems fascinating, and this understanding of TDP-43 seems really promising for understanding and beating ALS.

I'm just a humble undergrad, and had not heard of cryptic exons until today. Do they play a part in cellular physiology of other systems besides the nervous system?

I was also wondering if there was any evidence to suggest that a failure of cryptic exon repression might play a role in other neurodegenerative conditions?

Thanks so much for your time, and for doing this AMA!

SgtGhoul

I'm just a humble undergrad, and had not heard of cryptic exons until today. Do they play a part in cellular physiology of other systems besides the nervous system?

That's because they weren't really a thing until today. It's entirely new splicing biology. From our unpublished results, it appears that cryptic exons are everywhere but also contextual based on transcription / splicing factors.

I was also wondering if there was any evidence to suggest that a failure of cryptic exon repression might play a role in other neurodegenerative conditions?

Yes, in particular TDP-43 can be found in Alzheimer's disease 30-50% of the time but it's at a much lower frequency of neurons affected as compared to ALS or FTD. TDP-43 can also be found other rare diseases like inclusion body myositis and paget's disease of bone. There are also other classes of cryptic exons that we're beginning to identify that may be involved in other diseases outside of neuropathology but that's separate work.

What about C9ORF72? Hexanucleotide expansion of this region is currently being touted as the gene most frequently associated with ALS as well as FTD.

thepombenator
C9orf72 was indeed a big discovery but it only explains +/- 30% of familial cases (depending on where you look, it's also been found in some previously "sporadic" cases).

It's thought that some really attractive Viking with this mutation was the initial carrier and the mutation spread throughout northern Europe. C9orf72 is actually pretty rare in places like Asia and when it is found, it can usually be traced to European ancestry.

C9orf72 patients also show lots of TDP-43 inclusions, so it does seem like there's some kind of convergence onto TDP-43.

Are there any theories or speculation about what causes TDP-43 to clump up in the exo-nuclear area of the cell? Do we know what the root cause of the disease is?

TheRealNicCage
A large chunk of the TDP-43 protein is enriched in arginine and glutamine amino acids, which make it look like a "prion-type" protein. It's reasonable to assume that under certain conditions, this prion-like region misfolds and becomes really self-sticky.

Most of the genetic mutations in ALS point to trash clearing mechanisms so that's the general thought process believed right now. Certain proteins clump together in neurons by accident and the cell cleans it up. If it can't clean it up, the clumps get bigger and more out of control.

We're still not 100% what's driving this process though.

What would be the protein which mimics TDP-43? I'm not knowledgeable in biology so I'm interested in finding out how scientists create proteins which can perform the same function as others. Since we're able to do this, could we theoretically just replace all the malfunctioning bits in our body?

SadAsparagus
TDP-43 is basically divided into two parts:

1. A targeting part (think of claws that can grip stuff)
2. An exon repressor part (think of a porcupine's quills)

I guess you can think of it like a porcupine.

So when the cell goes along and tries to incorporate a cryptic exon, TDP-43 binds to the cryptic exon and sticks out its back with all its quills. The cell goes, "oh hell no" and moves on.

What we did was cut off everything except the targeting part of TDP-43 (maybe the porcupine was a bad analogy...) and put back another known exon repressor. It might seem obvious to do this now, but before, we didn't really know that this was TDP-43's primary function.

Now, you have this protein that can target all of TDP-43's original locations but has a shiny new exon repressor that doesn't clump together.

In the neurons of ALS, these TDP-43 porcupines can't do their jobs because their quills end up sticking together for some reason.

Hope that helps!

Dr. Ling,

I'm as excited as anyone to read your article and hear your description. I hope this demonstrates itself into an actually actionable treatment for people with such a horrible disease.

However, like most terminal illnesses, if I Google or even pubmed search "breakthrough" and "als" I get hundreds of results, most of which did not pan out. I certainly understand excitement for your discovery, but for patients with this disease, family members who have watched it claim livelihood and life, and physicians who have painfully "managed" als I wonder if you've given any consideration from toning down the rhetoric? Research like yours needs to be replicated and developed into treatments, and to me, the breakthrough will be if we can improve the life of someone with als with your discovery.

Sorry if I seem like a buzzkill, I mean this respectfully. Do you not think its premature to use the phrase "breakthrough?". It's new, exciting knowledge that opens a path of research. But if it turns out that this accumulation is just the end point of a disease process (not a middle, or initiating), then this is actually an interesting fact rather than a breakthrough -for patients-.

Thanks for considering a very nuanced question, and congratulations on your years of work showing us a new pathway of exploration .

DijonPepperberry
Hi,

I completely understand what you're saying and it's valid point, as I am also quite a skeptical person when it comes to science. But honestly, I do think this is a breakthrough because it's very, very mechanistic.

In principle, our current approach to studying neurodegenerative brain diseases centers around observation. We observe pathology or we look at genetic mutations. Somewhere in between, we try to understand clinical symptoms with the two pillars of pathology and genetics but it's still very observation based. Even if you look at current therapies for Alzheimer's disease, they're not mechanistic in nature. We've struggled for decades to understand what amyloid beta does and we still don't really know. Now, the field is migrating towards tau pathology I'd argue we know even less about how that spreads. The point is, we're desperate to find strategies to treat Alzheimer's that even though we don't know how everything works, reducing amyloid beta in the brain might be a good strategy because that's what we see in the genetics and pathology.

I think this is why a lot of results don't end up being meaningful. It's not due to willful negligence, but rather due to incomplete understanding.

I think our discovery is different. It points to precisely why TDP-43 is needed for the cell. If the rescued stem cells did not grow or even if there was a "partial rescue", I would not be nearly as enthusiastic about this discovery. But these cells did grow with TDP-43 and it was because of a carefully designed experiment that provided strong evidence for our hypothesis.

It's also a breakthrough because we can narrow our focus on something tangible. Often times researchers meander their way through the darkness, unsure of which direction to go. For the past decade, the focus on TDP-43 has dwindled because little progress was made, little lights that briefly drew the attention of researchers but ended up not being replicable or true. With this discovery, however, we've replicated it already with many collaborators offline and it appears to be the real deal because it makes so much sense.

Our lab is not the only one working on TDP-43 and millions of dollars around the world are being spent studying this protein. This research money will now have a much clearer focus, something that's really important for scientific progress.

But if it turns out that this accumulation is just the end point of a disease process (not a middle, or initiating), then this is actually an interesting fact rather than a breakthrough -for patients-.

This is absolutely valid, but the counter-point is that there's literally no way to be sure until we test it. That's the issue here, we're perpetually working with limited information and just have to make the best educated guess forward. But briefly, I'll outline a few reasons why the field believes that TDP-43 is central to ALS.

1. Mutations in TDP-43 cause ALS
2. TDP-43 pathology is not in every disease and in fact, is pretty rare. The only other diseases that show TDP-43 pathology are inclusion body myositis (a rare muscle inflammatory disease) and Alzheimer's Disease, where inclusions are exceedingly rare (<1% of neurons will show inclusions in 30-50% of the cases). In this respect, TDP-43 pathology appears to be very specific to ALS-FTD so something really weird would have to be going on in order for TDP-43 not to be involved in the pathogenesis of disease.
3. Many genetic mutations linked to ALS show TDP-43 pathology. I.e. the common factor for many different mutations seems to be this TDP-43 pathology.
4. Finally, TDP-43 is an essential gene. If you knock it out embryonically, all higher order organisms die (flies, zebrafish, mouse). Many labs have also deleted TDP-43 from neurons specifically and observed neuron loss. Coupled with our observation of cryptic exons and the TDP-43 nuclear clearing pathology, it definitely seems reasonable that putting TDP-43 back into neurons could help.

You bring up a good point and I want to try to convince you that we've given the term "breakthrough" a lot of thought. Let me know what you think.

Sigh, I came to this thread in wide eyed hopes that practical treatment or a cure had been found. Of course it's never that simple or speedy. My uncle was diagnosed three years ago and I'm not sure how much longer he'll have left so I was sad to read the "2-3 years for clinical trials" as it looks like time won't be on our side. I understand there's a million factors for why this kind of thing can't just start today.

I'd just like to thank you and your team for your research and breakthroughs for this horrible disease. I hope more than anything that you can save or improve the lives of others in the future.

Uhhbysmal
It really breaks my heart everytime someone mentions this. I'm really sorry for all the patients that are

currently dealing with ALS, but we are trying our hardest to get this to clinic. Please don't get too sad, your stories inspire researchers like us to work harder so in a way we're all curing this together.

Thank you for the warm wishes.