Potent Biology: Stem Cells, Cloning, and Regeneration (2006)
Lecture Four—Stem Cells and the End of Aging
Nadia Rosenthal, Ph.D.

1. Start of Lecture Four (00:17)

[ANNOUNCER:] *From the Howard Hughes Medical Institute... The 2006 Holiday Lectures on Science. This year's lectures, "Potent Biology: "Stem Cells, Cloning, and Regeneration" will be given by Dr. Douglas Melton, Howard Hughes Medical Institute investigator at Harvard University, and Dr. Nadia Rosenthal, senior scientist at the European Molecular Biology Laboratory. The fourth lecture is titled... And now to introduce our program, the president of the Howard Hughes Medical Institute, Dr. Thomas Cech.*

2. Welcome by HHMI President Dr. Thomas Cech (01:07)

[DR. CECH:] Welcome to the final presentation in this year's Holiday Lecture series on stem cells. You may have noticed a common thread running through these lectures. The tools and findings of basic research provide a powerful springboard for medical advancement. Doug Melton's love of developmental biology is leading to better understanding and hopefully new treatments of diabetes. Nadia Rosenthal's commitment to understanding muscle is leading her towards breakthroughs in preventing and treating heart attacks and discoveries made in mice are being used to translate to strategies for human therapy much more quickly than ever before. In this talk Nadia will extend our thinking about stem cells in regeneration to include how stem cells may play a role in reversing the process of aging and perhaps even extending life span. And now a brief video to introduce Nadia.

3. Dr. Nadia Rosenthal on teaching (02:22)

[DR. ROSENTHAL:] As a child I really was just a naturalist so we spent a lot of time just foraging around and essentially looking into rock pools and having a good time, but I didn't think of myself as a scientist at the time, in fact my parents were from the theater, so if anything I was considered a budding artist because I liked to draw and I think my parents are were quite astounded that I might take on something as bizarre for them as a biology career. So in this case it was the opposite. "Why don't you become a sculptor, why don't you "become an artist?" and instead I said, "no "I want to be a biologist." There's something exciting and almost obsessive about being a scientist. There's never an end to the questions you can ask and I see absolutely no distinction in my students between that bug being caught by men or women it's absolutely equal and I am convinced that this is merely a question of teaching women how to be competitive and how to ask for what they need to get their science done. What's really great about having stuck it out this long in science, with that initial passion still intact is that our lab is actually now able to answer some limited questions about how organisms form and I'm absolutely secure that the next generation of scientists, the young students and post docs in my lab are going to be the ones who will really be able to unravel that in a way that will satisfy what I was looking for namely a quantitative way to approach the somehow intangible nature of beauty and pattern and form. And I think that this is probably going to be the reason that I want to work on aging research because I want to stick around long enough to see that happen.

4. Physiological characteristics of aging (04:21)

Well, buon giorno again. Today we are going to follow some of the themes that we started hearing about in Doug's lecture about degenerative diseases that often affect the aged and how these diseases are in some cases diseases that we could model or perhaps even treat with stem cells. Today I'm going to focus on not the diseases that are associated with aging but the actual aging process itself. So we're all getting old, that's the bad news. And what's worse, if you look on the left at some of the attributes of youth, robust organs high

tissue turnover, wounds healing very rapidly with less inflammation associated with that healing and less scaring. All of those youthful attributes are somehow blunted and compromised as we get older so on the right, the old gentleman, this wonderful drawing of Leonardo Da Vinci showing youth and age facing each other the older man is compromised, frailer organs lower tissue turnover, wounds healing more slowly and when they are wounded, older people tend to heal less well so that inflammation sets in and scaring can often compromise the function of the tissues and organs. So the question is how much can stem cells actually address these natural but rather alarming side effects of getting older?

5. The stem cell pool and ability to regenerate tissue (06:02)

Let's have a look at how stem cells might contribute to different tissue types because different tissue types actually age at different rates. So if we consider tissues in different categories according to their capacity to turnover at the top, high cell turnover, high regenerative potential they include blood cells we've heard a lot about those, gut epithelium and the epidermis and that's because we believe there are abundant stem cells associated with those tissues. Now in the medium category we see such tissues as liver which can actually regenerate quite well and skeletal muscle less so. But it's better than some of the lower regenerating tissues such as the brain, the kidney or the heart which we'll hear about later. And in each case the capacity for regeneration appears to at least roughly track with the contribution of stem cells.

6. Three way stem cells could affect aging (07:02)

So how could it be that stem cells has an effect on aging? Maybe they're just tracking with aging. Is it possible that they actually cause it? If so there are a few different ways in which we could imagine this could occur. One would be that we simply have less stem cells as we get older. Or perhaps we have the same number, but they're less good at doing what they normally do. Or perhaps the environment which they find themselves in our aging bodies, has shifted so that they're all right but where they are is not. So let's look at each of these possibilities and see whether there's any truth to them. The first question is whether there are less stem cells as we grow older. And to look at this question we're going to look at a particular tissue type, which I particularly like to work on, skeletal muscle which is sort of in the middle of the road. There are stem cells, those tissue cells we talked about yesterday, the satellite cells but the question is, are there the same number of satellite cells as the muscle ages?

7. Capacity to rebuild muscle decreases with age (08:10)

Now I'd have to say one thing about muscle. You all know that we can change the size of our muscle rather voluntarily by going to the gym or by working out in other ways, and in fact muscle is one of the most marvelously responsive tissues in that sense and yet at the same time as we grow older no matter how much we work out, no matter how much that master athlete runs, we end up losing up to a third of the muscle mass in our bodies by the time we're 75 or 80 years old. And that really leads to a lot of problems in society. Older people are frailer, they tend to fall and then their bones are brittle so they tend to break and in general it's an enormous cost to society but it's also a real problem for quality of life as you grow older. So these aren't just trivial academic questions these are questions that really could have an effect on society. So let's look at what happens when a muscle is injured and regenerates. As you know from my previous lectures we talked about these stem cells called satellite cells that sit within the muscle bed. They're normally quiescent, but when the muscle is injured they're induced to proliferate by activating signals and this allows them to produce more cells that can then replace the tissue that is damaged or lost so that repair occurs and in fact the muscle is as good as new.

8. Older muscle has fewer satellite cells (09:34)

So the question then is does this effective replacement during aging change and the answer is, it does. This graph shows the number of satellite cells in fibers in mice that are three to four months old out to 28 to 33 months old. Now for those of you who aren't mouse specialists I can tell you that a 28 to 33 month old mouse is a real geriatric mouse, about 85 years old. That mouse is not moving fast and if you see the number of satellite cells in their fibers perhaps that's a clue why that is. So we can then conclude at least for a mammal that older muscle has fewer stem cells and if that's the case then perhaps we just can't replenish our muscles as effectively.

9. Satellite cells from aging muscle are still potent (10:26)

But there's a bit of a conundrum in this because if a muscle stem cell, a satellite cell, is removed from a young muscle fiber as you see in the top panels in the middle there is a little nucleus that's lit up to show that that is in fact a stem cells nucleus, not a regular muscle nucleus. If that muscle satellite cell is removed from the muscle and is put in a dish and allowed to proliferate, slowly but surely it will make muscle and it doesn't do it any better or worse than an old satellite cell. So there's something about the capacity to make muscle that appears to be retained even though these cells are less in number.

10. Aging reduces satellite cell signaling (11:09)

However that's not the only thing that a satellite cell has to do. It has to actually respond to injury and it has to proliferate. So are there changes in the capacity of these satellite cells to do that job? And for this we turn to a number of different well-known signaling mechanisms within the muscle system. In this case we're going to talk about one such signaling mechanism that has actually been associated with aging because it declines in old muscle and that's the Notch- Delta signaling mechanism. So I'll tell you in detail how it works but first the reason that it was considered was it's required for proper formation of the first skeletal muscle in the embryo and it's also important in regulating satellite cells in the adult and it has been shown to decline in old muscle. So what is the Notch-Delta signaling pathway and could it be a clue to the decrease in the capacity for skeletal muscle to regenerate as we get older?

11. The Notch-Delta molecular signaling pathway (12:11)

So here's a picture of what Notch-Delta looks like. Notch-Delta consists of two sub-units. Both are membrane associated proteins both have a little part of them that sticks outside of the cell and another part inside. Delta is in the top in the satellite cell in this particular picture and Notch is in the bottom. These are both receptors in the sense that they receive signals and the way they do that is to interact. When they interact there's a relay of events which includes clipping the two sides of the dumbbell of Notch apart so that that pink part the intercellular part can move to the nucleus and start transcription of RNA. So in that case we have a way of telling the cell on the outside to do something on the inside and that's exactly what signaling does.

12. Notch-Delta response differs in young and old muscle (13:06)

In a young muscle, after injury we find a very robust increase in the number of Delta molecules sitting on those satellite cells and that is probably the reason why in fact satellite cells are so responsive to injury because they increase the signaling rev it up and are able therefore to go into another proliferative round. And interestingly in old muscle that response is absent so that the Delta increase doesn't show up. Now in some real-life pictures from Tom Rando and his colleagues, here you see what muscle young and old looks like after it's been injured as it pertains to the Notch- Delta signaling system. On the left is a young muscle that's been injured and the injured fibers here are artificially lighted up in a sort of pink/yellow scheme and you can see that the same injured cells are in the old but there's a difference, no green. What's the green?

Green is Delta. So Delta is being highly upregulated in the entire vicinity of the injury in a young animal but somehow that response is absent in the old animal.

13. Changing Notch levels affects muscle repair (14:19)

Now again this is one of these observations where you could say, well this is an obscure set of receptors that's interacting and it changes during aging. How do we know it has any causation? Maybe it's just going along for the ride. Now in order to test for that scientists have to perturb the system. So we have to change something about Notch-Delta signaling and see if that changes the way the muscle can regenerate. And that's exactly what Tom Rando and his colleagues did with some very clever tricks. First of all I have to explain that there are ways in which you can inhibit the Notch signaling pathway as well as increasing the Notch signaling pathway and I won't go into the details of how we do this, but just suffice it to say that if you treat a muscle with an inhibitor and you injure that muscle, in a young animal you see that in the top left panel a normal muscle starts to degenerate in a way that is much, much more reminiscent of what an old muscle looks like if you inhibit Notch. That is to say if you compare the inhibition of Notch in a young animal, it looks much more like the way an old animal responds to injury that's in the lower left. Now let's say in a complementary study we activate Notch. Can we actually turn an old muscle into a young muscle in its capacity to regenerate? And the answer is we can. So here you see that an old muscle is looking much much more like a regular young muscle in its regenerative capacity. So these sorts of experiments begin to give us clues as to what molecular changes are going on during muscle aging and how we might intervene in those changes. Now

14. Does the stem cell's environment change during aging? (16:07)

finally, I'd like to talk about the possibility that the stem cells have their own intrinsic problems during aging, but the environment is aging as well. So the question here is, if we change the environment of an aging tissue, will the resident cells in that tissue respond in a different way? Let's look again at what happens in an injured muscle. In a young injured muscle we have a very robust signal that seems to go through the entire muscle activating the satellite cells. It involves Notch, it involves a lot of other things as well and what that does is produce in the end, a proliferation of the stem cells. In the older muscle a similar injury is capable of... in activating those same responses but they're blunted in some way as if there was some negative damping down of the response to regenerate and in that case what you see then is less satellite cells. So would it be possible that there is an environmental effect here on the way these satellite cells are responding and could there be some way that we could expose the young muscle to an old environment and the old muscle to a young environment?

15. Response of old mouse stem cells to young mouse environment (17:27)

Now short of transplanting muscles from young animals into old animals which doesn't really give you that much information Tom Rando came up with a very ingenious scheme. In this case he asked the question "Where's the fountain of youth?" Is the young animal capable of reprogramming old muscle to respond to injury in a more effective way or is it possible that the old animal if exposed to a young environment will actually in some way damp down the capacity for the young muscle to regenerate? To do this he made parabiotic parings between mice of different ages. Now how this works is a small suture is made in the side of the animal's skin. In both cases in these pairs they are sutured together as if they were mini Siamese twins. Not their whole body, but just a small portion of their body is connected. At this point what happens is that the circulation of the two animals begins to run into each other and eventually after a couple of days of this the animals actually share the same circulation. Now they share the same blood cells but they also share all of the factors that are floating around in the serum of your blood,

16. Young-old "pairing" improves repairing ability of old muscle (18:40)

and the question then can be asked, what happens if you injure the young animal or the old animal in these, what we call heterochronic pairings, or partners? So I'm going to show you a complicated slide which gives the answer to this question and although it's complicated it has a real dramatic punch line here. So let's go through it very quickly. We're going to look first at the capacity for a young mouse to sustain injury when exposed to a young mouse's environment. That's a control because in fact we assume nothing's going to change. And in fact if you see at the top, you're beginning now to be muscle experts, you can tell that that muscle there is actually regenerating pretty well. Now at the bottom you see a stain for red and red means new muscle forming. So where you see red on the lower panel that means regeneration is occurring so this is just a very easy way to see whether there are going to be changes. Now you see here that if you injure the young animal and the young animal is partnered with the old animal, it doesn't seem to affect the young animal the young animal continues to be able to regenerate just as well. Now let's look at an old animal who is paired with another old animal, again another control which tells us that the actual procedure itself isn't changing anything about the way in which these animals can regenerate. The punch line is here, if the old animal is injured when paired with the young animal, it regains the capacity to regenerate its muscle. So that's pretty astounding. That means that there's something about the young animal's environment which is affecting the old animal.

17. Serum factors can activate Notch pathways in old cells (20:30)

Okay, so the obvious answer you're all thinking I'm sure is, well that's easy, they're just stem cells in the young animal that are going over into the old animal and doing the job. I mean that's the obvious answer to this so in order to test for this we looked in detail at the capacity for the young animal to actually both activate the Notch pathway and to deliver cells to the old animal. So first what we're going to see here is that in fact the Notch pathway is activated in old animal cells if cultured in a dish with young animal serum. Now the serum does not have any cells in it it's just the stuff that your blood cells float around in. It's full of factors and those factors are capable of activating the Notch pathway in the old stem cells. So that tells us that it's probably not cells and here's just the data to suggest that that's the case. Here you're looking at young cells on the left with serum from young and old same number of Notch positive cells. The old cells that are exposed to either young or old serum either have less Notch as we see in the previous slide, but they also have the capacity to activate Notch with young serum. Now

18. Old muscle revival is due to young molecules, not young cells (21:56)

finally in a real proof of concept in the actual animal you can mark the cells in the young animal by using a transgenic animal and that allows you to follow the cells if they do move over at all. So the way we do this is to use a young animal that has a green fluorescent protein transgene in every one of its cells and then we look in the old animal after injury and see whether in fact there are any green cells in that old animal and the answer is there are not. So although the cells are moving around those muscle cells are actually controlling the regeneration of the old animal's injury in response to young factors. So before we take questions then, we'll just review what we've seen so far. We can attribute some of the decrement in function in aging to decreased number of cells, at least in skeletal muscles. Those cells don't work as well and the environment in which they find themselves is not as conducive.

19. Summary of factors involved in aging and stem cell response (23:04)

So probably what we're looking at here is a mixture of different factors, lower stem cells worse environment and we're now poised in the field to ask the questions, if we change the environment can we change the number of stem cells? Can we actually rejuvenate ourselves if we understand more about the fountain of youth, the famous fountain of youth which appears to be floating around in all of your blood streams and less so in mine. So obviously we'd love to know what are those factors. And now we're in a wonderful

position to be able to answer that question with a systematic approach. We have a test and we have a question in which we can couch that test. So finally then we can ask the question for the next part of the talk, are there factors that we already know about that could perhaps be some of these candidate factors and are there ways in which we can improve both the capacity to regenerate our own tissues, but also perhaps to improve the capacity for stem cell therapy to work better in older people

20. Q&A: How do muscle cells "know" that they are injured? (24:13)

and I'll stop there and I'll take questions. Yes.

[STUDENT:] I was wondering how muscle fibers actually know if they're injured or not in a molecular sense.

[DR. ROSENTHAL:] Well that's a very good question and the problem is that this is a field that I love and so you are going to have to stop me from warbling on and therefore what I'm going to say in a very short hopefully succinct answer is that there is a mechanical effect on muscle which is to literally destroy cells and when cells are burst apart there is an immediate response in the surrounding tissue of any organ and what happens among other things is that signals are sent out from that injured area to call in important other cells from the blood stream such as the inflammatory cells the cells that might be there who need to actually clean up the mess, to take away the dead cells and make sure that those dead cells are appropriately degraded and to couch, if this is a surface wound to somehow close it to keep it from getting infected. Each one of those populations of cells that comes into the area is a potent source of factors itself so what we're trying to figure out now is, what are the factors that are coming from the injured tissue versus what are the factors that are coming in to help clean up the mess. Just like that, concentration. Yes

21. Q&A: High regeneration tissues have medium stem cell numbers? (25:50)

I'm going to save this one for you.

[STUDENT:] Why is it, despite the amount of stem cell contribution, that the amount of regeneration is the same?

[DR. ROSENTHAL:] In which case?

[STUDENT:] Like in the chart, we had a triangle decreasing the amount of cell contribution?

[DR. ROSENTHAL:] That's right.

[STUDENT:] There's no higher regeneration.

[DR. ROSENTHAL:] Oh, I'm sorry, in that middle part. In those middle tissues. We believe that those tissues have been somehow programmed to be able to regenerate because they're constantly in very heavy use. So if you think about the two tissues that were mentioned, skeletal muscle and liver, that are in there these are tissues that are not in high turnover mode. That is to say they're not actually being sloughed off the way the intestine or the skin is, but they are also prone to injury. In the case of liver it's because of toxins or waste products that could actually injure the cells. In the case of muscle you actually injure your muscle when you go to the gym, that's why it's highly not recommended, but at any rate you can actually feel, "no pain, no gain," that is injury and that means that your muscle has to have a way to rejuvenate itself. However we believe that in those cases the amount of turnover is not as great as it is in things like the skin and therefore we're just in a sort of intermediate stage, but this is somewhat of a hypothetical gradation and

obviously each tissue type has a different way of doing this repair process. Okay. And this one is an easy one.

22. Q&A: Old stem cells revert if young environment is removed? (27:30)

Yes.

[STUDENT:] If we do happen to make the old muscle cells look young by changing their environment or by just changing or putting the young cells into the old cells wouldn't it be most likely that it would just relapse back into the way it was before we changed its environment just because cells are so just comfortable with their beginning environment?

[DR. ROSENTHAL:] Well if in fact the argument is that the environment can change the cell or perhaps a better way to say it is that the cell has no intrinsic reason not to be able to respond to the environment not to be able to turn on its Notch signaling pathway or to proliferate in a way that it needs to do, clearly the minute that we take out the stitches and separate those two mice the old mouse will go back to being weak again, there is no question about it, so this is not a solution. Do not get any old person to allow you to suture yourself to them because you'd have to stay there for the rest of your life and you'd get old too and then it wouldn't work anymore. So basically we're looking to think about ways to use that proof of principle to isolate the molecules involved. Now those molecules might be things that we can actually deliver as therapeutic in a much more consistent and lengthy way to stave off some of the problems associated with aging.

23. Q&A: In rapid aging diseases, do stem cells appear old or young? (29:06)

The lady with the dark jacket and the red scarf around her neck.

[STUDENT:] I've heard of a disease, I'm not sure what it's called but it's where young kids age really fast but their age is still the same and their personality is still young... but, don't they still keep their stem cells from when they were young or do they also lose their stem cells?

[DR. ROSENTHAL:] That's a really good question and this is a very rare but extremely distressing disease called progeria in which there is premature aging, in fact the progeria phenotype is one that has been actually modeled in animals looking for ways in which to induce premature aging in a mouse, to try to understand what it is about humans with these afflictions that we can understand and perhaps cure, and it's a long and complex field and would be a wonderful subject of another Howard Hughes lecture, but I'll leave it to say that we're basically at the point where we know a little bit about the disease, it's multi-faceted and stem cells in certain cases are involved. So I think I'm afraid I'm being told to move on to the second half of the lecture and so I'll obviously take more questions after that.

24. Can we improve the heart's ability to regenerate? (30:36)

So I'm going to finish this lecture series with a major challenge and that is to address the issue of how we might be able to regenerate an organ that we all depend on, minute by minute, but that is one of the most intractable organs to regenerate in our entire body and that's the heart. So the heart, of course, as you know is a very complex organ that forms very early in development and continues to function throughout our lives and yet if you find it on our chart here you see that it is one of the lowest regenerating tissues in mammals and it has some of the lowest cell turnover outside of the brain. Now what is the function of a heart? Well it's a pump and it pumps in two different ways. Seen here in the animation,

25. Animation: Heart structure and function (31:32)

we're going to first look at the right ventricle atrium connection in which cells are being brought out of the low oxygen-starved body and pumped into the lungs, and if we look at the right hand side of the heart, oxygen-rich cells are being brought out of the lungs and being pumped back into the body, and if we look at the two sets of two chambers together we see this marvelous coordination which allows us to take our blood out of our body and put it back into our body in a completely different oxygen state.

26. Heart function is crucial, but the heart is a poor regenerator (32:05)

So with that kind of extraordinarily important function which goes on from the minute you start to be a human being, somewhere in that embryogenic gray zone, when you heart starts to beat till the day you die, you can't miss a beat well a few, but that's about all. And so that heart has to go on and on and on and wouldn't you think that that would be the one place that would be full of stem cells and yet it's not.

27. Demo: Cause and effect of a heart attack (32:33)

It's so bad that when people get heart attacks which means a cessation of the function of the heart due to an injury, it's often a lethal or eventually a lethal condition. So what is a heart attack? I'm going to show you on this little model here. So that's about the size of a human heart, about the size of your fist and the two chambers that we saw before are more or less here, the two big chambers that essentially drive your heart and if you see how much muscle there is, it's a bulging muscle, I mean it's essentially all muscle it has to be because it's a pump, it's pumping and pumping. The only way that you can keep this heart in this incredibly ready state where it can give a beat every minute or 60 beats a minute or whatever it is is if these veins and arteries called the coronary circulation are fully open and operating. So the heart not only pumps for the body it pumps for itself and the way it does so is to take a little bit of blood out of the highly oxygenated blood that's coming through the aorta and divert it into its own muscle to hold that muscle in a state of eternal readiness to work. Now if their is any problem with this circulation you essentially deprive the heart muscle of oxygen and as you know without oxygen tissues can't live. And so heart attack is usually caused by a problem in one of your arteries. So assume that this is one of your arteries running through your heart. This artery is probably my artery after having spend five years in Italy eating too much rich food. All of this is yellow stuff is called plaque and it's a collection of cholesterol which builds up in the inner sides of your blood vessels over time and this can be a genetic cause or it can be a lifestyle cause but at some point during this period there's the danger that some kind of a rupture can occur through some kind of a mechanical injury in that plaque and blood can flow out into your blood vessel that actually causes, because it's an injury, a clot. And that clot, to close the injury against the outside which is in fact the blood vessel, can dislodge and gets stuck in one of these descending arteries right here, and then what happens is that you see what we call an infarct.

28. After a heart attack, cardiac tissue is lost (35:22)

On the left here you see the blocked artery has caused an area of damage, shown here as a sort of black smudge. What's going on there? The dying myocytes, shown in purple are of course not able to contract anymore and the ones that are surviving around the side try to divide but myocytes aren't very good at dividing, and what happens eventually is that the scar tends to actually grow and the reason it grows is that the myocytes around the scar start to expand and become larger and hypotrophic because they have to still do the same job for the whole heart that that healthy part did. Yet those large myocytes eventually get to the point where they can't get any bigger and yet the heart is still having to pump. At which point they actually end up dying. What eventually happens is that this original often minor injury that people don't even know happened, then turns into a major problem because the heart starts to fail. It cannot actually pump anymore and then you have heart failure.

29. Other organisms can regenerate heart muscle (36:31)

Now this is a really important problem in the Western world. And obviously it's something that many people have considered as a prime target for therapy, in particular stem cell therapy because we are looking at a loss of tissue that's exactly what a heart attack gives you it gives you the loss of the myocardium. However scientists, biologists have noted for a long time that this incapacity for the mammalian heart to regenerate is in fact not something that it shares with some of the lower organisms. And here we see again, regenerative capacity on the left and evolutionary scale on the right and again we see that some of our lower vertebrate cousins can do a much better job. So let's have a look at the fish. The fish is capable of regenerating its heart in the most remarkable way. And for that we're going to see a video.

30. Animation: Zebrafish heart regeneration (37:26)

Zebrafish, which any of you who are fish fanciers keep in the fish tank, can get to be about two inches long. It's got a little heart with just two chambers one atrium and one ventricle and that pumps blood throughout the body past the gills which are the equivalent of the lung in a fish. The heart is very muscular just like our heart, inside there are very thick muscular walls that allow the heart to effectively pump throughout this very complex series of veins. Now let's see what happens if we cut off the tip of the heart. Ouch! Don't worry, the fish is okay. It's okay! The fish immediately closes up that wound within seconds the clotting process starts up. Now in our heart what would happen is there'd be a big clot, and eventually that clot would heal over but nothing more would happen. But Ken Poss and his co-workers have recently noted that, aside from the fact that there are cells within the zebrafish myocardium in the red muscle that can actually activate like satellite cells and start to divide to make new muscle there's also this slowly proliferating, engulfing layer of single cells on the outside of the heart called the epicardium which is really a magic layer of cells because it engulfs the whole scar area and then in a really remarkable series of events that recapitulate developmental biology of the heart a series of growth factors in this case a growth factor called fibroblast growth factor is produced by the heart as it's regenerating and this fibroblast growth factor or FGF docks into epicardial cells set on the edge of the heart and those epicardial cells with the signal know to march into the myocardium. So there are they are marching in. Now, the next thing they do obviously now we're in a macroscopic picture is a absolutely fundamental part of making new muscle which is to vascularize it. So with the new veins in there, the fish ends up with a brand-new heart with new muscle and not only new muscle but all of the appropriate coronary vasculature and the fish can actually survive and swim away.

31. Potential cell therapy for heart failure (39:57)

So we can't do that! Boy would we like to be able to do that. I mean I don't want my heart cut up but if it ever happened I'd like to be able to swim away like that. Now what's the difference between fish and mammals really then? Well let's just look at it from a very simplistic point of view. Hearts can regenerate with tissue replacement in a fish. Several different layers of cells seem to be involved. There seem to be stem cells within the myocardium and then this magical layer which is the origin of the coronary vasculature in development for the heart seems to recapitulate that same program and become again capable of making new blood vessels. In the human heart failure is caused by tissue loss and no replacement. So what are the possible ways that we could address this problem? Well we could study the fish. And that's exactly what people are doing. What makes it so possible for a fish to be able to do this? But we can't wait as human beings to understand exactly what's happening in the fish, we'd like to be able to start treating the patients who are in the clinics right now.

32. Evidence that the heart can incorporate circulating cells (41:02)

Can we replace heart cells to treat the disease? Well this idea actually has its origins in some very intriguing observations early on in heart transplant medical practice. So in a transplant, a truly failed heart that can no

longer work is removed and a healthy heart from a donor is put in its place and you literally sew the veins and the arteries onto the new heart and the new heart can function more or less as the old heart did. This is a very complex procedure, it's very expensive and the problem is there aren't that many hearts floating around to use for the many, many people who need them. Nevertheless the fact is that when you put a heart into a foreign host you can ask interesting questions about that heart. Does that heart get any cells from the host? Does it incorporate cells from its host? Now if in fact the heart that was put into a patient comes from a female into a male patient, the male patient in each one of his cells has a Y chromosome that the whole heart that was transplanted does not. So scientists have probes that we can use to show whether a cell has a Y chromosome or not. So we can actually go into autopsy situations after the patient has eventually died, and look at that transplanted heart that often was living in that patient, that female's heart was living in that male patient, could be for decades, and look to see whether there were any male cells that ended up in the female heart, and miraculously there are quite a few. So this means that a normal functioning heart can actually pick up cells from its environment. So if that's the case, how is it doing it? Is it picking it up from neighboring vessels? Or is it picking it up from the bone marrow because of course the bone marrow is full of male cells and the blood's going through the female heart everyday? We just don't know the answer to that but it suggests that the heart is capable of picking up cells that are circulating.

33. Using bone marrow stem cells to rebuild heart tissue (43:09)

So with that encouraging piece of rather arcane information in hand, scientists have begun to think about how to take stem cells from a patient namely bone marrow stem cells from a patient that's healthy in their bone marrow, but has a horrendously failed heart and see if they can actually help the patient that way. Now remember that these progenitor cells have to do two things that I showed you the zebrafish does effortlessly. Number one, they have to make new muscle cells and number two, they have to be able to put blood vessels through that muscle to give it the appropriate oxygen. So I'm going to tell you a little bit now about where we stand with these sorts of trials because they're ongoing. For patients that have had acute myocardial infarction and these are trials that are based entirely on bone marrow stem cells from the patient themselves going into themselves, so these are what we call autologous transplants. These can be extracted from the patient's bone marrow, and cultured, purified, depending on the protocol some of the trials have used certain subsets of bone marrow cells called endothelial progenitor cells or EPCs. Others have reasoned that muscle cells might be possibly useful even though they're not heart if they contract maybe they would work as well and these cells are then introduced in various ways into the heart of the patient. Now in the case of the left hand protocol there's an infusion balloon that runs through the aorta down into the patient's heart and delivers the cells that way. So catheterization of the circulation in human patients is something that is very well understood and is quite routine. Alternatively you can use needle injection with a catheter and a flexible needle so that you can actually put the cells into the myocardial wall or you can just open up the patient's chest and jam the cells in, *Pulp Fiction* style. Now

34. In recent trial, stem cell heart therapy had mixed results (45:12)

I'm going to tell you about one recent trial that was just published about a month and a half ago in which that first method was used intercoronary infusion. In this case the patient was an acute myocardial infarction patient, heart- attack-recent patient comes into the clinic is and is chosen to go either into a placebo group, or into a group that's going to get the cells. So what this means, and these are very complicated trials to set up because you also have to blind all the people who are doing the trial to whether the patient is getting cells or just serum, just saline because you might be able to pick up all sorts of effects based on perception, and so you want to get rid of all of that. So these are now trials that are called double-blinded placebo-controlled trials. These trials mean that many patients come in some of them are secretly in a placebo group, some of them are secretly in the group that's going to get the cells. They're all treated as any patient should be treated for myocardial infarction but there's just this added extra treatment, and then the question is what happens. Now this was a trial, very large trial and many patients were enrolled and the results just came out and the

answer is encouraging on one level but a little bit disappointing on another. As you see from the numbers here, patients that received the bone marrow got a 5.5% increase in their function as measured by ejection fraction which is the amount of blood which is ejected out of heart. So the better the ejection fraction, the stronger your heart is. Now of course some people recover spontaneously from heart attacks and you can see that by the number of the placebo which is 3%. So a 3% increase in the capacity for the heart to do better is what you would get anyway. Now this is not going to cure anybody in the long haul. So clearly, although it's a proof of principle that there's some effect, beneficial effect, we are far from the optimal protocol. Clearly we have a lot of work to do.

35. Using an IGF-1 mouse to see if heart muscle can make new cells (47:20)

So what are the other ways in which we can address this? Maybe we can stimulate the heart itself to make new cells. And if that's the case maybe that would help us to address the issue. So in our lab we've tried a technique in mouse to see if that would work and you're familiar with this story because of the IGF 1 growth factor treatment I told you about yesterday for muscular dystrophy. In this case we tried the same trick, except we addressed the question of heart regeneration. To do this we made a mouse that overexpressed this transgenic IGF 1 in the heart and we did so by injecting a cardiac transgene into the heart of a mouse and then we followed that mouse as the progeny developed that had the gene expressing this growth factor within the heart and looked to see whether we had any effect at all on the heart or whether these animals developed abnormally because we're putting a lot more of this growth factor into these mice and they have this growth factor from conception onwards. And miraculously we found that the animals regulated very well. They saw the growth factor, they didn't seem to mind the growth factor. The only thing we did see is that if you look at these pictures of mouse hearts as they get older at two months of age the mice had slightly larger hearts if they had IGF 1, but it was just a precocious growth to an adult stage because we found larger cells, which in this case was what we expected, but those cells never did anything other than get to the size they normally would be in a six month old mouse, and then the mouse seemed totally normal. So on one hand we were very happy and on the other hand we wondered if we had just wasted our time.

36. Damage to cardiac tissue heals in the IGF-1 mouse (49:07)

So we decided to try to mimic an myocardial infarction in a mouse. Now mice actually don't like McDonald's, they don't eat rich food. It's really hard to get them to go into some sort of atherosclerosis. They're not really fond of high cholesterol, they like carrots, unlike Doug, and therefore you have to do something else. So what we do is we put a little string around the coronary artery and tie it down a bit as if there was a clot in there and that gives them the equivalent of a myocardial infarction as seen on the right. Now we then can follow to see whether the animals that had the growth factor do better than the animals without. So on the left you see a mouse heart that looks normal with a big left ventricle in a cross section. And on the right you see a control in which we opened up the mouse and gave it a myocardial infarction closed it up and waited for a couple of months and then looked at it again, and you can see that this mouse has very nasty heart failure. That thin wall is exactly what patients look like after they've had a myocardial infarction and waited a long time. Now a myocardial infarction, the same myocardial infarction technique on one of these animals that we had engineered to express the growth factor, didn't appear to have the same response at all and as you can see in the lower left hand panel there was a rather miraculous recovery. In fact it reminded us, perhaps hopefully of the way the zebrafish regenerates. And so we believe then that the way in which the growth factor works is somehow to retain some of the tricks of evolution that we've lost and go back to being able to regenerate the heart

37. Cellular basis of IGF-1's role in cardiac tissue regeneration (50:55)

and if you look at the actual cellular basis of this I've done it as a cartoon here. On the left you're familiar with this picture, it's a scar, it's going to eventually cause problems for this mouse. On the right we have

brand new cells, we have a vessel going through it and essentially we're on our way to a complete recovery. Now how could IGF 1 be helping? Well one thing that we noticed is that

38. IGF-1 animals express signaling factors that aid in regrowth (51:23)

IGF 1 overexpressing animals appeared to express a number of molecules that we associate with homing and there are growth factors and all sorts of other ways in which we can find tracks of cells that are preferentially drawn to regions of injury and in fact that's exactly what we saw, that these attractive molecules called chemokines were expressed at quite high levels in these animals.

39. Tβ4 found to reduce scarring in heart damage (51:51)

Finally, before I finish I'd like to just mention a very, very new result. Literally two weeks old. So I threw these slides together literally over the last two days to show them to you because I think it's very exciting and it brings home a point that warms the cockles of my heart and that is an experiment that was done by Paul Riley recently in which he tested the capacity for a molecule called thymosin beta 4 which is actually a molecule that helps cells rearrange their cytoskelton, their shape, to help with the capacity for a mammalian mouse heart to regenerate and what he found in a word was that injecting this molecule into the mouse's heart created a much better environment. In fact it looked a bit like what happened when we over expressed the IGF 1 gene

40. Tβ4 and FGF create a response similar to zebrafish regeneration (52:42)

and when he looked in detail at how this actually occurred, it was really astounding because the thymosin beta 4 was activating that epicardial layer, just like the zebrafish does. And so it appeared that we were beginning to have a pathway here that we could actually start to imagine looks like a zebrafish regeneration pathway that we could artificially induce in a human heart. So we imagine that thymosin beta 4 might activate the epicardium to make new blood vessels. We know that FGF is another factor that we can deliver that might actually then turn those newly-activated epicardial cells into cells that can actually make blood vessel cells, endothelial cells and eventually then, hopefully we can understand how with the IGF 1 gene we might be able to build new heart muscle itself. So in fact we believe that there might be ways in which we can replace cells, we might be able to stimulate the heart to make new cells and we can even keep it alive by improving the way in which the hearts revascularize their tissue using this rather amazingly reminiscent protocol that looks for all the world like a protocol that the zebrafish has used

41. Stem cells for potential heart repair (54:00)

by definition. Anyway, just to finish off then, here are some of the ways in which cells might be used to cure heart disease and in many ways you can think of many organs standing in for this heart because the ideas are the same. The ideas come from the possibility for cells within the tissue itself to regenerate and how can we activate those. Cells that might be available that we're not even aware of in a tissue that could be cajoled into helping out and finally cells that might come in secreting different factors that could help us to convince the heart that it actually could regenerate in a better way.

42. Acknowledgments (54:41)

Now before I close and take questions I'd like to just acknowledge the fact that I have a rather extraordinary group of people to thank, which are my own lab, and I have to tell you that they're watching this from Italy. They called me yesterday and told me that I was a disgrace for the T-shirt episode. **[laughter]** And that if I didn't do better I wasn't allowed back. But I mean in all honesty that they've been wonderful in letting me out of school for a couple of days to come and talk to you and it's really a pleasure to have young people in

our laboratories who get as excited about science as we do and I'd just like to acknowledge all those wonderful people in a rather salubrious environment in a small trattoria in Rome,

43. Q&A: Are blood types a factor in heart transplants? (55:28)

and now I'll take questions. Yes.

[STUDENT:] With heart transplants, do blood types still play an important role in them?

[DR. ROSENTHAL:] With heart transplants blood types do play an important role and in fact the capacity to hold onto a transplanted heart is largely dependent on how well that heart is matched at a number of immune levels. So thanks all very much and I'm going to now turn the podium over to Tom Cech.

44. Dr. Thomas Cech announces speakers for next Holiday Lectures (56:01)

[applause] [DR. CECH:] Nadia, thanks for another great lecture. I want to thank everyone for a very successful Holiday Lecture series. Thanks to the Howard Hughes Medical Institute staff to our production team, especially to you the student audience for all of your great questions and let's have one more round of applause for our terrific speakers. **[applause]** Now how about next year? Well, our topic for next year's Holiday Lecture series is HIV and the global AIDS epidemic. Hughes investigator Bruce Walker from Harvard Medical School will be joined by his colleague Bisola Ojikutu from South Africa and they will present four lectures talking about how the AIDS epidemic got started, what the virus is like, how it became an epidemic and what we can do in terms of prevention and treatment. So we hope to see all of you next year. Until then have a great holiday season. **[applause]**