[MUSIC PLAYING]

**SPEAKER:** Well, it's great to be here today. I want to start with my disclosures. I don't have any financial disclosures. Some of my work is supported by the NIH. After the talks yesterday I realized I have some additional disclosures I need to include.

First of all, I'm not a pulmonologist. In fact, I'm not a physician. I'm one of the PhDs you were warned against hiring yesterday. But perhaps my most important disclosure is that I am a telomere biologist meaning I see the world as a bunch of different telomere lengths and it undoubtedly influences my own research.

But I just want to make the point really quickly, I didn't-- in research, sometimes you can't pick what you study. I didn't start my career to study lung disease, but I had to make a series of choices to change what I was doing to chase after something. And I'm going to try to illustrate that.

Here's what I hope to cover today. But I'm going to jump right into something that happened a little while ago. We're approaching the 50th anniversary of a paper from a molecular biologist named James Watson who was observing T7 bacteria phage replicating by EM.

And noted that as he watches, he said the following, I see no simple way, this is from his paper, for the three prime to five prime growth to reach the three prime end of the template. Each such cycle of replication would produce smaller and smaller progeny molecules.

Now, I reviewed that paper again this week just to make sure he didn't say anything about pulmonary fibrosis. But he didn't. And who would have predicted when he spotted and named the end replication problem, I don't think the next thing he thought is a-ha, individuals with mutations or problems with end replication will develop lung disease.

So sometimes unexpected things happen and science takes time. This was 20 years after Dr. Watson's other discovery, and it took another 10 years to figure out the mechanism of how the end replication problem is solved, and it took 15 more years to recognize it may have clinical implications in cellular senescence, and it took another 10 years after that to find the first mutation. So it takes time and it takes tough choices.

OK. Briefly, these are telomeres. They're the ends of chromosomes. They have one job, to block the DNA damage response. This looks like a broken piece of DNA. It's covered by these proteins that say this is not a broken piece of DNA.

When a telomere becomes too short they can't bind and you get a DNA damage response, that depending on the context can lead to cellular senescence or apoptosis. The end replication problem is solved by a remarkable enzyme, as Watson correctly pointed out.

As replication occurs telomeres shorten due to the end replication problem. This problem is solved by a remarkable enzyme telomerase. It has a reverse transcriptase, TERT in an RNA component TR, and this enzyme functions to add new telomeres onto the end of chromosomes.

Now, remarkably telomerase is one of the most tightly regulated genes, certainly that I'm aware of, and perhaps in the genome. Small changes in the levels of telomerase lead to disease. Too much telomerase and a cell becomes immortal. The single most common recurrent mutation in cancer are mutations in the promoter of telomerase.

And on the other hand, if you, I'm sorry, too little telomerase, telomeres will gradually shorten over generations and lead to disease, a classic case of the Goldilocks effect. You need exactly the right amount. Because I want to make a quick point and I make this often is, it's not the mutations in these genes themselves that cause disease, for most of these, certainly in the case of TERT and TR.

It is telomere shortening and leads to this one of a kind genetic pattern where it's not inheritance of the mutation that matters, but the telomere length and also yields this one of a kind clinical presentation that I think makes it one of the trickiest to spot in that in each generation oftentimes, not always, depending on the mutation you'll see a different presentation of disease. Such that a single family, grandparents could be presenting in the pulmonary clinic, parents could be presenting perhaps in the liver clinic, and grandchildren in the bone marrow failure clinic, presenting clearly a unique problem.

People who take care of these individuals must be aware not only of lung disease, perhaps bone marrow failure and also unusual phenotypes, brain calcifications, which occur, and it does present a unique challenge.

This was nicely summarized by Dr. Molina. There have been numerous mutant genes that have now been identified in these patients. I just want to point out the ones that have been identified in pulmonary fibrosis, and note there are a few diseases that have so many mutations in the same pathway.

Some of these have unique clinical presentations. These patients with CTC1 mutations, they were identified in a eye clinic as causing Coats, this very unusual disease Coats plus. And it's really interesting. So people think CTC1 mutations cause Coats plus, but if you look in the supplements of their paper, many of these patients die from pulmonary fibrosis. If we don't typically consider it a pulmonary fibrosis disease, perhaps because of the context it was described was in an eye clinic.

So all of the mutations in all of these genes can cause pulmonary fibrosis. I also want to point out that two of these genes PARN and NF1 were introduced to the telomere community by the lung community. So these genes were found in pulmonary fibrosis and then linked to telomere biology.

OK. As I said, it's not the mutations themselves but telomere length that matters. So this was a study we did a few years ago, and I want to point out this was also mentioned by Dr. Molina that these were 52 consecutive patients we measured telomere on, they had the sporadic form of IPF. This was done by flow fish.

And what's remarkable about this is not how short that-- in fact, there were only two patients in this entire population that were average. The entire group itself was shifted down. And this was remarkably reinforced by a recent study by Genentech in collaboration with several academic centers where they hold genome sequenced more than 10,000 people and also measured telomeres.

Now I want to point out their controls in this experiment were individuals with age associated diseases like Alzheimer's disease, rheumatoid arthritis, colon cancer, asthma. And only one of these diseases was associated with short telomeres, and that's lung disease IPF. There's a unique connection between the telomere and the lung.

So we recently spent a lot of time characterizing whether or not measuring telomeres was useful in the clinic. And I just want to point out some of our I think quite unexpected findings. So here are patients organized by their first clinical presentation and their telomere length.

So across the top here is primary immunodeficiency. They have super short telomeres. This on the y-axis here is the change from the age adjusted mean. Patients who present with bone marrow failure also have fairly short telomeres, if you have a combination of bone marrow failure, liver disease, and so on, this IPF and emphysema, so some lung presentation.

And you can see that these have amongst this group the longest telomeres. But if you graph this another way what you can see is really it's age dependent. So there's an age range that when you're telomeres get short, it can determine what your presentation will be.

So if you're a newborn and you have short telomeres, you're going to have primary immunodeficiency. If you're 50 years old and have short telomeres, you're likely going to present with lung disease. Now, I want to point out something here. Short telomeres are not sufficient to cause lung disease.

I'm glad many of my colleagues are here, I'm going to tell you because they think that I think everything in the world from common colds to pulmonary fibrosis are caused by telomeres. But this suggests and shows that there's something else. If short telomeres were sufficient to cause lung disease, all of these patients would present with lung disease. But in fact you need something else.

You need 50 years. So you must have short telomeres plus 50 years. And if you look at it this way, here's a group of patients that we measured and their genetic mutations that they had, I want to point out, all of these mutations were either functionally verified or verified by genetics. And you can see that there appears to be a threshold where you get sick,

So it's not being below the 1st percentile per se that causes disease, but it appears that there is a length at which, and this makes sense from a molecular biology, if your telomere is six kb long, so long as it can prevent the DNA damage response, you're fine. But when it gets down to that level where it can no longer take care of its function, it will cause disease.

So you can see all of the patients that present here you can almost draw a straight line here. Now, there's a problem because the population is aging and we're all headed toward that straight line. This is a form of DNA damage that you can't escape.

Now interestingly, if you look back at some of the autopsy studies that have been done on centenarians. As much as 30% of centenarians have evidence of pulmonary fibrosis, suggesting that this might be, in a sense, a normal part of aging, and this line as it continues to come down and we approach this threshold when our telomeres become unable to carry out their primary function.

So this is just showing it by different genes predominantly, not true for all, but DKC1 and perhaps 10f2 are the only ones we've seen that cause significantly shorter telomeres, likely because of the DKC1's X linked. So these males are essentially null for this gene and, wow, that's a typo, 10f2 because it's a component of the sheltering component.

OK. So I just want to reiterate again that it takes advanced age plus short telomeres to get fibrosis. So there's something else, and this can be nicely illustrated looking at a family, for example.

So here's a family we found where the grandparent here presented at 68 with IPF, had two daughters, one at 55 that had emphysema presenting at 44 years old, a sister that had combined emphysema and fibrosis, and a grandchild that had bone marrow failure. So I think that this family, at least what I took from this, is in addition to short telomeres there are these other things in the world besides genetics, namely the environment, that can make significant contributions to the development of these diseases.

In these two sisters, for example, one had a significantly longer smoking history, and perhaps that particular environmental exposure pushed her toward this disease presentation. But I think it points out a problem here in that a single genetic predisposition can develop clinically multiple phenotypes depending on environmental exposure.

So we've been trying to study this for some time by modeling it in mice briefly. Without getting into it, mice are a tricky model to study telomeres because they have such long heterogeneous telomeres. We came up with a model where we cause telomere uncapping, so by removing one of the telomere binding proteins.

And due largely to work done by the surfactant community, we and Dr. Beer's, we've chose to focus on type two cells when we induced telomere dysfunction. And the idea was this was going to be the perfect model. I let the mice become adults. This was an indusable system. I could give them tamoxifen and induce telomere dysfunction when they were adults and they would develop pulmonary fibrosis or emphysema.

When we did that, in fact, what they got was a very non impressive inflammation. And it was chronic and it persisted. And so I just want to point out something again that we've never seen a patient three years old with pulmonary fibrosis, and these mice may not live long enough to develop the same phenotypes that humans do over 50 years.

So there are profound defects. So this was a inflammation. I mean, it's an issue. I'm not saying it's not a problem. But when you take these cells out, again, we also bred in a lineage trace so that we could pull these cells out. These two mice appear identical, but when you challenge these cells to grow in vitro and form these alveolar spheres, we've never seen a single alveolar sphere yet in these mice, even though by all appearances they look fine.

Their capacity to proliferate and their epithelial capacity to repair is severely compromised. And this again can be observed if you challenge these mice. So here's a [INAUDIBLE] challenge, and sure enough they showed profound sensitivity. So this model has led us to the hypothesis that this telomere dysfunction can limit the epithelial or other cell types inability to respond to an injury.

If you have a reoccurring injury by say breathing or catching colds, over time that can accumulate and eventually exhaust the potential of the lung epithelium to repair. And this process over time can lead to a fiber optic response. So I just want to take a moment here, and I thought Dr. Molina did an excellent job of saying this matters clinically. And so my training before I was a team leader biologist.

I was studying hematology and I was astounded when I switched fields that hematologists tend to sequence frequently. Somebody shows up with low blood counts and they'd send them for genetic testing, and for genes that are quite rarely mutated. So the question is, is it useful? Does this information help us out at all or should we just keep going?

So I just want to point out some recent studies that were done. So Dr. Molina nicely showed that these patients have troubles after lung transplant and that they can have a more rapidly progressing disease. There were two studies published just a few weeks ago that demonstrate some of the mechanism of why that is. So this is one study from Mary Armanios' group, just showing that these patients have a predisposition to these opportunistic infections and they have real teese problems in their immune responses; and a very nice paper from Pittsburgh from the McDior group here and Julia Papeskew, showing that in these patients they have compromised ability to respond to viral infections.

And that matters in the context of lung transplant when there is often CMV mismatches and these patients are subject to relapsing by uremia if their immune system is compromised. So while I tend to focus on the lung I think these studies highlight that these patients have a systemic disease. It's not just the lung that's the problem, but they have a host of other problems that if not recognized can cause problems.

So I'm going to conclude here. I want to acknowledge first of all, the Alder lab, we're a small group right now. Oh, I skipped ahead looking at my screen and not this one. I just want to conclude. So telomere dysfunction really is one of a kind and genetically I think it has to be the most interesting genetics pattern out there. It presents a very significant challenge in the clinic because as was noted sometimes these patients present with normal hematopoietic-- they may appear normal by all measures, yet when challenged will develop clinical problems.

Telomere dysfunction by itself is not enough. It requires time, and usually about 40 or 50 years at least before disease. So there's another component that adds to this. It's unique amongst age associated diseases. This was one of the most interesting things I thought that I've read this year is that I think that telomeres cause all age associated diseases. But turns out I was wrong.

It appears predominantly to affect the lung. Certainly our model suggests that it can inhibit the lungs ability to repair after injury. And I think importantly there's good cause to incorporate this into the workup for lung transplantation. There'll be a talk later today I think from Dr. Madayer that will talk about some potential approaches that could be taken. But I think the reality is, and I'm stealing from one of the after school cartoons I grew up on, and that knowing is half the battle.

I remember when they did a clinical trial for patients with bone marrow failure due to telomerase mutations. And in that clinical trial they gave them myeloablative suppression or regime before they did the transplant and all the patients died. And they had a choice. They could have said, don't transplant patients with telomerase mutations. But I think there's an opportunity then to develop modified protocols, and now there are lots of examples of successful transplants in these patients. But they have to know before they could make those adjustments.

And I'll just finish by acknowledging Mark Roth in my lab. We're a growing group. We're always looking for good people. This group here at University of Pittsburgh is truly remarkable in helping move this research along and other collaborators elsewhere that have helped in my research along the way. And none of this would have been done without funding from these institutions. Thank you.

[APPLAUSE]