Introduction to Mechanobiology
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Week – 06
Lecture – 30
Mechanobiology of Diseases: Atherosclerosis & Hypertension

Hello and welcome to today's lecture of Introduction to Mechanobiology. In the last few lectures that started discussing about Mechanobiology of diseases and in that context I had discussed Cancer.

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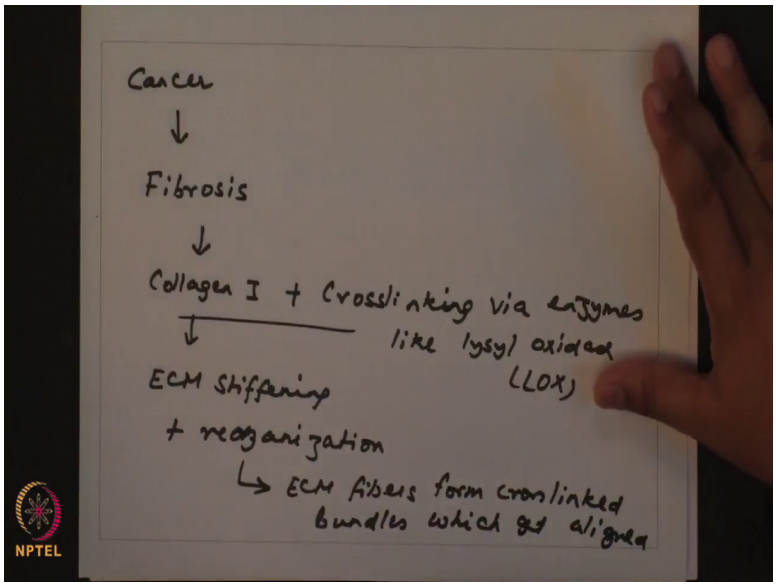

And shown that in cancer you have this response of Fibrosis, marked by increased deposition of Collagen 1, this leads to ECM Stiffening; Collagen 1 and plus cross linking we are enzymes like lysyl oxidase also represented as LOX.

Both these things lead to ECM Stiffening and reorganization by ECM reorganization where you form where you have ECM fibers form cross linked bundles which also are aligned.

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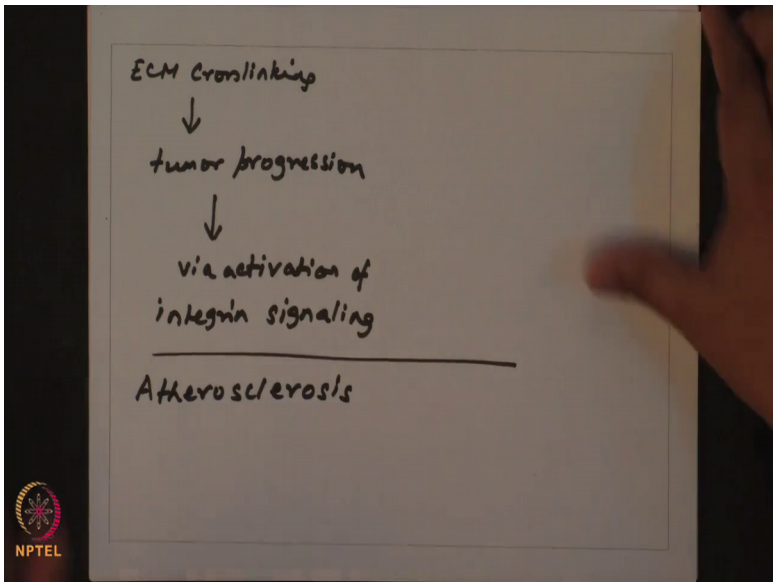

All of these responses in the cell level, what we found by discussing the cell paper we showed that this ECM cross linking is capable. So, your ECM cross linking is capable of inducing tumor progression.

So, you will lead this leads to and this tumor progression is via activation of integrin signaling. Today we will discuss another disease again marked by fibrosis which is called Atherosclerosis.

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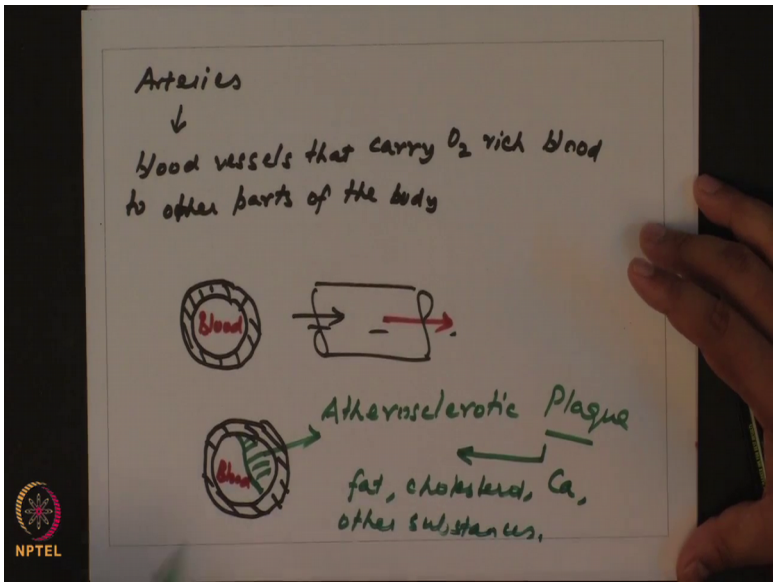

What is atherosclerosis? So, all of you know that you have Arteries within our body and these are blood vessels that carry oxygen rich blood to our organs to other parts of the body.

If you see the cross section of a blood vessel you have this nice structure, where you have blood flowing through these channels. So, the cross section is circular. So, in side view if I draw and I have blood flowing through this channel, Now what happens in atherosclerosis the same blood vessel now looks here you have blood flowing the green zone is called an atherosclerosis plaque.

This atherosclerosis plaque essentially it restricts the blood flow. So, instead of flowing through the entire artery blood flow is restricted and this plaque is made up of multiple constituents including fat, cholesterol, calcium and other substances.

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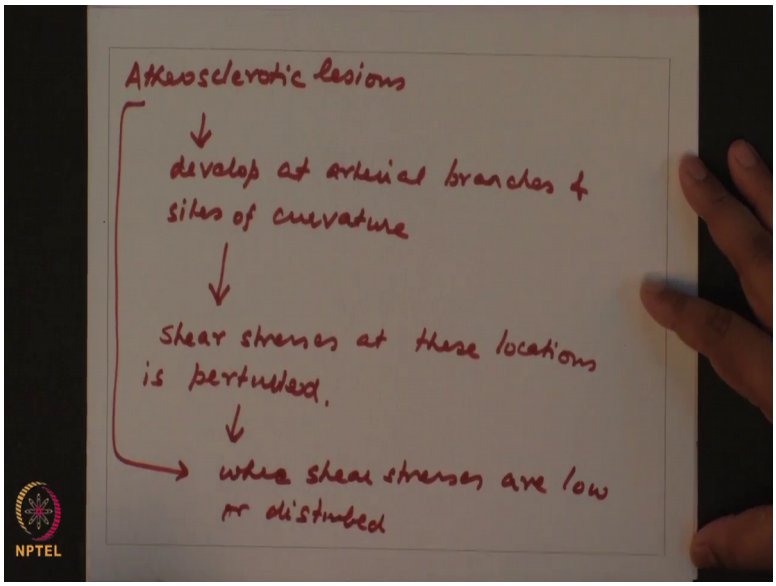

So, over time this plaque becomes overtime your plaque stiffens and restricts blood flow eventually causing a heart attack your blood flow is completely gone.

When you do these tests doctors say that you have 98 percent blockage in arteries or veins so on and so forth. Though the initiation of this atherosclerosis is attributed to that the circulating factors like IDLs or low density lipoproteins and triglycerides. There are other changes in the flow pattern in the way the blood flows through these arteries and intriguingly people have observed that atherosclerosis lesions are typically localized

They develop at arterial branches and sites of curvature. So, what is the reason for that? So, with the help of fluid mechanics it has been demonstrated, that the type of shear stresses at these locations is perturbed. And this and when I say it is perturbs what you have found that these lesions are formed at locations where shear stresses are low or disturbed.

So, suggesting that the flow profile itself has a role to play in this and subsequently people have shown.

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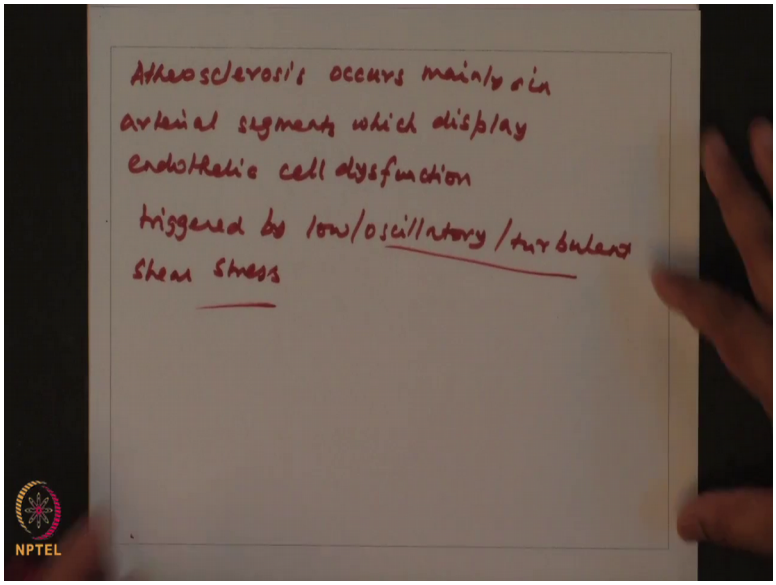

That Atherosclerosis occurs mainly in arterial segments which display endothelial cell dysfunction; endothelial cell dysfunction triggered by low oscillatory or turbulent shear stress.

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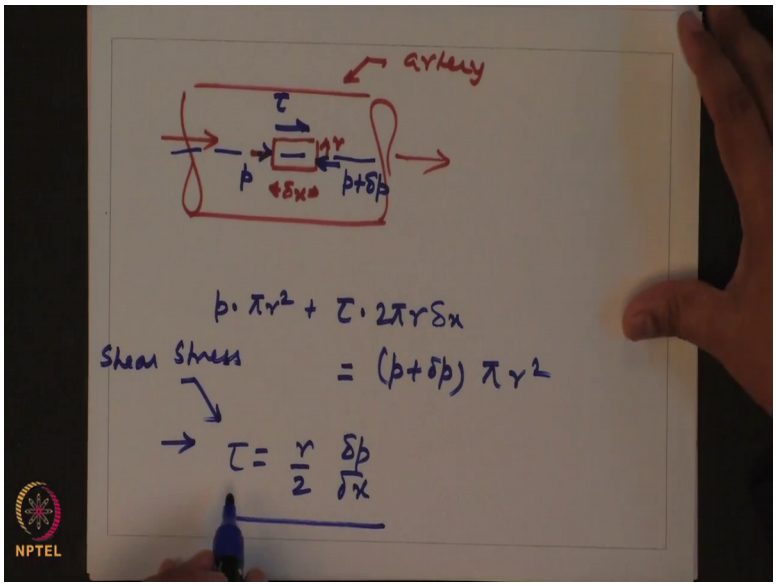

Let me briefly explain what is this. So, if you take a pipe if you take a simple pipe. Let us say this pipe is equivalent to your artery your blood is flowing in a given direction. If you take a small element just this there is a line of symmetry down the center. Let us take a small fluid element in the center. What are the forces that are that this fluid element is subjected to? Let assume that this length is delta x and this radius. So, because it is the pipe it is axial axially symmetric.

So, you have this radius r. So, on this wall you have a pressure force p. So, because this is only an elemental length of delta x which is very small I am putting a pressure of p plus delta p here. And you have a shear force tau. So, shear as you as you know from my recall from our discussion that shear is tangential forces.

You have these forces and the force balance equation looks something like that. So, pressure into the cross section area is pi r square plus tau into the area because it is the tangential length tangential area. So, 2 pi r is the circumferential length into delta x is the total shear stress and this must be equal to p plus delta p into pi r square. So, from this equation you can establish a simple relationship like that p is r by 2 del p del x.

So, tau is my shear stress tau is my shear stress. This varies that r by 2 del p del x, which means that tau max must be at the walls. So, the walls are subjected to maximum amount of shear force and from this equation you can derive the flow.

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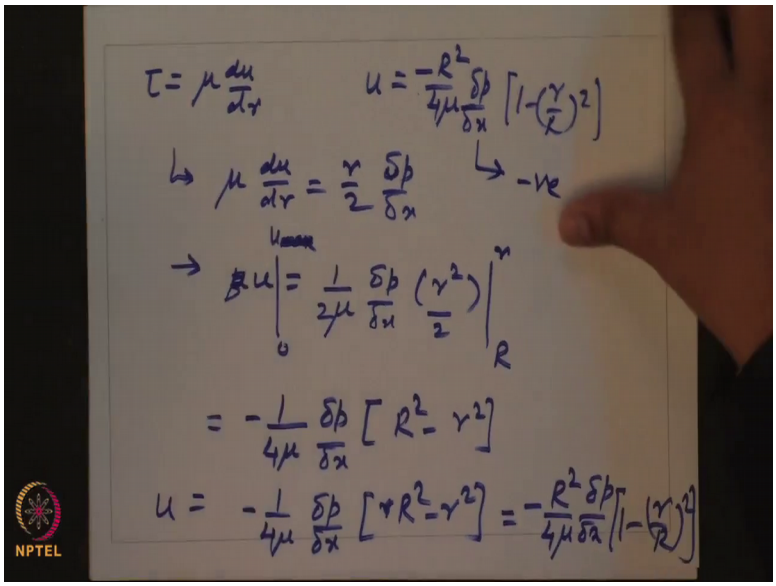

So, because tau is given by mu du dr this is Newton's law of viscosity, you can put this equation mu du dr equal to r by 2 del p del x, you can take a you can integrate this equation so you can find out that mu into u is r if I remove mu from this side is 1 by 2 mu del p del x common r square by 2 and if I integrate from 0 at the walls to maximum at the center. So, R from capital R to small r or u can integrate from 0 to u then you would get this equation 1 by 4 mu del p del x into R square minus r square. So, minus 1 by 4 mu del p del x is minus into R square minus r square. So, you can take out R square common.

When r is equal to 0 you have u max is, this is your velocity profile let me write down the equation here is minus r square by 4 mu into del p del x into 1 minus r by r whole square. There is a minus sign here for the simple reason because del p del x is negative. So, negative into negative is positive. So, if you plot the velocity profile the velocity profile will be something like this.

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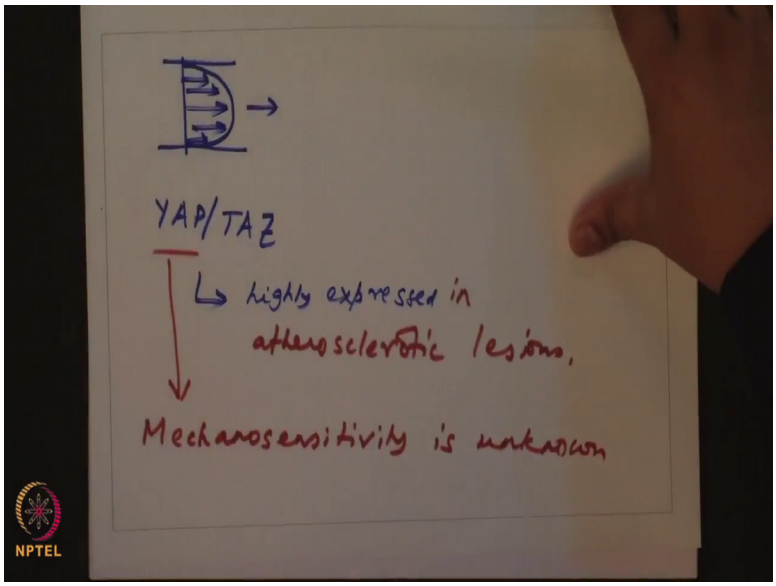

So, this is your pipe this is the direction of flow you have maximum velocity here. So, this velocity profile is parabolic in nature. Now what has been observed is there are 2 transcriptional factors YAP and TAZ; ace associated protein and TAZ these are highly expressed in atherosclerosis lesions.

Whether there is any association between YAP TAZ and Mechano sensitivity is unknown the link is unknown.

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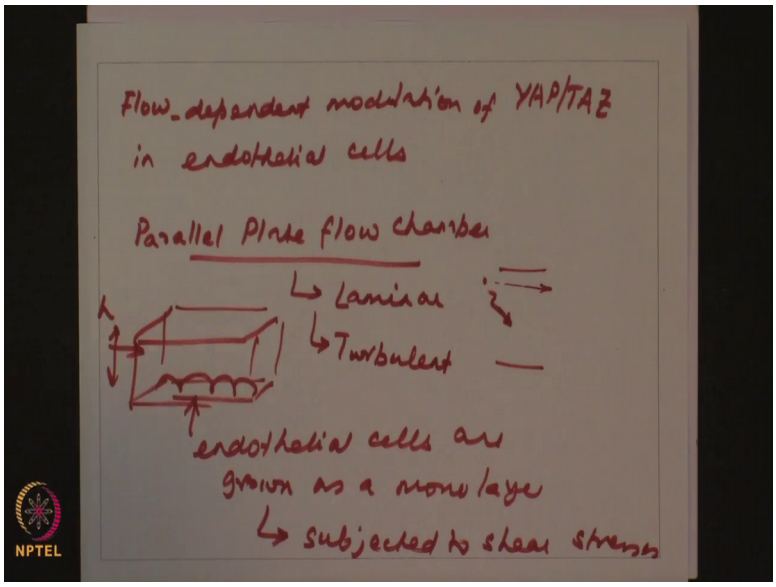

Today I will discuss one paper where they have we have described the Flow-dependent modulation of YAP/TAZ pathway YAP/TAZ pathway in endothelial cells. So, for doing this for doing this they use a parallel plate flow chamber.

Where again like a pipe, you have a parallel plate flow chamber. If you look at the base you have endothelial cells are grown as a mono layer and then you have flow. So, this has a given height. So, you have a flow profile being imposed on the cells. The cells are subjected to shear stresses.

So, using this you can vary the type of flow as steady our laminar. In laminar flow when you have flow there is no mixing. So, in the pipe if you add a small amount of dye at a given point the dye will travel along that direction and will not diffuse in other direction. So, this is what is meant by laminar flow in contrast you can have turbulent flow where there will be mixing of these particles across this radial distance.

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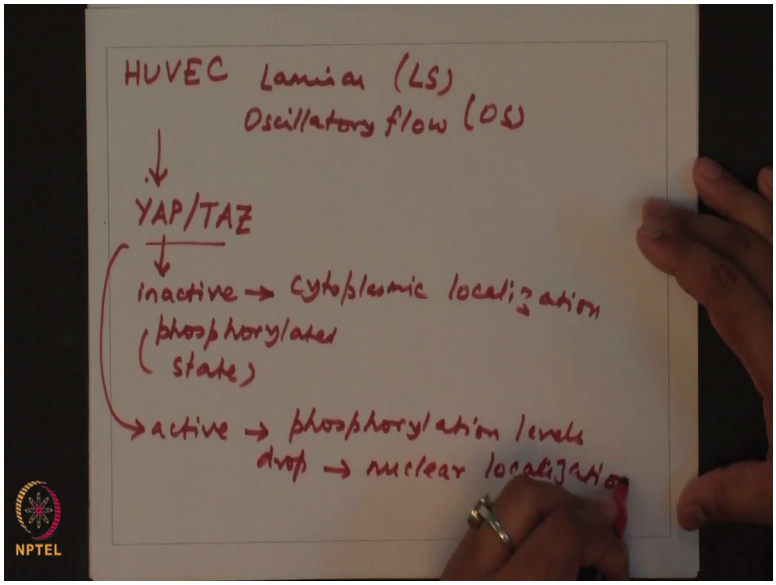

What they found was they used HUVEC cells this is human umbilical vascular endothelial cells and then they subjected to Laminar or O scillatory flow. This is laminar shear stress oscillatory shear stress and what they found was under laminar conditions.

They checked under these conditions what is the profile of YAP and TAZ now these transcription factors exhibit this particular behavior that when it is inactive is inactive it

in phosphorylated state and it exits exhibits the cytoplasmic localization, but when we get active it is phosphorylation level drop and leading to a nuclear translocation nuclear localization.

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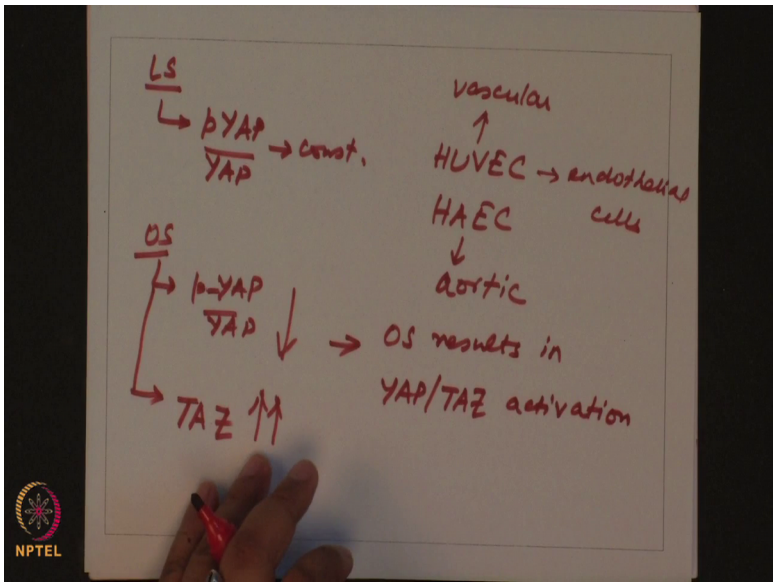

When they did this experiment what they found was under laminar shear stress their phospho YAP levels were constant. So, phospho YAP to total YAP was constant, but under oscillatory shear stress this decreased phospho YAP to total YAP kind of decreased and under these conditions TAZ the expression profile of TAZ increased.

So, this was this they observed not only in HUVEC cells, but also in aortic endothelial cell. So, this is aortic endothelial cells and this is vascular endothelial cells EC stands for endothelial cells. In both these cases they observed this particular profile suggesting that, they suggest that OS results in YAP TAZ activation and they also confirm this by the localization signature.

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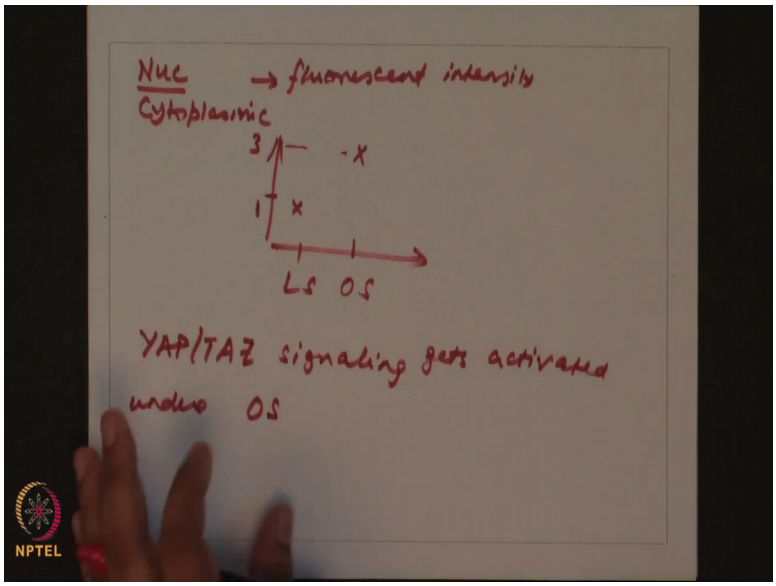

What they did was they tracked the Nucleus to Cytoplasmic intensity signal, fluorescent intensity signal And then they found was compared to laminar stress conditions, where you had a given profile, which was lesser than a comparable to 1 under these conditions this increased in OS this was significantly increased to values 3.

So, this confirmed that YAP TAZ signaling gets activated under oscillatory shear stress.

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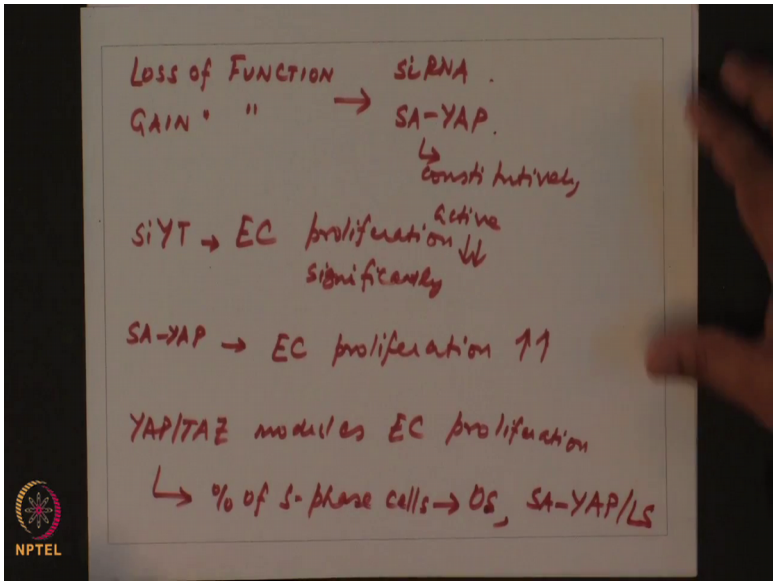

What they then did was performed GAIN of function and loss of function experiments. So, there for loss of function there is SIRNA they knob down the expression of the YAP/TAZ and for gain of function they over expressed YAP.

This was always constitutively active and what they found was under these 2 conditions when they knock downed YAP/TAZ signaling endothelial cell proliferation dropped significantly. And when the activated YAP this led to dropped EC proliferation increase significantly.

So, this suggest that YAP/TAZ signaling modulates EC proliferation and mechanistically what they found what the percentage of S-phase cells was significantly increased under oscillatory shear stress as well under when YAP was over expressed even under laminar shear stress conditions.

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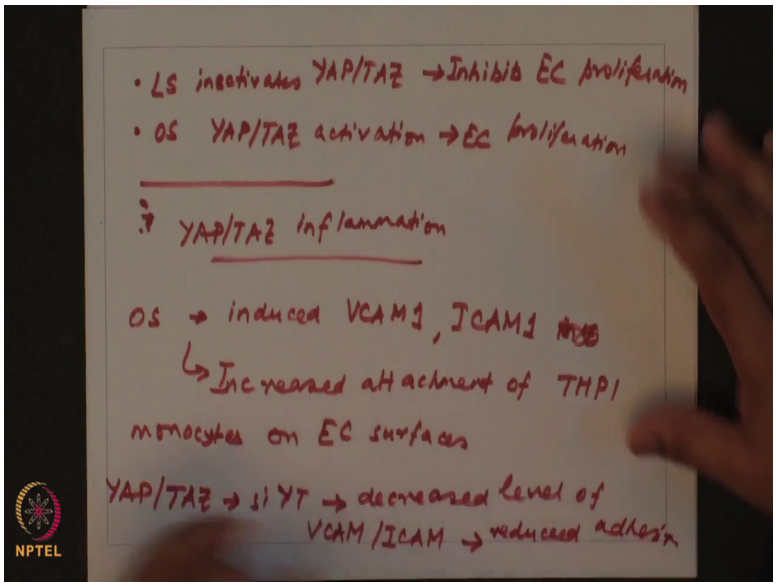

Together what they concluded was under laminar shear stress inactivates YAP TAZ signaling.

While oscillatory results in YAP/TAZ activation and this leads to EC proliferation. Further similarly what you have under inactivates YAP/TAZ and inhibits EC proliferation what they then checked was what was the role of YAP/TAZ in mediating inflammation.

What they found was oscillatory shear stress it induced, expression of VCAM 1 vascular cell adhesion molecule 1 and ICAM 1, in ECS and this led to increased attachment of THP1 monocytes, on EC surfaces; parallely when the knock down YAP/TAZ with siyt YAP/TAZ they saw decreased level of level of VCAM and ICAM expression leading to reduced adhesion.

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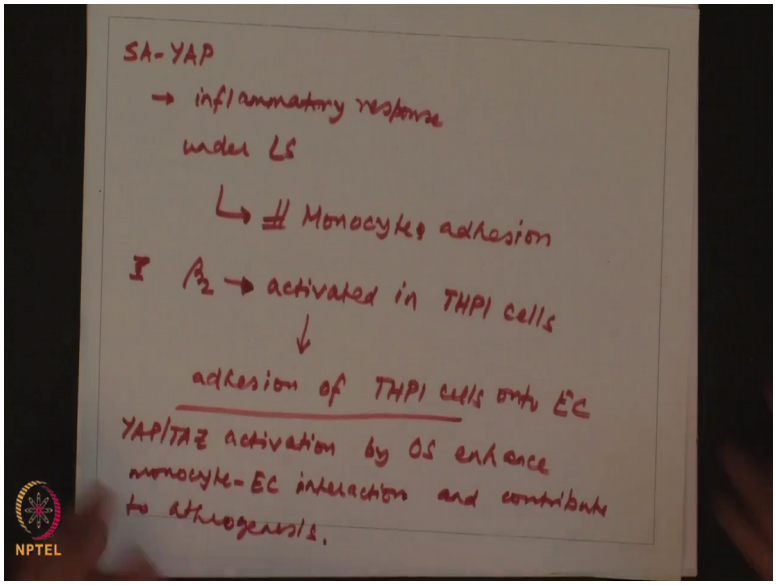

Also when they over expressed using SA-YAP considerably active YAP this increase the inflammatory response under even under static conditions; So, even under laminar shear stress conditions you had inflammatory response when you over express they say yap as quantified by the number of monocytes which got adhered adhesion and all of this was made possible via expression of integrin beta 2.

So, what they found was integrin beta 2 expression led to beta 2 expression was activated in THP1 cells. And this is the mechanism which explained the enhanced adhesion THP1 cells onto the ECS. Together what the authors concluded was that YAP/TAZ activation by oscillatory stress, this enhance monocyte-EC interaction and these can contribute to atherogenesis this can initiate atherosclerosis.

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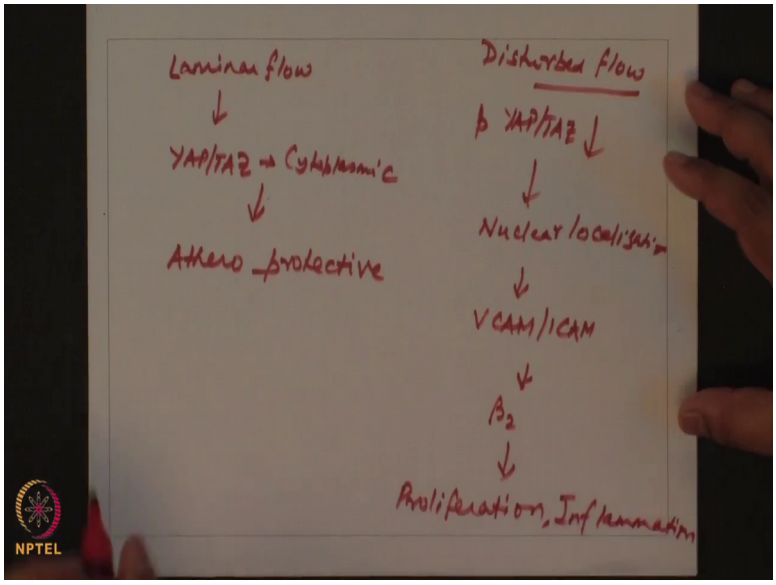

Together the authors came to the following conclusion. So, whenever you have laminar flow your YAP/TAZ exhibits a Cytoplasmic localization and as a consequence there is the reason the overall situation is athero protective, but under disturbed flow YAP/TAZ phosphorylation goes down that leads to nuclear localization up regulation of VCAM and ICAM expression in ECS and integrin beta 2 expression in these cells.

So, overall these leads to proliferation and an inflammation signal. So, this shows you that other than soluble factors you can have this mechanical flow induced inflammation created in zones where flow gets disturbed in either one direction or where the shear stress is low that leads to buildup of these atherosclerosis blocks or if the flow is disturbed in wider direction in oscillatory then also you can get these zones with that I

Thank you for your attention