**Introduction to Mechanobiology**
**Prof. Shamik Sen**
**Department of Bioscience & Bioengineering**
**Indian Institute of Technology, Bombay**

**Week - 07**
**Lecture – 31**
**Mechanobiology of Diseases: Muscular Dystrophy**

Hello and welcome to today's lecture of introduction to mechanobiology. In the last class I discussed about Atherosclerosis.

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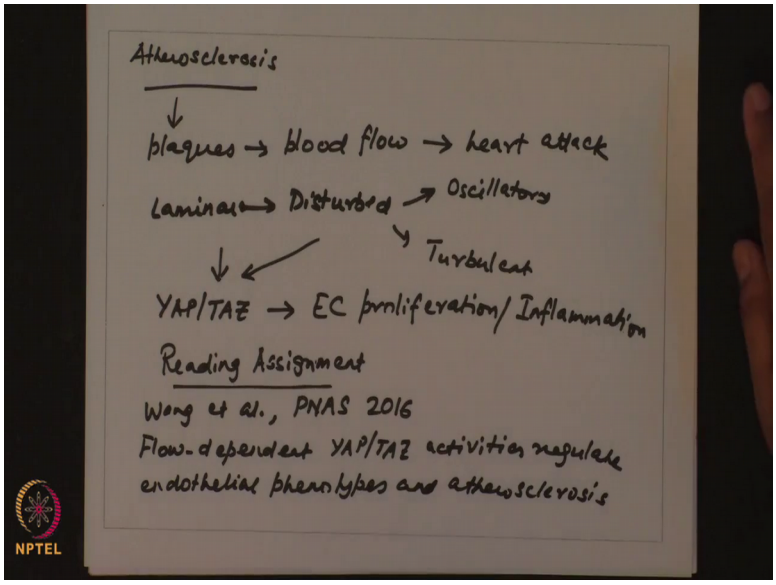


And I so, in this disease you have build up of plaques which limit blood flow through the arteries and eventually might manifested itself as a heart attack and flow is completely stopped. And I showed you how by if the flow profile can change from laminar to disturbed, as part of being disturbed can be oscillatory or turbulent.

And describe this paper which showed how yap taz activation by disturbed flow led to endothelial cell proliferation and inflammation. So, as part of my reading assignment we have this 2016 paper. So, you have this paper as reading assignment flow dependent yap taz activities regulate endothelial phenotypes and atherosclerosis.

So, today I will discuss about another disease muscular dystrophy.

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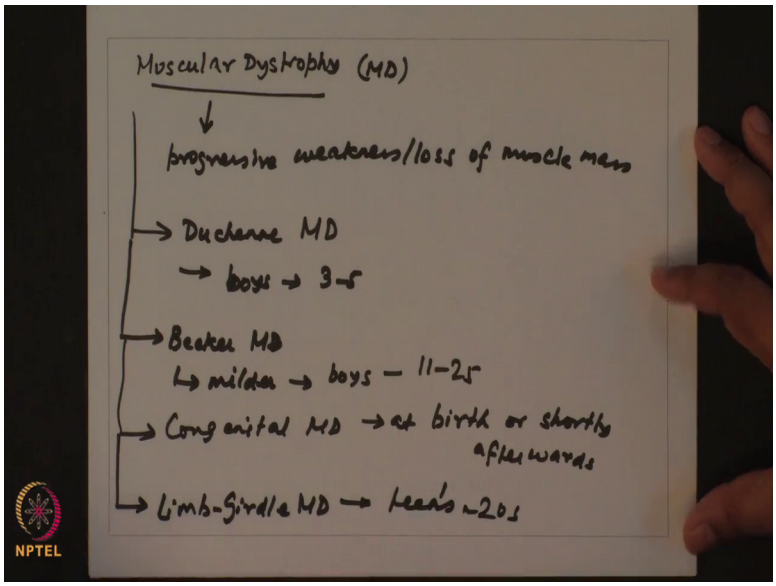


So, there this disease is a group of disease that cause as you have the term muscular dystrophy. So, you have progressive weakness and loss of muscle mass. So, there are various types of muscular dystrophy, among them the most common being duchenne muscular dystrophy MD ok.

So, this affects boys and start between the ages of 3 to 5. You have Becker muscular dystrophy. It is much milder than duchenne muscular dystrophy it also affects boys, but in the age group 11 to 25. So, it is affect start to manifest slightly later. You have congenital muscular dystrophy as the term suggests it starts at birth or shortly afterwards. You have limb girdle muscular dystrophy, typically starts in the persons teens at the age 20s ok.

So, there are various other muscular dystrophies including Emery dreifuss muscular dystrophy, typically again affects boys around 10.

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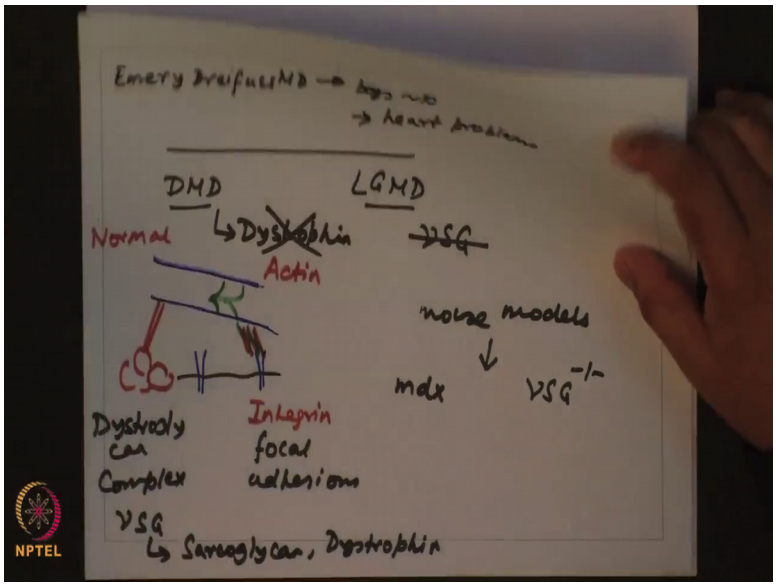


And these have lead to heart problems. So, I will discuss about 2 of them duchenne muscular dystrophy and limb girdle muscular dystrophy. So, in duchenne so, if I draw so, if I draw the muscle structure in most cells ok.

So, imagine if this is the plasma membrane. So, on the outside you have and you have this bigger complex which are linked. From here so, in normal muscle you have 2 sets of adhesions which link the actin cytoskeleton to the outside. You have the integrins based focal adhesions, if the integrin based focal adhesions and you have the what is called the dystroglycan complex ok.

So, in DMD or duchenne muscular dystrophy. So, as part of the dystroglycan complex there are multiple protein, like gamma soarcoglycan am just writing soarcoglycan as well as dystrophin. In DMD due to mutation dystrophin expression is completely gone. So, the dystrophin complex is missing, and then limb girdle muscular dystrophy gamma soarcoglycan is missing. So, what has been observed ok.

So, to study the effects of these proteins what people have done is they have created mouse models, where they have deleted the expression of either dystrophin which is called an MDX mouse where the dystrophin protein is missing. Or you have gamma soarcoglycan deficient cells. So, what has been observed is in gamma soarcoglycan deficient cells.

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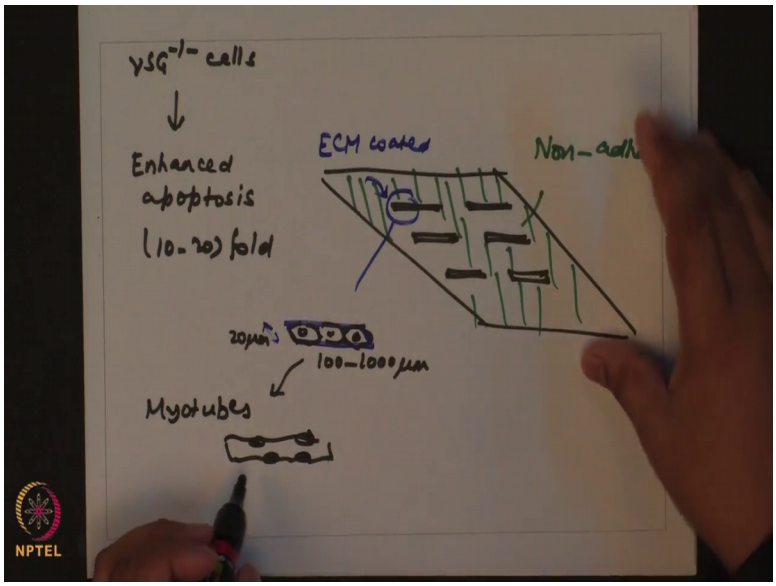


These cells compared to normal wild type, these cells exhibit enhanced apoptosis. And this is nearly 10 20 times 10 20 fold increase in apoptosis ok.

To study the effect what underlines this mechanism, what others have done is they have created these patterns. You have so, in 2D you will have these strips of adhesion islands, you have these are ECM coated. So, if you zoom in you will get these islands which are roughly 20 microns in width, and 100 to 1000 microns in length and the remaining area, the remaining area outside this is non adhesive ok.

So, later in lectures I will show you how you can make use a micro fabrication to generate these structures. So, this allows you so, when you plate cells the cells will go and populate these patterns. So, you have myoblasts which are aligned and they populate these patterns, and these myoblasts which finally, fuse to form multinucleated myotubes. You can generate these multiple structures.

So, what people have done is then on glass you have these substrates where you create this bottom myotube.

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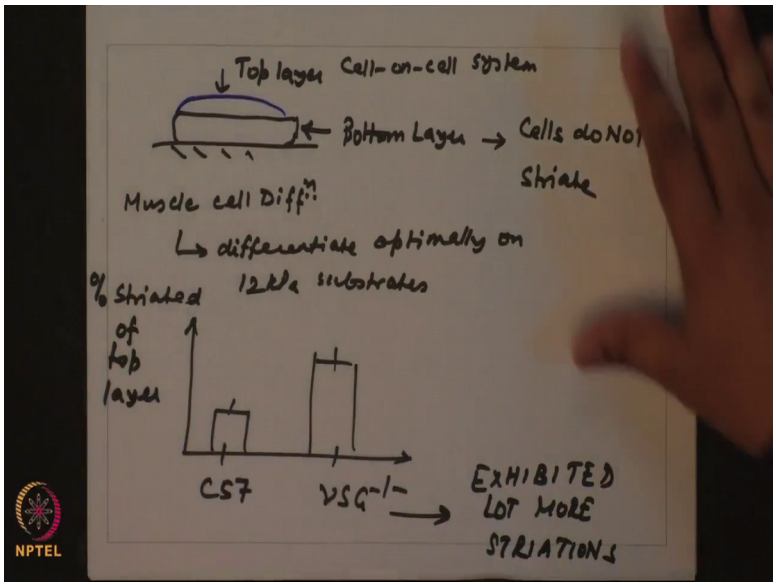


And on top of which you add a second layer of muscle cells. So, you get the top myotubes. So, in a sense you have created a cell on cell system. And so, this is a top layer of muscle cells or which finally fuse to form myotubes, and this is the bottom layer ok.

So, I had previously discussed the effect of stiffness or muscle cell differentiation. So, these guys they differentiate optimally on 12 kPa substrates. So, because these cells are cultured the bottom myotubes on glass cells never straight do not, but the top cells straight. So, when these striation was compared in wild type in C57 wild type mice cells isolated from C57 wild type mice and cells isolated from gamma soarcoglycan deficient mice.

So, what was observed the top layer if this was the level of striation these cells this is only the percentage striated of top layer. So, these cells exhibited lot more striations. So, striation is linked to contractility right. So, since formation of striations is indicative of a formation of sarcomeres functional sarcomeres.

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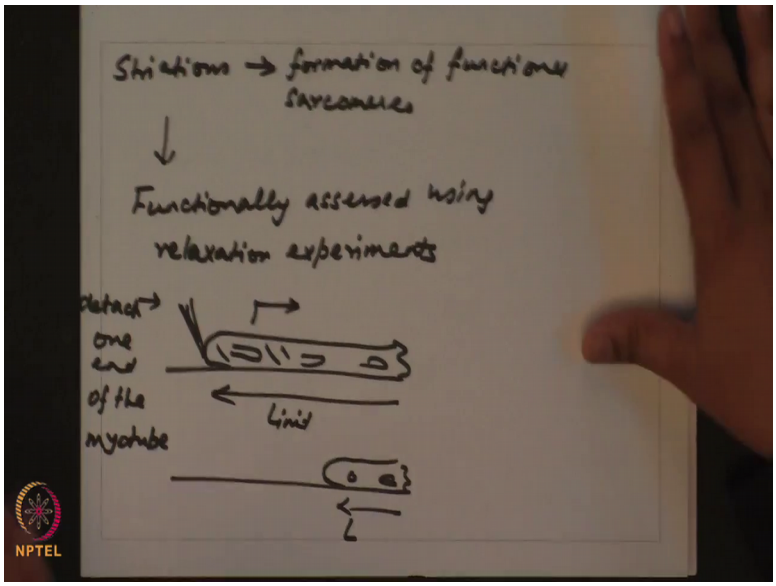


So, this was assessed functionally using a relaxation experiments. The idea is very simple. You have let us say you have a long myotube on a substrate these are your nuclei. And imagine you come down with a pipette and detach one end of the myotube ok.

So, what will drive is this so, when you detach the force balance is perturbed as the consequence of with this myotube will relax. And finally, come to some other state. So, if this was the initial length, this is some other final length. And you can track this length as a function of time.

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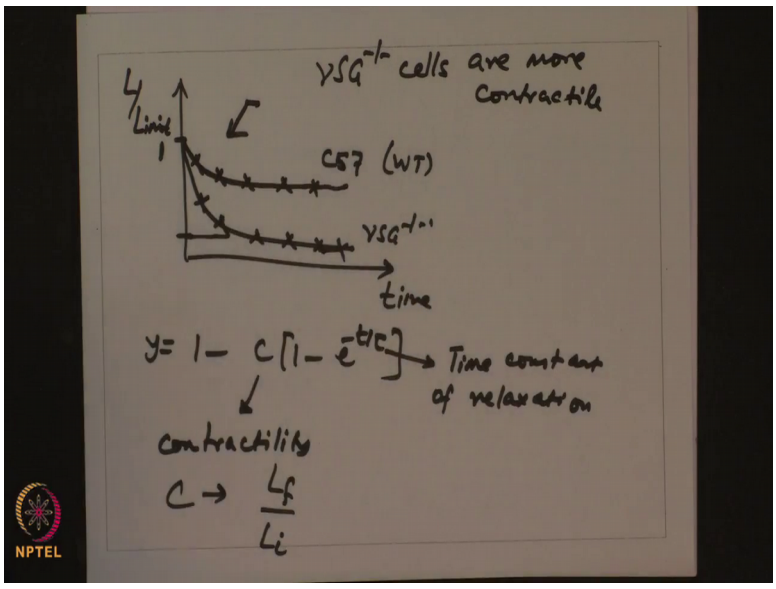

So, L as a function of time t. So, if you plot L by L initial, you will start from a value of one and then you will get a curve like this ok.

So, what they observed was for C57 cells, which is wild type. Whatever with the relaxation curve for gamma soarcoglycan deficient cells the curve was somewhat like this. So, this suggested so, since this final saturation point the saturation point. So, what you can do is you can fix these curves you can fix, you can fit these curves using an expression, let us say y equal to 1 minus C into 1 minus e to the power minus t by tau ok.

So, let us say you use. So, these are your actual experimental points. You use this you fit it with the following expression you can backtrack 2 values one is C and one is tau. So, tau is the characteristic time constant of relaxation. And C is an estimate of contractility. So, as t tends to infinity let us say if let us see this expression as t tends to infinity, this value will give you a value of 0.

So, 1 minus C is the value. So, in a sense C is nothing but the final ratio to the initial ration. C is if you take the cell the L final by L initial is what is C. So, it gives an estimate of how much it contrasts. So, if the if contractility is less then L final will be comparable to L initial and you will get a lower value of C. So, in this case so, compared to C57 the gamma soarcoglycan deficiency cells are more contractile.

So, what you can conclude from this experiment is cells are more contractile. And if you look at these cells in culture. So, what you would see is many of these cells. So, this is actually the pattern imagine that this is the outline of the pattern on which the cell was fitted.

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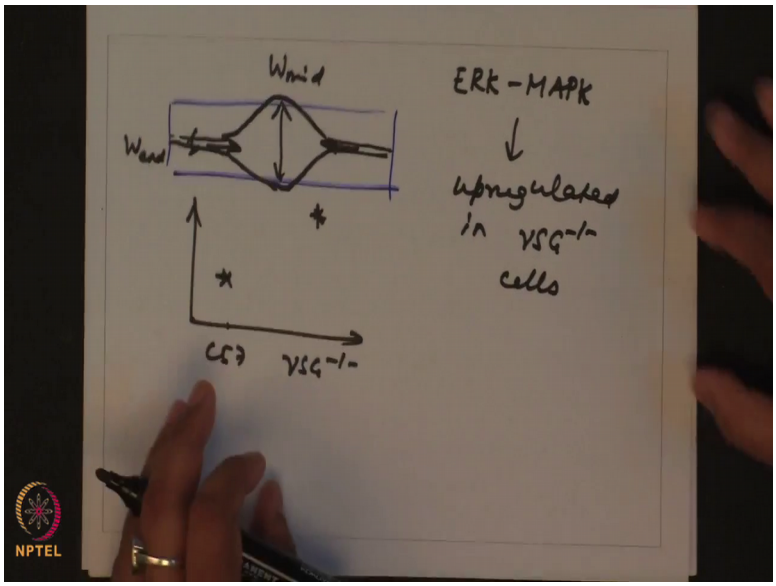


So, many of the cells exhibit a profile like this, where the center if you compare the central width to the end width, You would have compared to control cells if you had 10 percent of cells.

Whatever be the percent of cells exhibiting this phenotype in case of C57 cells in gamma soarcoglycan deficient cells it is significantly higher. Now what might lead to this effect? One is the contractility in which the cell is pulling on itself the cell is executing this increased amount of forces as a consequence of which it is detaching under the effect of it is own force ok.

So, in terms of the signaling pathways which were activated, what was observed was the ERK map kinase pathway was up regulated in gamma soarcoglycan deficient cells. So, this is the status in gamma soarcoglycan deficient cells. One more thing which was common so, this kind of phenotype. So, increased apoptosis was not seen in MDX mice.

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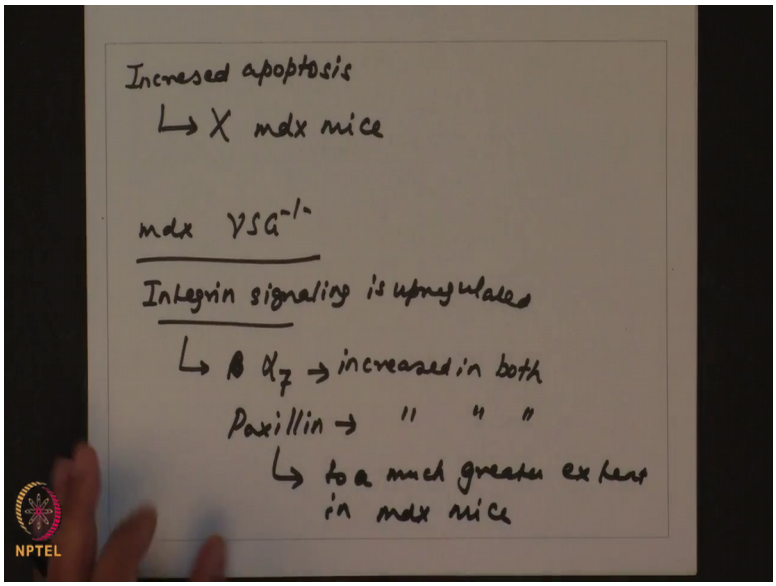


MDX mice are muscular dystrophy mice, they do not exhibit this increased apoptosis. But there is a commonality between MDX and gamma soarcoglycan deficient cells across both of these.

So, compared to control integrin signaling is up regulated and when I say integrin signaling as part of these. So, integrin beta 1 sorry, alpha 7 was increased in both. Paxillin which is not the integrin signaling molecules was also increased in both, but it was increased to much more to a much greater extent in MDX mice.

And so paxillin has 2 phosphorylation sites a tyrosine 1 1 8.

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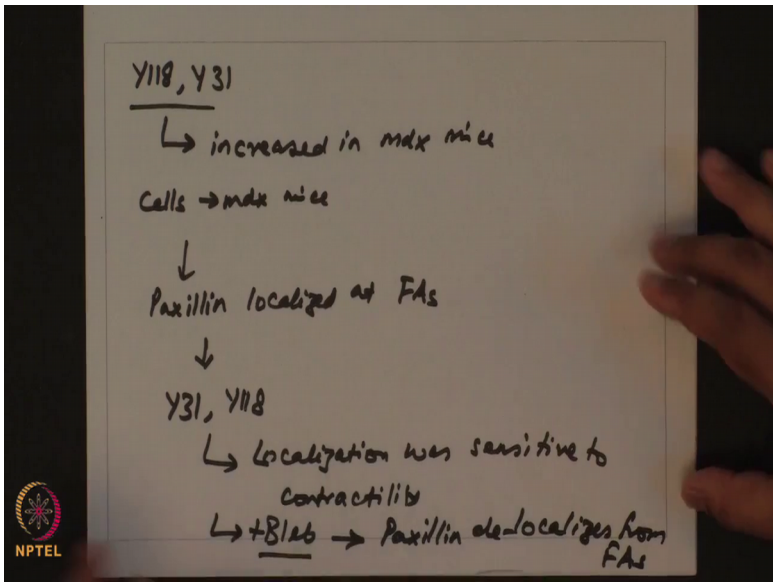


And tyrosine 31 these are increased in MDX mice. So, phosphor screen revealed that increased amount of paxillin signaling in MDX mice. So, what is paxillin doing in these cells? So, what was observed was in MDX cells in cells isolated from MDX mice paxillin localized at focal adhesion was phosphorylated.

So, you had wrote Y31 and Y118 phosphorylated and more importantly it is localization was sensitive to contractility. So, when you treat it with Bleb which is the inhibitor of myosin 2 then you have immediate delocalized. So, paxillin delocalizes from focal adhesions. And if you remove then again they come back to the focal adhesions ok.

So, this amount of turnover of focal adhesions can be assessed using frap experiments or fluorescence recovery after photo bleaching.

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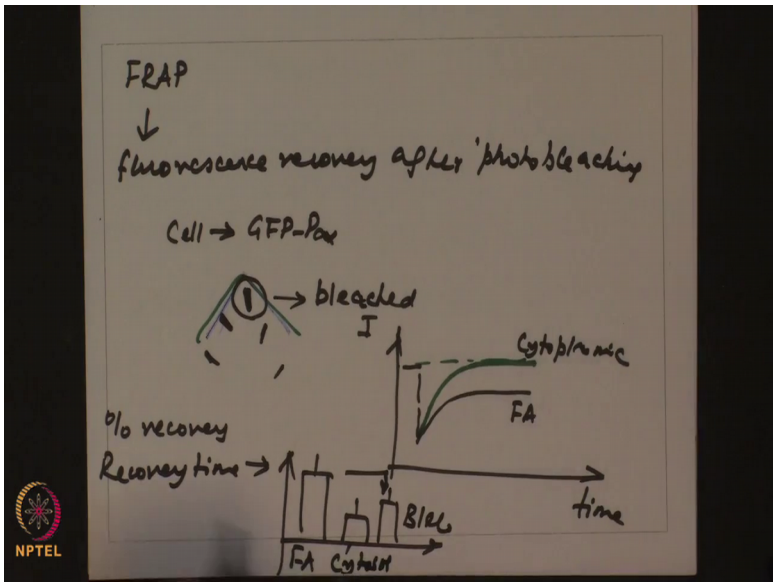


So, what you do is you take a cell which is transfected with let us say GFP paxillin let us let us say this is an outline of a cell, say this is outline of a cell. And you have GFP paxillin adhesions. You focus on one small adhesion and you shine light So that it is locally bleached. And then you track what is the intensity at this point as a function of time ok.

So, if you plot the intensity you will have a curve something like this. So, you can backtrack what is the percentage recovery. So, in this case for example, if you had a protein which was fully mobile then after you bleach it will come down come back to the same value. But at focal adhesions at focal this was observed in the cytoplasm where the protein was freely diffusing in focal adhesion it is recovery in what less ok.

So, if you track the time to recovery and the recovery time. So, what they observed, was compared to focal adhesions the recovery time. So, this recovery time was much higher at focal adhesions compared to cytosol, and when you treated with blemish statin the recovery time decreased. So, you have in a decrease in blemish statin, and this is in cytosol. Suggesting that paxillin is interacting with other proteins at focal adhesions.

Now, what was also observed were was when paxillin was over expressed.

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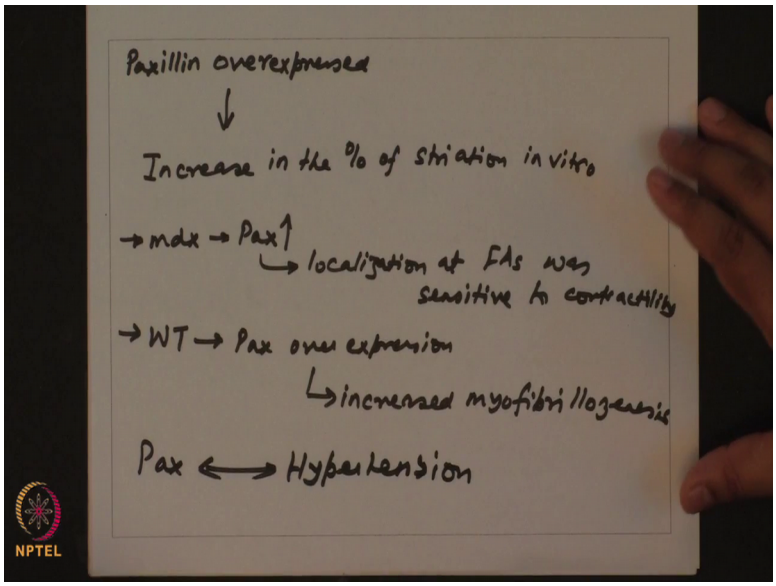


There was an increase in the percentage of striation in vitro. So, you use the cell and cell geometry to track how much how many myotubes up straight it. So, there is in MDX mice there is an increase in paxillin, paxillin localization was at a phase was sensitive to contractility. And when you took control a wild type if you over express paxillin. So, this led to increased myofibrillogenesis ok.

So, this suggests this these experiments suggest a link between paxillin and contractility or hypertension. So, you can assess it functionally using experiments like the relaxation experiments that I suggested.

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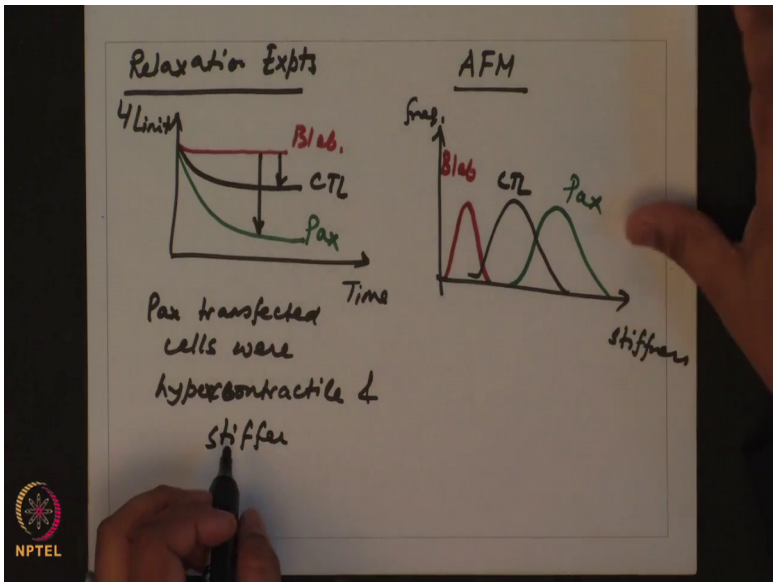


And what was observed, what was observed if this was the curve for control cells, when you over express paxillin.

So, paxillin over expression. So, this is L by L in it this is time. They relaxed by a lot amount. So, you can track and when they treated with blemish statin right blemish statin inhibit is contractility, when they treated with blemish statin this was negligible, because blemish statin relaxes all contractility. So, you can track this difference compared to blemish statin how much is the increase in this length. And what was observed was paxillin lead to increase relaxation.

So, paxillin transfected cells where hyper contractile. And this can also be indirectly estimated using atomic force microscopy. So, later in the course you will have a detailed discussion of how we can use afm for probing mechanical properties of cells. And what was observed was So, if you track the stiffness, if cells if this is the distribution you get for control cells when you over express paxillin the curve shifted to the right is to the right means cells became stiffer and for Bleb treated cells.

So, this is with Bleb this is control and this is with paxillin over express cells. So, this suggests that paxillin transfected cells are hyper contractile and stiffer. So, the same experiment was done between so, all these experiments were done with C57 cells transfected with paxillin. When the same experiment was done with C57 and MDX what was observed was Compared to the response of C57 cells ok.

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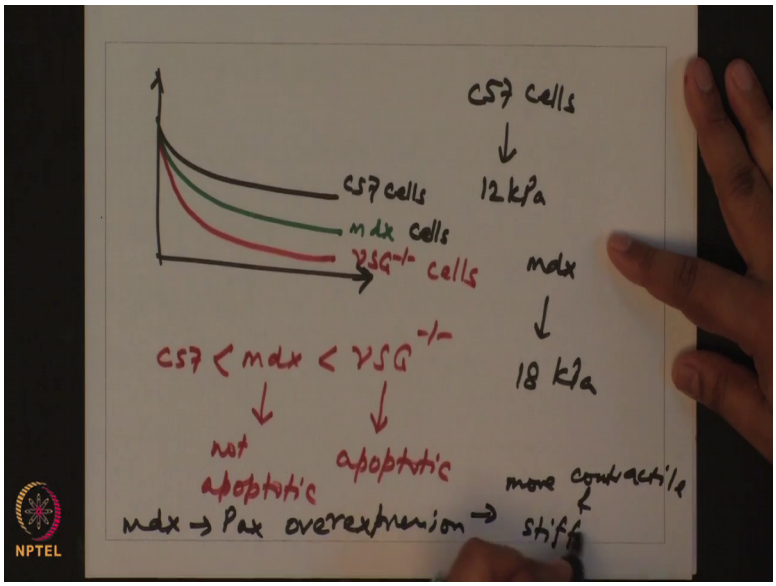


So, with in MDX cells they were more contractile, but they were less contractile than gamma soarcoglycan deficient cells. So, you have a situation in terms of contractility C57 less than MDX less then gamma soarcoglycan deficiency cells. Now these guys are apoptotic, these guys are not apoptotic ok.

What was also observed was compared to C57 cells tissue from C57, but the average stiffness was order 12 kPa is similar to normal muscle, MDX muscle tissue was nearly 1.5 fold stiffer at 18 kPa. So, like previously MDX which exhibit paxillin over expression are contractile are more contractile and stiffer compared to C57.

So, what is the implication of this? Now what has been observed is in the clinic MDX expressions are often given this drug called prednisone.

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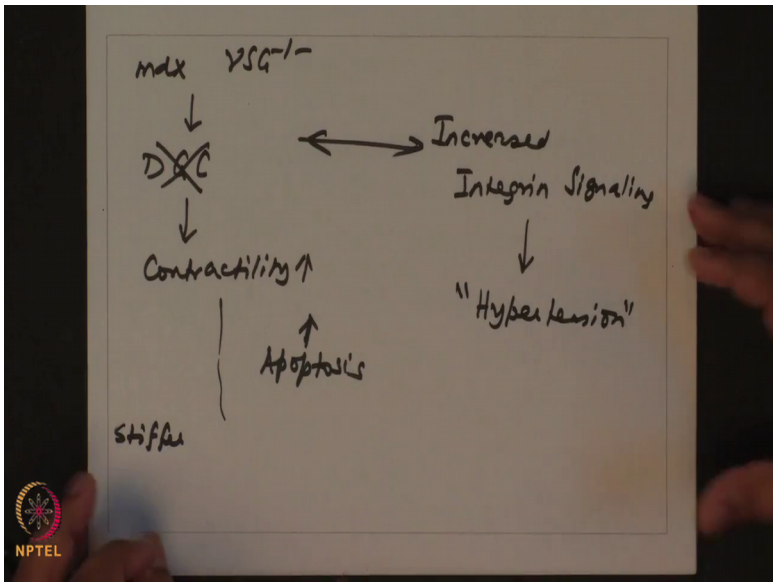


Prednisone, this is a glucocorticoid. So, this is used only as a palliative, but how it works is not known. So, experiment was performed in which you had control cells if you had treat it with pdn or prednisone and asked how does this stiffness strange ok.

What was observed was pdn treatment made cells softer. So, suggesting what has been done is using this drug you are making the you are reducing the level of tension. So, in a sense what you saw is your MDX or gamma soarcoglycan deficient in both these cases your dystroglycan complex is being perturbed which leads to a contractile increase in contractility of these cells, in one case it leads to apoptosis ok.

In gamma soarcoglycan deficient cells and in both the cases the tissue is stiffer. So, in both these cases this is associated with increased integrin signaling. But the problem is this integrin signaling is in turn driving hypertension, promoting contractility and driver hypertension. So, this is the problem though the integrins try to compensate the effect of dystroglycan by over expressing integrins and integrin signaling in a sense it leads to hypertension.

With that I stop here for today as part of reading assignment.

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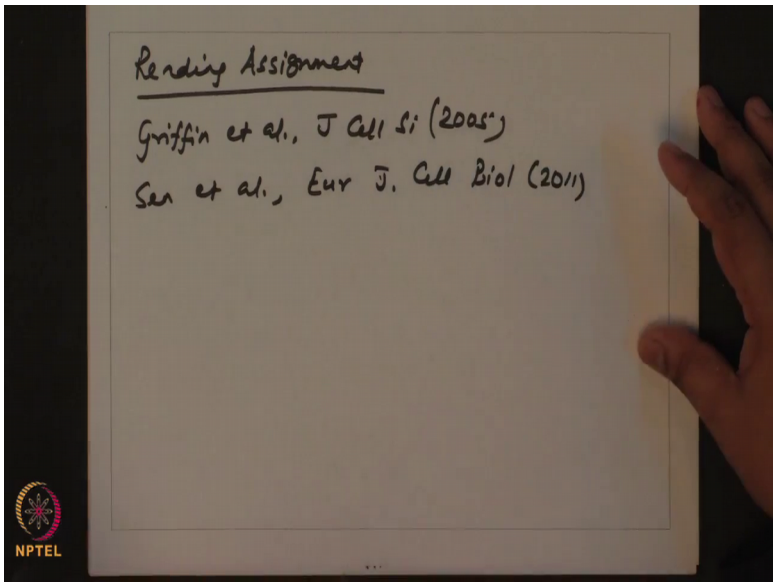


I give you 2 papers; one is Griffin Et AL journal of cell science 2005. The other one is Sen. Et AL European journal of cell biology 2011 ok.

Thank you for your attention.