

Hello. I'm Monte Westerfield and this is USH Talks. I'm a professor in the Institute of Neuroscience at the University of Oregon. Our laboratory studies Usher syndrome, and today I'd like to talk with you about research that we're doing to develop therapies to preserve vision in Usher syndrome.

Those of you that have viewed previous USH Talks already know a lot about Usher syndrome, that Usher syndrome is caused by gene mutations. We currently know that mutations in any one of 11 different genes-- at least 11 different genes-- can cause Usher syndrome. These Usher syndrome genes produce different kinds of proteins, and it's thought that these proteins bind together to form protein complexes. And hence they work together. And that's why mutations in different Usher genes produce pretty much the same symptoms in Usher patients.

We also know that Usher proteins function in the retina in the eye and in the inner ear, and that's why when there are mutations and problems with these proteins, it results in vision and hearing problems. We also know that Usher gene mutations ultimately can cause retinal and inner ear cells to die. Previous USH Talks have talked about gene therapies that are being developed. These are very exciting research avenues that ultimately may produce cures for Usher syndrome.

But we also know that for gene therapies to work, we need to save cells from dying. Because the gene therapy can't work if the cells are already gone. And this really provides the motivation for the research that I'm going to tell you about today to try to develop therapies to slow or halt the loss of cells in Usher syndrome to provide time for development of gene therapies, and to provide patients with longer-term vision. Also it's possible that these therapies that protect cells may well also enhance the efficacy of gene therapies. In other words, they could be used as a co-treatment.

So let's talk a little bit about the cells that are affected in Usher syndrome. So in the ear, the cells that are primarily affected in Usher syndrome are the mechanosensory cells that are specialized to detect sound. The primary cell type in the eye that's affected are photoreceptor cells. These also are specialized cells. And they're specialized to detect light and provide us with vision.

So the Usher proteins are found to be localized in these specialized cells in the regions as indicated by the arrows in this diagram, in various specialized subcellular locations in both the mechanosensory cells and the photoreceptors. So to try to develop therapies for preventing cell death, we use zebrafish as a model system for Usher syndrome. Zebrafish have been described in a couple of previous USH talks, so you probably remember that they have a variety of advantages for studying Usher syndrome.

In particular, zebrafish retinal and inner ear cells are very similar to humans. Zebrafish have the same Usher genes as humans. And zebrafish Usher gene mutations produce retinal and inner ear cell death, just as in humans. And importantly, zebrafish have advantages for drug screening. They're a small aquatic animal, and it's easy to apply compounds and drugs to the water and then test whether or not they are able to delay cell death.

So how do we measure vision and hearing in zebrafish? We have a variety of methods for doing this. For vision, one simple way is to look behaviorally. So this is a system where a fish is placed in a dish, and the dish is placed inside of a chamber that has alternating black and white stripes.

So when the chamber rotates, the fish's eyes follow the movement of the black and white stripes as shown here. The fish on the left is a normal fish, and you can see the eyes following the black and white stripes, which are not shown in the image here, whereas the mutant fish on the right that has reduced vision does not follow the movement nearly as well.

Similarly, we can examine balance in zebrafish as shown here in looking down on a dish with some Usher gene mutants. There's one fish here in the center who is upright. That's a normal fish, whereas the other fish have obvious balance problems. They lie on their sides, and when they try to swim, they oftentimes swim in circles.

Similarly, we can measure hearing by simply tapping on the side of a dish. This startles the fish, and they respond. Whereas fish with Usher gene mutations do not hear as well. And when they do respond, they have these obvious balance problems.

So how do Usher gene mutations result in cell death? We know that Usher genes produce different kinds of proteins and that these Usher proteins bind together to form protein complexes in photoreceptors and mechanosensory cells. So when and where in cells is there a problem with these proteins? And how does this lead to cell death? So our strategy was to study the mechanosensory hair cells and the photoreceptors and examine when and where the Usher proteins form and where the protein complexes form to try to figure out where and when the problem occurs.

So just to review briefly how proteins are produced, so proteins are produced from the genes that are located in the chromosomes and the DNA in the nucleus. And the first step in this process is that the DNA produces an RNA. And then the second step in the process is that the RNA travels from the nucleus out into the cytoplasm in the cell body to a structure called the endoplasmic reticulum, which we refer to as the ER. And it's at the ER that the RNA is translated into protein.

The proteins, then, are further transported to their functional sites. In this case, this is a mechanosensory cell in the inner ear. So we look to see where along this pathway the Usher protein complexes form and what goes wrong when there is an Usher gene mutation. What we discovered is that the Usher protein complexes actually form at the level of the ER, the endoplasmic reticulum. And the complex as a whole is transported from the ER to its ultimate functional site.

So what happens, then, in an Usher mutation? When there is an Usher mutation, we found that the Usher protein complexes fail to form at the ER even though the mutation may affect only one of the proteins, none of the other proteins bind together to form a complex. And the proteins accumulate in the ER. They're not transported out to their ultimate, functional sites in the cell.

So when proteins accumulate in the ER, this produces an abnormal response in the cell that's referred to as ER stress. And we measure this in a variety of different ways. Two methods are shown here on this slide, where the higher the bar, the higher the level of ER stress in the cells. And these are in the case of Usher IC mutations.

So ER stress in general is not a bad thing. Cells can oftentimes compensate for it. But with the long-term problem that proteins are either not being made, or are being made in an incorrect form because of the Usher gene mutations, this ultimately results in cell death. The cell can not compensate to the ER stress over long periods of time.

So this was a pretty exciting result, because it indicated that ER stress is probably the proximal cause of cell death in the retina and the inner ear in Usher syndrome. We also know that a variety of environmental factors can increase this type of cellular stress. So we examined exposure to toxic compounds that produce ER stress, and we also looked at exposure of the animals to bright light. Because bright light is also a stressor, particularly for the retina.

And as shown here in this experiment comparing normal animals to Usher mutants-- in this case, Usher 2A mutants-- the two bars in the left here indicate the normal in green and the mutants in red when the animals are living in relatively low-light levels in our aquarium facility. And the two bars on the right show the animals

when they are exposed to bright light. This is not intensely bright light, but light that would be equivalent to sitting in the shade on a bright, sunny day outside.

And what you can see here is we counted the number of dying cells in the retinas of these animals. And for the normal animals, there's really no significant effect of increasing the light, whereas in the Usher mutants, exposure to this bright light really increases the number of cells that are dying in the retina, indicating that probably the light exposure is contributing to the ER stress in the cells that's already being produced by the mutation. And that then results in much more rapid cell death.

So in summary, what we've been able to show is that ER stress is a proximal cause of cell death in at least some forms of Usher syndrome. We haven't examined all of the different Usher genes yet, but the ones that we have examined all produce ER stress. And this is an exciting result, because there are already drugs that are approved, and also drugs in development, to reduce ER stress in more famous neurodegenerative diseases like Alzheimer's and Parkinson's disease, where neuronal degeneration in these diseases is also, to some extent, due to ER stress.

So we think that these drugs that are in the pipeline or already approved for patients in these other diseases may be appropriate for use in Usher syndrome. So we plan to use our zebrafish models of Usher syndrome to test these and other potential therapies. We can screen many, many small compounds and other potential drugs to see whether or not they can reduce ER stress and save cells from dying.

Also, our results point out the effect of light. Sunglasses are probably a good idea for everyone, but our results would suggest that sunglasses are probably very important for Usher patients to protect the retina. And it's a real simple thing that everyone can do, so take care of your eyes.

Finally, I'd like to acknowledge people that have done this work. Our group at the University of Oregon, and we are also collaborating with the Radboud University Medical Centre group. And the bottom are our funding sources, people, and agencies that are supporting our work. And thank you very much for your attention.