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Science AMA Series: Hi, reddit! I'm Will Mair, assistant professor of genetics and complex diseases at Harvard T.H. Chan School of Public Health, and my lab recently found a causal link between RNA splicing and aging

WILLMAIR R/SCIENCE

Hello, reddit!

My name is William Mair, and I'm an assistant professor of genetics and complex diseases at Harvard T.H. Chan School of Public Health. My lab recently published a paper in Nature which, for the first time, reveals a causal link between a process known as "RNA splicing" and aging. This research sheds important light on when and how our cells deteriorate over time. Aging is a key risk factor for a variety of chronic diseases, and our lab is working to identify what's happening at the molecular level in various organ systems that allows these diseases to occur.

What is RNA splicing? In order for bodies—and cells—to maintain youthfulness, they must also maintain proper homeostasis. At the cellular level, that means keeping the flow of biological information, from genes to RNA to proteins, running smoothly and with the right balance. While a considerable amount is known about how dysfunction at the two ends of this process—genes and proteins—can accelerate aging, strikingly little is known about how the middle part, which includes RNA splicing, influences aging. Splicing enables one gene to generate multiple proteins that can act in different ways and in disparate parts of the body.

To find this link, we designed a series of experiments in the roundworm Caenorhabditis elegans to probe the potential connections between splicing and aging. Because the worms' cells are transparent we were able to use fluorescent genetic tools to visualize the splicing of a single gene in real-time throughout the aging process. After five days, some worms showed a youthful pattern of splicing while others exhibited one indicative of premature aging. We were able to use these differences in splicing (reflected fluorescently) to predict individual worms' lifespans prior to any overt signs of old age.

We still have much more to learn about this, but the findings open up an entirely new avenue of investigation that could help us understand how to live longer and healthier.

I'll be here to answer your questions from 11:00 AM to 1:00 PM EST; Ask Me Anything!

EDIT: It's 11:00 AM and we're getting underway. Thanks for all your questions so far! I'm also joined here by/u/carolineheintz, the first author of the paper.

EDIT: It's 1:17 PM and we have to stop, but thank you for your great questions! If you want to learn more,you can visit our lab website.

CORRESPONDENCE:

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Hi Will, and thank you for doing this AMA! I have a few questions for you:

1. I was a bit surprised to see you saying that this is the first time someone has causally linked RNA splicing to aging. I can think of at least a few previous studies that highlight the important role of RNA splicing in longevity assurance (e.g. splicing defects driving the progeroid syndrome HGP, splicing defects in age-related diseases such as cancer, mog-5 regulates lifespan in daf2 mutants etc.). That said, this is very interesting work. I was hoping you can expand a bit on which genes/pathways seem to be most impacted by changes in splicing, and similarly, are some tissues more likely to show deviations from homeostasis in splicing than others?

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2. Have you looked at human and mouse transcriptome data? There are hundreds of studies comparing 'old' and 'young' in GEO -- if you look in these datasets, do you see that the 'older' groups typically have less splicing than the 'younger' groups?

SirT6

Hi, thanks for your questions, /u/SirT6, I'll respond in two parts:

1. Absolutely, there are many links between dysfunctional RNA splicing and specific age-related diseases (neurodegenerative disorders etc) and some progeria disease models. In our work we were interested in trying to find links between RNA splicing or splicing defects and innate aging in non disease models. Using a nematode worm that lives and ages in about 3 weeks in the lab, we used in vivo fluorescent reporters that allowed us to visualize splicing defects with age in individuals in real time across a population. Using these reporters we could predict which animals were aging fast or slow within a population. We also slowed the aging process via dietary restriction (reducing food intake without malnutrition) and were particularly excited to find specific components of the splicing machinery that were required for this lifespan extension effect. Increasing levels of these splicing factors could then slow aging and mimic dietary restriction in animals that were eating normally. So in short, absolutely there are links between RNA processing in diseases of aging and now our data show that defects in RNA processing with age may drive the innate aging process itself and may therefore be a factor underlying the central question of my lab 'Why are we more likely to suffer chronic diseases when we are old than when we are young?'

Hi Will, and thank you for doing this AMA! I have a few questions for you:

1. I was a bit surprised to see you saying that this is the first time someone has causally linked RNA splicing to aging. I can think of at least a few previous studies that highlight the important role of RNA splicing in longevity assurance (e.g. splicing defects driving the progeroid syndrome HGP, splicing defects in age-related diseases such as cancer, mog-5 regulates lifespan in daf2 mutants etc.). That said, this is very interesting work. I was hoping you can expand a bit on which genes/pathways seem to be most impacted by changes in splicing, and similarly, are some tissues more likely to show deviations from homeostasis in splicing than others?

2. Have you looked at human and mouse transcriptome data? There are hundreds of studies comparing 'old' and 'young' in GEO -- if you look in these datasets, do you see that the 'older' groups typically have less splicing than the 'younger' groups?

SirT6

Hi, /u/SirT6, here's part two:

We are beginning to look through these studies to compare them to the data we identified in our experiments. There are many datasets showing changes in RNA splicing with age in mammals, especially in the brain, and even expression of some splicing factors the correlate with lifespan in different mouse strains. Our new data reveal RNA splicing changes in long lived dietary restricted animals and in animals on DR that are no longer long lived due to inhibition of 'splicing factor 1'. So these data allow us to try and filter out changes that are causal to the DR effect and then compare those to changes seen in mammalian and human studies.

Aging is a key risk factor for a variety of chronic diseases.

I've heard a lot of futurists taking the angle that aging itself is a chronic disease.

How do you feel about scientists that are approaching aging itself as a disease to be cured? Is this a fool's errand or an interesting approach?

AlexanderDeLuca

I don't think that aging itself is a disease. That being said there are reasons to think that a traditional biomedical approach to curing diseases of aging may not have the impact we would like on adding disease free years at the end of life. A simplistic distinction between public health and medicine is the former is focused on prevention, the latter on cure. The success of a 'disease-centric' approach to aging is limited by high levels of co-morbidities in the elderly - in the US over half of people older than 65 have two or more chronic conditions. Curing one disease in its entirety does not add disease-free years to the end of life if other conditions remain. An alternative approach is applying a public health strategy, namely prevention, to basic science by reducing the extent to which age is a risk factor for disease. Potential for such an approach was first shown using the nematode C. elegans, where single gene mutations dramatically prolonged lifespan and maintained animals in a youthful state. Identical genetic modifications prolonged healthy lifespan in fruit flies, mouse, and have now been linked to long-lived human populations suggesting that, unlike chronological age, physiological age is malleable, and mechanisms that modulate it can be exploited to reduce overall disease risk. So, I do not think that studying the basic science of aging is a fools errand, it's an alternative and complementary approach to discovery for disease therapeutics.

Hi Will

This is fascinating research indeed - thanks for doing this AMA. Ageing and longevity is one of my pet interests (not in any academic sense, I just follow the research on it).

I have two simple questions as a non-researcher in this space:

1. What's the process that controls how dietary restrictions affects RNA splicing itself as it pertains to more complex beings like humans as opposed to C elegans?
2. What's the role of telomeres in the process of your cellular homeostasis dogma of DNA>RNA>Protein? Because there has been a lot of research and popular interest in telomere length as a potential area of intervention to pause or reverse ageing. Again I ask from a position of ignorance.

Thanks again for doing this AMA. And keep up the good work! With any luck we may yet live long enough to step foot on a Mars colony because of your research allowing us the lifespan to see it happen!

mvea

If only these questions were simple, /u/mvea! We looked at some of the key nutrient and energy sensing pathways that link food intake to aging rate. One of these is the amino acid sensing and growth regulating pathway, the mTORC1 pathway. Drugs such as rapamycin that inhibit mTORC1 have been shown to slow aging in many species, from the C. elegans that we work on to fruit flies and mice. Strikingly, the RNA splicing factor we found that blocked lifespan extension by dietary restriction (SFA-1) in C. elegans also blocked lifespan extension by suppression of mTORC1. We are now focusing on how mTORC1 regulates the activity of SFA-1.

So far we have not looked at the link between telomeres and splicing. Telomeres play key roles in the senescence of rapidly dividing cells. Shortening of telomeres can lead to inhibition of cell division, and secretion of factors from those 'senescent' cells can negatively impacts cells around them. Recent work by others has shown that if you delete those senescent cells you can increase lifespan in mice. The cells in the species we worked on here - C. elegans - do not divide in adults. However, there are

some hints that telomere dysfunction can impact non dividing cells too, and because of the three dimensional folding of of DNA in nuclei telomeric sequence can in fact impact gene transcription. We have not looked at the interaction between SFA-1 and telomeres yet, but its a good idea.

In research with such a potentially infinite value for almost every human being, are there problems controlling the leaking of results (true or false)?
Must extra steps be taken to avoid a grey market appearing after each new communication of an advance?

This_Is_The_End

Hi, /u/This_Is_The_End, we are happy to have our research open to other scientists and to the public. Scientific advances are never done in isolation - they are additive, built on the discoveries that have come before. We hope that our research into the requirement for specific splicing factors in the regulation on longevity will just be an entry point, and will be followed up and furthered by us and by others. The ultimate goal of our work is not lifespan extension or immortality, but to try and reduce the number of years we spend in old age suffering from chronic and disabling conditions.

Hi, cool paper!

I didn't know about the link between TORC and splicing, which I guess ties in very well with the literature that TORC inhibition with rapamycin can extend the lifespan of mice.

Obviously it's hard to test whether this is the case in people, but I wonder whether you had any plans to? Rapamycin is obviously used to treat a variety of different diseases - I wonder if you could assess the splicing landscape (or even lifespan) of patients with some of those diseases, taking into account whether they got any TORC-inhibition or not?

jamimmunology

Hi, /u/jamimmunology, in our work we took advantage of the fact that C. elegans are transparent and used fluorescent reporters that allowed us to visualize splicing errors in live animals. We used these reporters to effectively predict which animals would live long and which would age faster. Of course in people this trick is not going to work! Instead, what we are working on is identifying specific RNA processing effects that we can identify using molecular biology techniques, which give the same predictive power as the reporters. If funding allows we will extend this work to mammals to find conserved RNAs we might use as biomarkers of aging. These experiments are more expensive than those in C. elegans, which is why we need to support funding to the National Institutes of Aging - the average dollar contribution for each of us annually to the NIA is about $3.57 - so for the price of one less coffee a year we could double our research investment into disease of aging!

Personal question. As expert in aging/longevity what are the top 5-10 lifestyle and health routines that you follow?

TorstenEndofMoney

Hi, /u/TorstenEndofMoney, Personally my interests are in why aging exists and why it evolved, and how we can think about the aging process itself to design new therapeutics for age-onset diseases, rather than how we might prolong lifespan. So no dietary restriction for me! That said my lab works on a key energy sensor names AMPK that plays roles in DR and is activated by, amongst other things, exercise. So exercise and a whole food diet certainly mimics some of the interventions that slow aging

in the lab.

Long term our goal is to develop therapeutics that might be used to reduce the extent to which age itself is a risk factor for chronic diseases. In just a hundred years we have added 25-30 years to average life expectancy in the US, with developing countries showing similar patterns. This trend is global and set to continue; by 2050 there will be 1.5 billion people over 65 worldwide. This rise in survival is overwhelmingly due to advances of public health, reducing childhood mortality and death from communicable diseases. However, success has come at a cost, as patient age is the single biggest risk factor for the majority of complex diseases. As a result, age-onset diseases including cancer, neurodegenerative diseases, type II diabetes and cardiovascular disease are generating a public health and economic burden that is rapidly becoming insurmountable. So our goal is to understand what it is that goes wrong in our cells in old age that leads to disease risk and find ways to prevent this occurring.

Hey Dr. Mair, thank you for doing this AMA!

Do you have any speculative theories on how the deregulation of splicing affects the aging process? Would it be due to improper gene lengths from incorrect splicing or more of genetic abnormalities (mutations) from erroneous splicing?

I hope to hear more from your team's research, this is quite fascinating!

Belsyre

Hi, /u/Belsyre, great question. Happy to speculate but you'll have to look out for our new work in the future to see the real answers to my speculation. One of the surprising results of the human genome project back when I was in college was how few genes humans have - about 24 thousand. Even more surprising is that the simple nematode we work on - which only has 959 cells - has almost as many genes as us. One of the key things that alternative RNA splicing does is it allows the generation of complexity from a limited number of genes- many of those 24 thousand genes are spliced up and re-arranged in different ways at the RNA level so that they can make more than one peptide. The more complex the species, the more alternative splicing they have. These different peptides or proteins than can perform different tasks in the cell. So one way the defects in RNA splicing may lead to age related decline is that an incorrect protein might made in an old cell - meaning the function of the original one is lacking and perhaps an additional process that might be maladaptive of cause a disease is activated. One finding we are particularly interested in came from our RNAseq analysis looking for specific processes affected by age in a splicing factor 1-dependent manner. We were excited to find significant gene expression changes in kegg pathways specific to fatty acid metabolism, and are excited to explore this link in more depth.

Do you believe that these 'splicing patterns' in the worms are similar to what we'd witness in humans? If you were able to look at the RNA of two 70 year olds, and one lived to 100 and the other to 73, would their RNA splicing pattern differently?

sasho8888

Thank you for your question, /u/sasho8888, we would expect to find different genome-wide splicing patterns between two 70 year olds with differing life expectancies. Our long term goal is to try and define which splicing differences would be able to predict which was which - i.e. can we use experimental approaches and machine learning to define splicing effects that are predictive of health outcomes. Alternative splicing is even more frequent in humans compared to C. elegans, reflecting the complexity of the proteome, but our hypothesis is that conserved families of genes and RNAs

indicative of healthy aging may be shared between the two. There are already some datasets showing changes in RNA splicing with age in mice and humans and correlations of splicing factor gene expression to human lifespan have been done.

I'm a layperson on this subject, but very interested. My question is: I seem to recall hearing that aging may be due to "lost information" during replenishment. Is this what you're studying (sounds like it might be), and is it true?

Why is aging always the case rather than a reversal or neutral effect? Or even something unrelated to longevity like color?

maori_kutta

The why questions are always the hardest! In fact aging does not exist in every species - some species such as hydra don't age, and of course some species can rejuvenate cells and limbs etc. So in fact aging evolved as a side effect of other things that are beneficial to us when we are young. Up until recently in our evolution those side effects were rarely seen as we tended to die of something other than old age - we don't see many old animals in the wild, we see them in zoos or in our homes as pets where they live protected lived. Animals that have low rates of predation or disease tend to live longer - bats live 30 years while mice live 3 because bats can fly to escape predators. Some of the longest lived animals are on an island where predation is low. Advances to public health have now given us those protective benefits, which is why we now see so many more age-related diseases now than in our history. For more on this I wrote a short piece last year

Hi Will and Caroline, Good stuff! I have a couple of Qs about the transcriptomics analysis

1. The GO enrichment for DE genes in AL, DR, and DR+sfa-1 RNAi showed over representation of metabolism related terms. However, GO enrichment for the gene with splicing changes is heavily dominated by immune response related terms. I would expect a similar trend in these two data sets, why this discrepancy?

2. The fluorescence splicing reporter showed beautiful correlation with sfa-1 levels and longevity. However, (Fig 2 b-d) for reads in intron % showed increase from 1% to max 3%. I am not sure how much contribution this small number of genes will have towards overall energy metabolism, especially when metabolism related genes are not enriched in this category. It will be helpful if you clarify your view on this. Lastly, are you looking into how pre-mRNA splicing homeostasis correlates with proteostasis in the context of Aging?

ayush_ranawade

That is an interesting observation, /u/ayush_rawande, and without further experimentation sadly I cannot give you an answer to 'why'. We were interested to see that there was enrichment of specific pathways/GO terms in both the DE genes and the differentially spliced, rather than broad changes spread across all genes/RNAs. To us, this suggests that SFA-1 is doing something more specific in DR than just generalized global splicing regulation, and we speculated that this may involve specific changes to metabolism under DR. As of yet, we have no data to causally link the metabolic changes regulated by SFA-1 to its effects on longevity. Lastly, absolutely: RNA splicing changes in RNA Seq data do not necessarily correlate with changes at the proteome level and this is something we are actively looking at, along with how RNA homeostasis and protein Homeostasis interact in DR.

Does the repression of a protein process slow or block production from that protein? At what limit do

you stop supplementing processes? I ask because if activating Sirtuins (via Resveratrol) represses CRTC-1 production, and CRTC-1 production is blocked in Alzheimer's disease (http://www.jneurosci.org/content/34/17/5776) , then can enough Resveratrol supplementation induce Alzheimer's by retarding or blocking CRTC-1 production?

SublimeGuy_1587

Hi, /u/SublimeGuy_1587, absolutely there are many feedback mechanisms in our cells that generally try and maintain homeostasis - keeping things the same. This may well explain reductions in efficacy of some drugs over time. Regarding sirtuins and CRTCs - the interaction there is somewhat tissue specific. In liver SirT1 inactivates CRTC2 by allowing it to be degraded by the proteosome. However, in some cells in the brain SirT1 can activate another CRTC family member - CRTC1. We have no data on causal interactions between sirtuins and CRTCs in the aging process but it would be an interesting idea to test.

What are some potential applications of this research?

ImNotImNotJesus

Hi, /u/ImNotImNotJesus, this can also apply to the question from /u/godshammgod15. The goal of our work is certainly not make nematode worms live longer - but to use them as a lab tool to expedite the discovery of new processes that link age to disease risk. Our work shows that deregulation of the RNA splicing process can predict animals that age faster, and that interventions such as mTORC1 suppression or activation of splicing factor 1 can slow aging and maintain animals in a youthful state for longer. The long term goal of our work now is to try and translate these findings to mammals to develop novel therapeutics for age-related diseases. We have already shown that some of the effects of splicing factor 1 in nematodes are also seen in human cells, and we are actively developing this line of research. This will take some time and effort, but the long term application to patients is therefore that we might use specific RNA splicing changes both as biomarkers of health and also as predictors of the response to a treatment. Lastly, if the effects of activating splicing factor 1 on health in C. elegans are conserved to mammals, this might open up new avenues for therapeutics.