RNA Biology
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Lecture - 57
Telomere, Telomerase and Impact on Genomes: Maintenance and Manipulation of Telomeres

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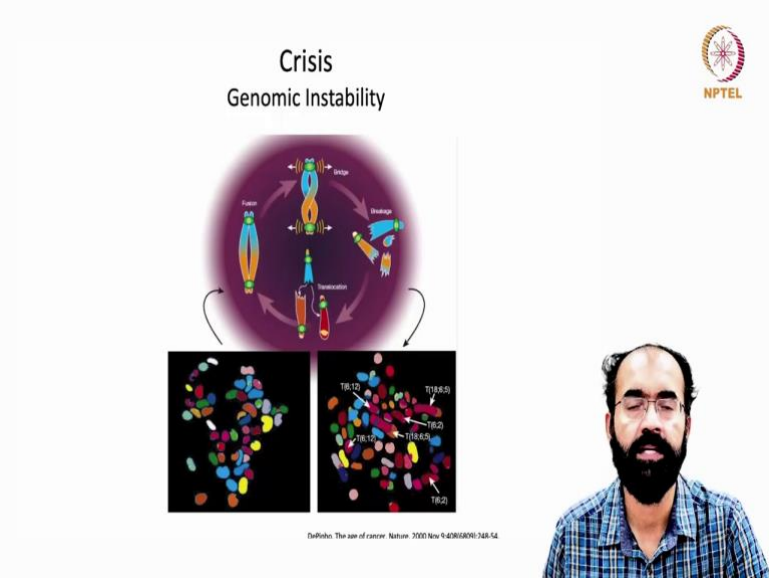

Hello everyone, welcome back to another session of RNA biology. And we were here in the previous class we are discussing how the chromosomal damage or how the reduction in telomere length can cause genomic instability means breakage of chromosome and can lead to damage in the genes. And allowing the cells to survive based on the availability of required genes for that cell survival.

During this process lots of cells will lose lots of genes, but that is ok for cancer cells because their goal is simply to survive. They do not bother about utilizing energy effectively.

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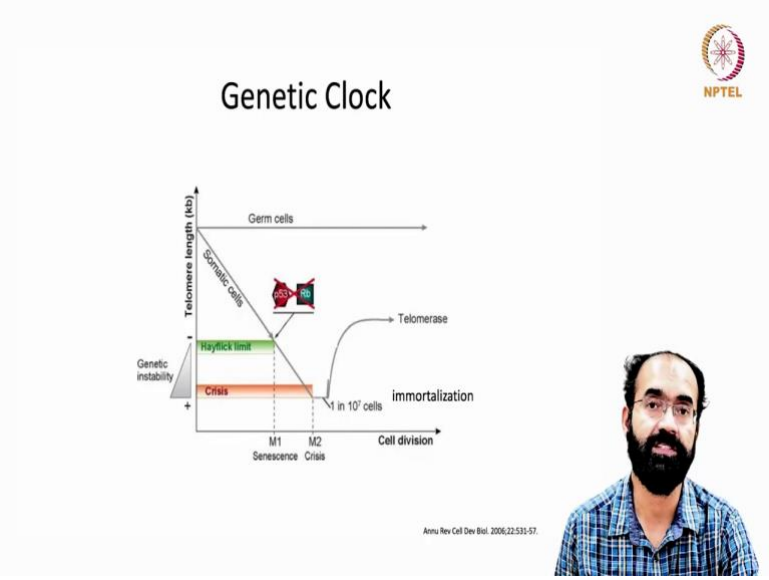

So, coming back to genetic clock the telomere length as it comes down it will reach a Hayflick limit, that is the limit in which the cells no longer able to divide. That means, until a critical length of the chromosome or the telomeres is tolerated. Once it goes below that it will eventually lead to a crisis phase.

In crisis phase the cells will completely stop dividing unless it gets a second chance in the form of cancer. So, telomerase activity has to be turned on if it has to make a comeback. So, normally if a cell is dividing say 10 raise to 6 cells means 1 million cells, 10 raise to 7 cells means 10 million cells.

So, 1 cell in 10 million will have a chance of reactivating the telomerase; that means, its spontaneous reactivation; that means the problem due to the Hayflick limit or crisis is exclusively due to reduced telomere length. So, telomerase is the rescuer. If it turned on spontaneously 1 cell in the 10 million total cells. So, that 1 cell will get a advantage survival advantage.

So, this can lead to immortalization. So, we discussed about immortalization of the cell sometimes done using viral vectors, sometimes it happens due to cancerous condition. Sometimes like this I mentioned here 1 in 10 million cells the telomerase can get activated and it can eventually lead to immortalization of that cell that means, the presence of telomerase ensures that it will not reach the Hayflick limit or it will not reach the crisis phase.

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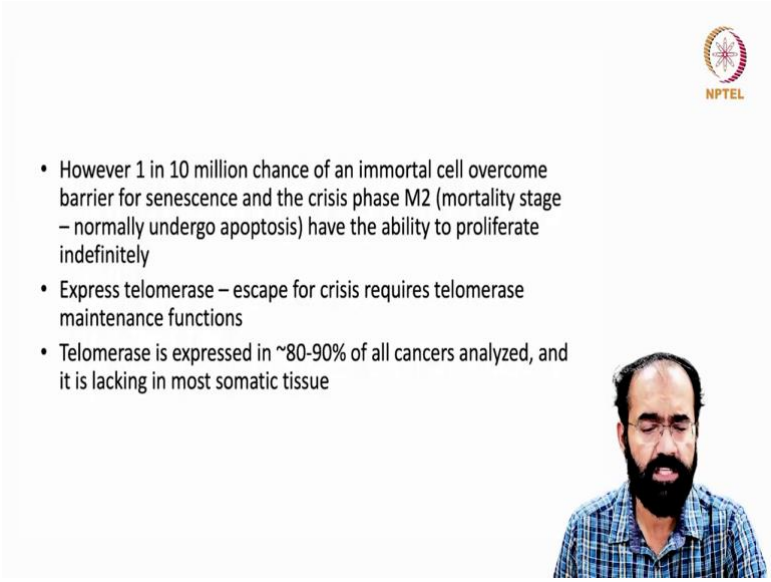

So, 1 in 10 million chance of an immortal cell to overcome the barrier for senescence and the crisis in M2 phase. M1 phase crisis is by the Hayflick limit that is the length of the telomere reaches. And once it reaches further low because of the prolonged or continued cell division it will reach a M2 phase. That is it is the second final phase of crisis. So, the mortality stage once it reached M2 phase this stage the cell undergoes death that is apoptosis.

So, once these cells 1 cell in 10 million have the ability to overcome this barrier the so called senescence and they will continue to proliferate. If it has this telomerase activity turned on, if it was able to withstand the crisis it will continue to proliferate indefinitely and we call this as a immortalization of the cell line or immortalized cell line.

So, they must express the telomerase, there is no way out other than having telomerase. Because the problem arised because of reduced telomere length has to be handled with a increase in the telomere length. So, they must express the telomerase escape the crisis and require the telomerase maintenance functions also.

Just today the telomerase expressed does not help much; it has to be maintained throughout. Because otherwise the cell will can again face the same problem of Hayflick limit and crisis stage and can undergo M2 phase and apoptosis. So, you do not want that to happen the telomerase should continue to be present.

The telomerase is expressed in around 80 to 90 percent of all cancers analyzed and it is lacking in all somatic tissue. So, a given cell in a tissue is turning cancerous one of the requirement is the up regulation or active reactivation of the telomerase. This will lead to the immortalization and in a host in a living organism this is nothing but cancer.

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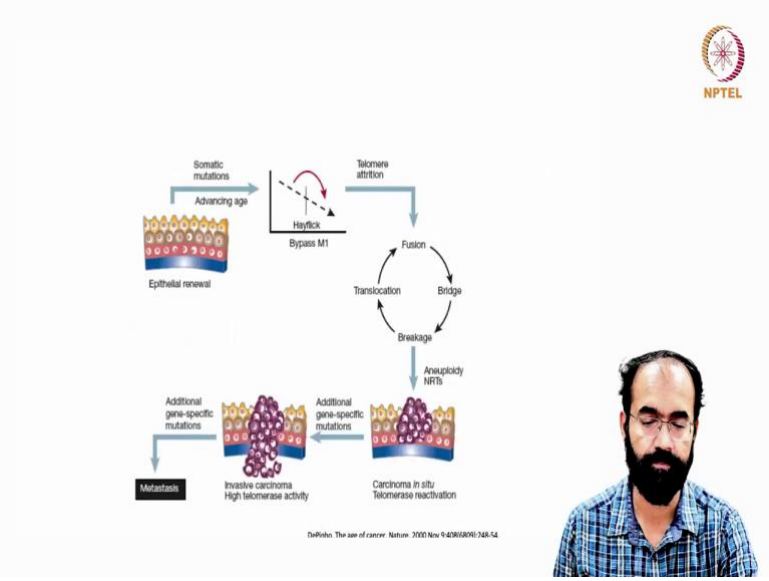

So, now let us see in a flow chart you have a epithelium and you have bunch of cells and this will welcome. So, many mutations that the changes can happen because your skin cells are exposed to you know sun rays, UV rays, etcetera or other toxins outside. So, they are always exposed to some DNA damaging compounds.

So, as long as the cell did not reach the Hayflick limit then they will not have too much of a problem with the bypassing the M1 will happen without too much of a problem. M1 is what? Hayflick limit. So, this will happen without too much of a problem because the length of the telomerase sufficiently long enough etcetera.

But what if the division is continuing? If the division is continuing, then there is a good possibility that it will face the M1 and M2 crisis faces. Then because telome as the cells continue to divide, if a DNA damage has happened on your skin cell naturally that cell will die and it has to be replaced by a new cell.

So, always there is a challenge that is occurring on the exposed cells, that is such as the epithelial cell and it will continue to renew. And if it bypassed the Hayflick limit

problem and the telomere length continue to get shortened and shortened and shortened and then now it is a phase where the genomic instability starts.

So, it can lead to chromosomal fusion or chromosomal bridge formation or breakage of the chromosome or translocation of this broken piece. It goes like a circle like a ah a circular motion it will continue. And this often results in aneuploidy. What is aneuploidy?

A change in the chromosome number, say normal healthy human cell will have 23 pairs or 46 number of chromosome. If 46 became 47 it is called aneuploidy. If 46 became 45 it is called aneuploidy; that means, a change in the number of chromosomes either increase or decrease. Both are problematic and we end up getting aneuploidy. So, this aneuploidy can trigger the formation of cancer.

So, carcinoma occurs in-situ and they will have to make a living by the reactivation of the telomerase. So, Hayflick limit and the crisis phase, genomic instability, reactivation of the telomerase. And this will further exacerbated by additional somatic gene mutations.

This is just like if you have petrol, you have fire matchbox does not cause a fire accident. Someone has to come and let the matchbox and put on to the petrol to have the real fire accident. Same way happens, some mutations that will facilitate the cancer progression.

So, genomic instability is there, it is broken and aneuploidy is there and telomerase got reactivated, but to become cancer you need further more mutation which is much easy to come. Why? Because this unstable genome is not performing the task the way it should be performing. There is no checkpoint is happening like G1, S G2 phase there has to be checkpoint specific checkpoints whether everything happened properly. So, nothing will happen effectively.

So, having a mutation acquired is much much easy in this kind of problematic cells or this kind of genetically unstable genome bearing cells. So, it can lead to invasive carcinoma and they always will have telomerase activity, rather if they do not have telomerase activity, they cannot make a living. So, that is a trade off. And additional gene specific mutations will reemphasize or they will reiterate the progression of cancer and you end up getting metastasis.

So, let us revisit again the somatic cells, get some mutations and it will reach the Hayflick limit and it will cross the barrier and the telomere length continue to be shortened. This will lead to fusion and then the bridge formation of the you know bicentric chromosomes etcetera.

It will lead to the breakage of the chromosome. And this broken chromosomes can again either form circular chromosome or it can fuse elsewhere or it can cause recombination, it can get translocated to another chromosome and it will continue to continue the cycle. And you end up getting aneuploid cells.

And aneuploid cells will establish a carcinoma, that is a cancer situation in real time. And some mutations are needed for them to become a very invasive carcinoma and further mutations are required from to do metastasis. Metastasis means they will start invading other organ a liver originated in a cancer originated in liver or a cancer originated in lungs is now going to kidney or going to brain or going to heart or some other organ or going intestine anyway or multiple organ.

Metastasis does not mean that it will go from organ A to organ B no; it can go from organ A to B, C, D and E. So, it can go to multiple places and may complicating the situation.

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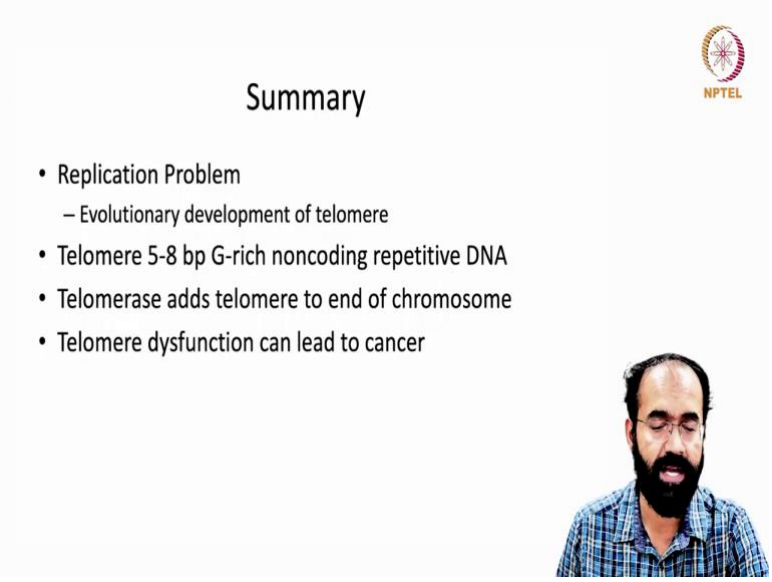

So, to summarize the replication problem is a evolutionary conserved phenomenon and this is broken, it is broken if the telomere length is not maintained. And the replication problem end replication problem is a fact and it is overcome by long telomeres and somatic cells lack the telomerase activity.

And telomerase remember it is a reverse transcriptase, it has got a RNA in their genome. So, it is a essential feature because of the replication problem the telomere is there, if there is no replication problem telomere is not necessary. And telomere does lots of good things preventing the genetic information, getting lost due to end replication problem.

The telomere is around 5 to 8 base pair, G-rich encoding repetitive DNA and telomerase adds telomere repeats to the ends of the chromosome in germ cells, cancer cells and stem cell, not in somatic cells. And telomerase dysfunction can lead to cancer. This is what we learned so far from the telomeres.

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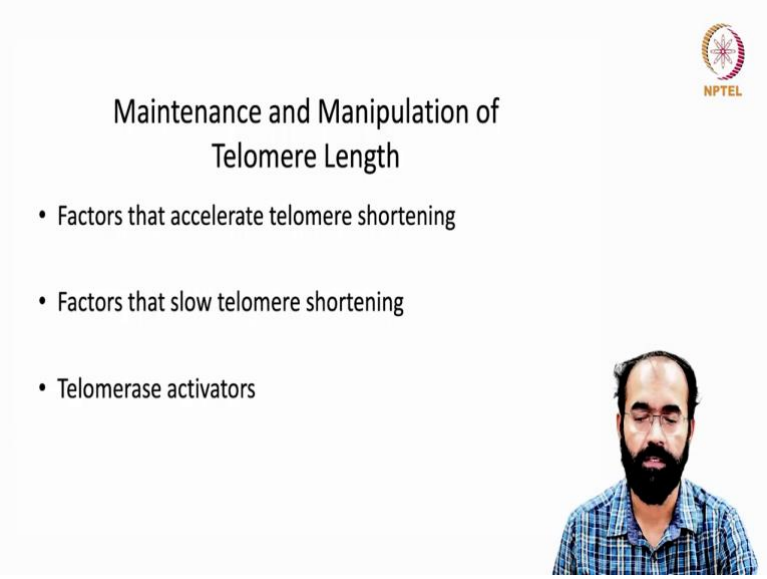

So, now let us understand little bit more about the maintenance and manipulation of the telomere length. Factors that accelerate telomere shortening can exist because like I mentioned if you are undergoing stress, stress can be mental stress, can be food stress, can be environmental stress, any stress leads to reduced lifespan of that cell.

So, if the lifespan of the cell is reduced it need to be replaced. So, in a given span if 5 cells should have been replaced if you are under stress, 10 cells have to be replaced. So,

they have shorter telomere because every replication of a cell is reducing the lifespan. Factors that slow telomere shortening is a good thing. If you can find ways and means in which the telomere shortening can be reduced that is good thing to fight against cellular senescence. And we can think of telomeres activators. Some molecules can activate or facilitate the telomerase activity.

So, what happens all those somatic cells lack telomerase activity? What if can moderately enhance the telomerase action or revive telomerase action that is a good thing. Because these cells despite losing chromosome ends in the form of telomere it is now able to bounce back.

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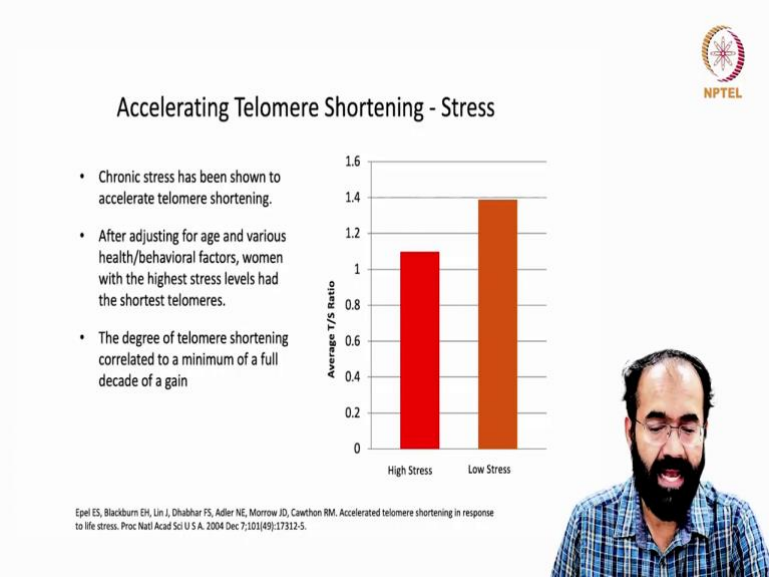

So, accelerating telomere shortening happens with stress. So, the opposite side of the telomere maintenance is the telomere loss. So, chronic stress has been shown to accelerate telomere shortening, chronic stress it can be anything.

Like people will be thinking over like in psychiatric terms people say brooding etcetera any kind of stress you do to yourself can lead to telomere shortening. After adjusting for age and various health and behavior and factors women with highest stress levels had the shortest telomeres.

So, what you understand women are vulnerable to shortening of the shortening of the telomere, credit goes to the stress. So, this is the normalized with age, various health

parameters and behavioural factors etcetera etcetera. So, the degree of telomere shortening correlated to a minimum of a full decade of gain.

So, what it indicates, the telomere shortening is quite well correlated, correlated means connected. It is just like you if a bird is sitting or a crow is sitting in a tree you just swing your hand as if you are about to throw a stone. So, you have neither stone nor you have planning to throw just a action is enough it will fly. So, this is called correlation.

So, you just swung your hand, crow understood something is not right some movement is happening. So, let me escape it will fly you from the tree. So, this is called correlative if you throw a stone, it flew that is an action, without any proper connection proper action simple one movement caused crow to fly, that is called a correlative observation.

But it has a connection, crow saw it and it sensed it as a danger and that is why it went. It happens with many like a fish in water also you just to push your hand towards the fish it will run the other way around although you never touched it nor have any plan of touching it. But that movement triggers an alarm. So, it sees your hand or action as a predator. That is a correlative observation.

So, degree of telomere shortening correlated to a minimum of a full decade of a gain. So, the gain can be whatever you are getting or whatever you are losing can take around 10 years to gain back. So, it is a slow process. So, they have a strong connection that stress have a strong connection to aging. People who are undergoing lots of stress they look aged than the others; credit goes to accelerated cell death and accelerated shortening of the telomeres.

So, you can see an average T S ratio T S ratio we already discussed, with the high stress people they have a shortened T S ratio whereas, low stress people have got a elongated or a longer T S ratio or larger T S ratio. So, high stress brings down the telomere length.

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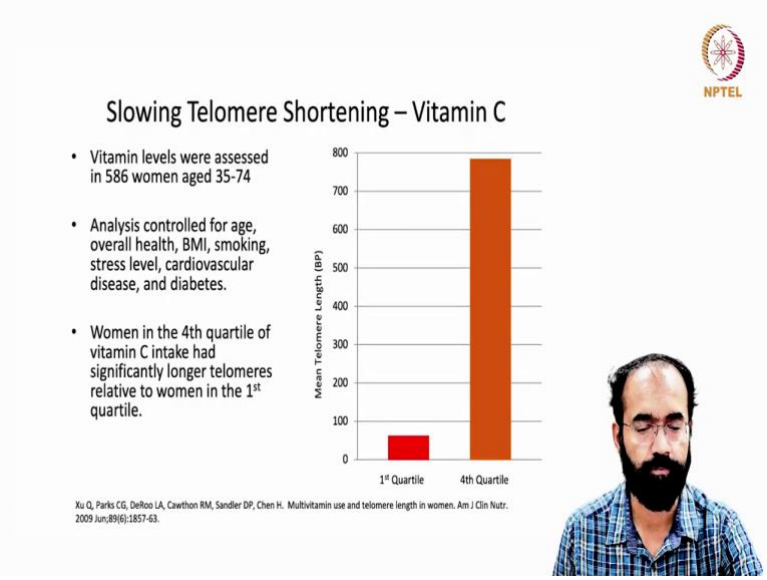

So, some examples slowing telomere shortening some role played by vitamins vitamin C. Vitamin levels were assessed in 586 women aged between 35 and 74. Analysis involved controlled for age, overall health, body mass index, smoking, stress level, cardiovascular disease and diabetes. It is been taken care of when you are collecting the samples or collecting the individuals.

So, women in the 4th quartile of vitamin C intake had significantly longer telomeres relative to women in the 1st quartile. So, what it indicates the amount of vitamin C, remember vitamin C is the one of the vitamins which our body cannot produce. Like primates cannot produce, even mice can produce, even lion can produce vitamin in their body.

It requires 4 enzymes we have only 3, the forth one is mutated. Because of that primates depend on fruits a lot fresh fruits, your body need vitamin C a lot it is a cofactor in various enzymes and it is present only in fresh fruits, fresh vegetables and it also helps in fighting infection.

If any of these animals like mice or lion or any animals when they are infected or their body have a pathogen or infection the vitamin C level increases by around 4 fold body starts over producing vitamin C. It helps a lot in fighting infection. But we cannot produce vitamin C, we depend on the food. So, that is why vitamin C becomes a

important component nor our intestinal bacteria also produce. Many vitamin B vitamins are produced by the intestine bacteria, but vitamin C has to come from fresh fruits.

So, you can see the mean telomere length in women who are in the 1st quartile of the vitamin C intake that means, that is in the lower quartile compared to the 4th quartile; that means, near completion. 4th quartile they had around 800 base pair higher than people who are in the 1st quartile. So, what indicates the mean length of telomere is longer in those people who have taken adequate vitamin C in their body.

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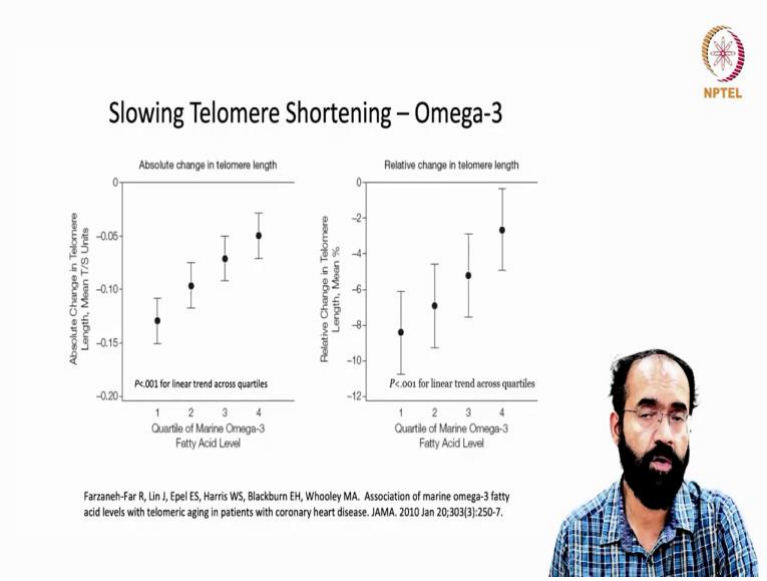

And of course, when your body is stressed naturally the telomere length becomes shortened. So, vitamin C kind of diet can enhance the telomere length. So, another example is slowing the telomere reduction in the rate of decrease in the telomere length that is slowing telomere shortening and the connection with omega 3. Omega 3 is a fatty acid which has got the omega carbon have got you know unsaturation.

So, the processing lead to the processing of these fats often leads to less harmful intermediate triglyceride intermediates and reduces the risk of cardiovascular disease. That is why you may have heard about omega 3 and omega 6 fatty acids a lot in diet and other chemistry we will not go into the details. But it has a good connection with telomere shortening.

So, this y axis you have absolute change in the telomere length and the mean T S ratio and x axis you have absolute change in the telomere length. But you can see here if you have different quartile of omega 3, you can see 1st quartile, 2nd quartile, 3rd quartile and 4th quartile if you compare it. You can see that who have taken adequate amount of omega 3, they have got a better T S ratio higher TS ratio means telomere length was better in them.

And you can also see in relative change in the telomere length that is in the means of mean percentage this is T S ratio here it is with mean percentage. Here also if you put adequate amount of omega 3 in your diet, you can see that the telomere length is adequate or much higher telomere length in those adequate omega 3 containing a diet or diet fed people have got better telomere length compared to those who are deficient.

So, these suggest that your diet contributes significantly to the survival of the telomere. And further cellular senescence they will reduce or prolong the lifespan and reduce the rate of cellular senescence.

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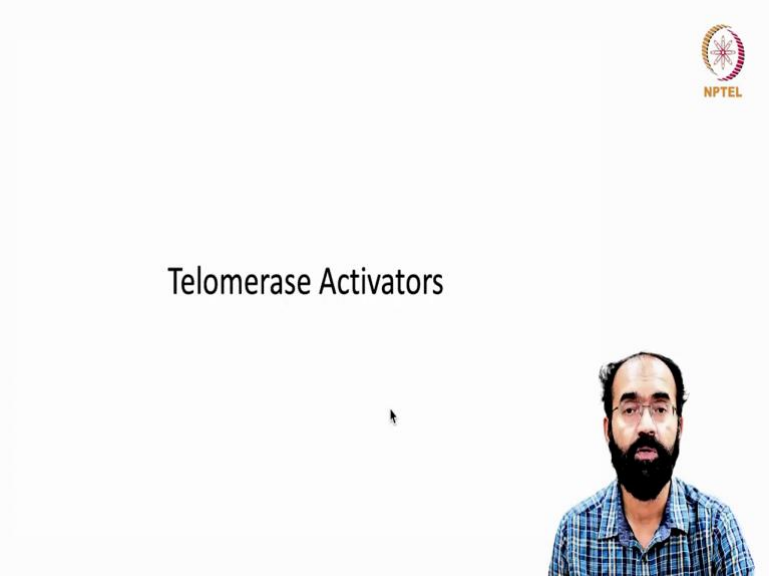

Let us think about telomerase activators, can you revive the telomerase?

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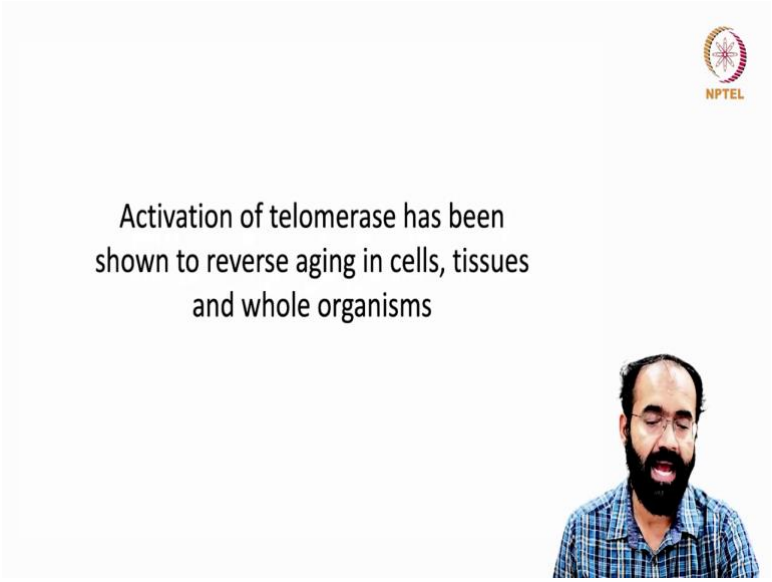

Activation of telomerase has been shown to reverse aging in cells, tissues and whole organisms.

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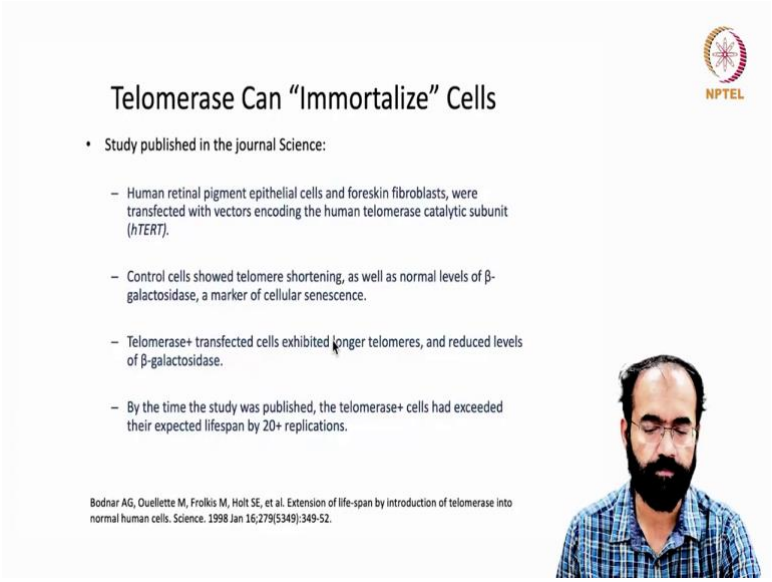

So, telomerase can immortalize the cells. A study published in the journal science have shown that human retinal pigment epithelial cells and foreskin fibroblast were transfected with vectors encoding human telomerase catalytic subunit, also known as hTERT when you express it what happens to them.

Control cell showed telomere shortening as well as normal levels of beta galactosidase a marker for cellular senescence. If you express telomerase, telomerase transfected cells exhibited longer telomeres and reduced levels of beta galactosidase; that means reduced cellular senescence. By the time the study was published the telomerase positive cells had exceeded their lifespan by 20 plus replications.

Say in 700 replications the cell should have completely stopped or completely stopped dividing. So, in the telomerase expressed one it went 20 plus replications more. So, it has a life for 20 more generations, the cell which should have stopped dividing. So, telomerase indeed is quite helpful in confirming their longevity.

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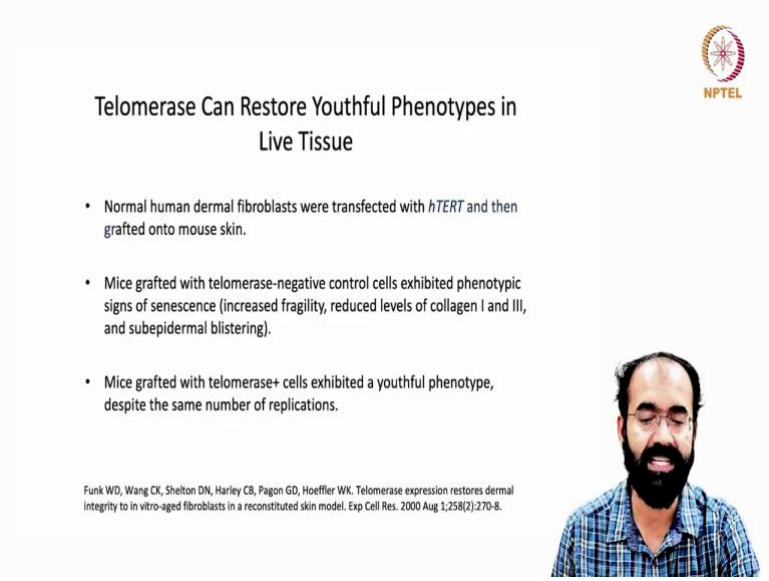

So, telomerase can restore youthful phenotypes in life tissue. So, normal human dermal fibroblast were transfected with hTERT and then grafted onto mouse skin. So, what happens?

Mouse grafted with telomerase negative control means telomerase missing normal cells exhibited phenotypic signs of senescence increased fragility skin like I mentioned in one of the previous class that old people who were 90, 98 they can peel off their skin. Because your skin tissue is more vulnerable, because they divide at a very rapid rate.

So, increased fragility reduced levels of collagen 1 and 3 and sub dermal blistering. So, this is a normal feature which you see in a controlled cells where telomerase is missing.

Mice grafted with the telomerase plus cells or telomerase expressing cells exhibited a youthful phenotype despite the same number of replications.

So, what you understand, your skin looks healthy and perfect, the credit goes to the telomerase activity probably in your skin stem cells or they are revived in some of your skin cells. So, that they look young and healthy. So, we will learn about more about the telomerase and also some other topic on the RNA biology in the next class.

Thank you.