Introduction to Mechanobiology
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Week – 06
Lecture – 29
Mechanobiology of Diseases: Cancer III

Hello and welcome to our today's lecture of Introduction to Mechanobiology. What the last 3 classes I had discussed 2 papers which link ECM Stiffness with Cancer.

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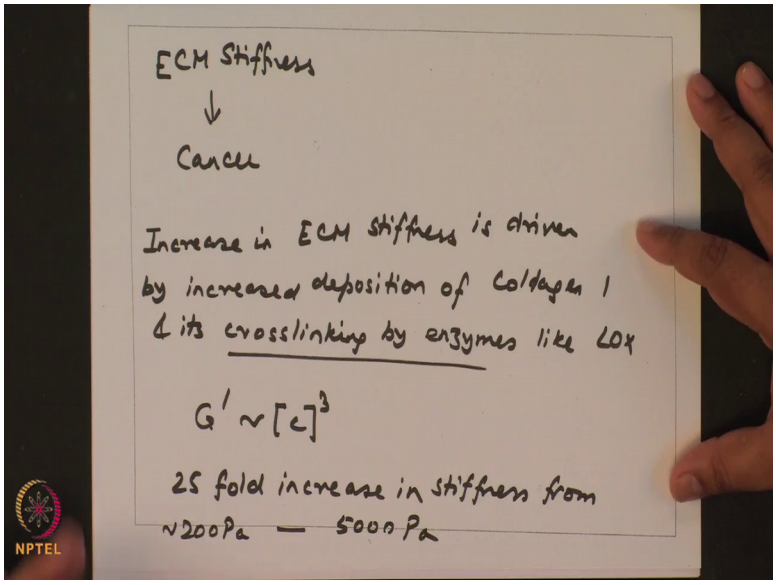

What these studies collectively demonstrated that when you have increase in ECM stiffness. Increase in ECM stiffness is driven by increased deposition of collagen 1 and it is cross linking by enzymes like LOX or like lysyl oxidase.

So, you remember earlier we had discussed how properties of collegen genes scale as concentration cubed. This tells you that increase in collagen density can trigger increase in bulk stiffness of the matrix. And when you have cross linking enzymes then this difference can further increase. This explains the nearly 25 fold increase in stiffness of mammary gland from roughly 200 Pascals to nearly 5000 Pascals you have a 25 fold increase in stiffness.

If you think of 3 dimensions on 2D I can keep on making the matrix differ and stiffer.

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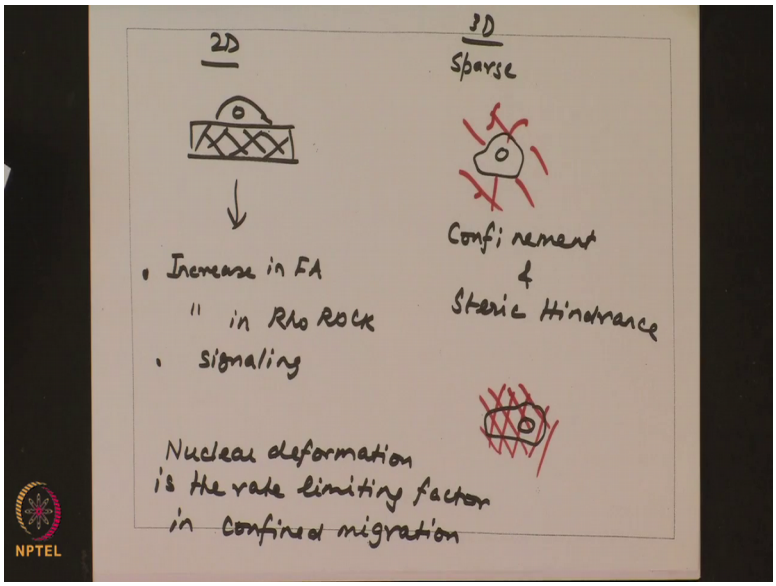

So, on 2D the cells will sense the higher stiffness of the substrate and this should drive. So, increase in focal adhesion and increase in Rho ROCK signaling. These are 2 key regulators of cancer in (Refer Time: 02:37). But the same cell if you think of a cell migrating through a 3 dimensional matrix when it is sparse; in 3D what matrix provides is confinement and Steric Hindrance.

In 2D steric hindrance is absent, but in 3D cells would have to continue with steric hindrance. So, in sparse matrices cells might actually pass through the pores; however in very dense matrices, when the matrix is very dense migrating becomes a rate limiting factor. And in a sense as we will discuss later Nuclear deformation is the rate limiting factor in confine migration.

So, how then with increase in stiffness how do cancer cells become more invasive? So, that is the question. And it turns out that increase in stiffness leads to stiffening of the matrix that leads to steric hindrance for the cells, have a way of dealing with that increased steric hindrance.

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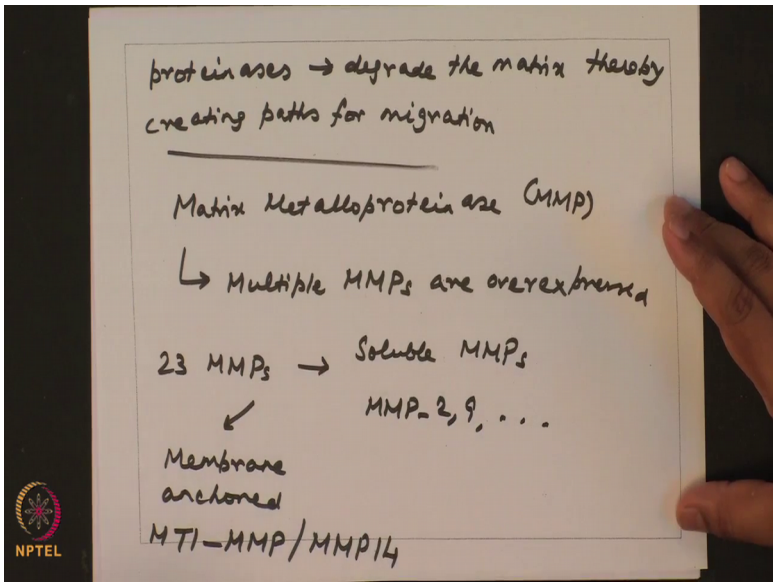

So, what they do is they make use of degrading enzymes of protein ases which degrade the matrix thereby creating paths for migration.

One of the most prominent family of you know endopeptidase is Matrix Metalloproteinase in short they are referred to as MMPs. And it has been observed that in cancer multiple MMPs are over expressed. So, in human there are 23 different MMPs some of these are matrix or membrane anchored.

One of the major MMPs which are membrane anchored is MT1-MMP is also referred to as MMP 14 or you have multiple soluble MMPs. Examples are MMP 2, 9 etcetera.

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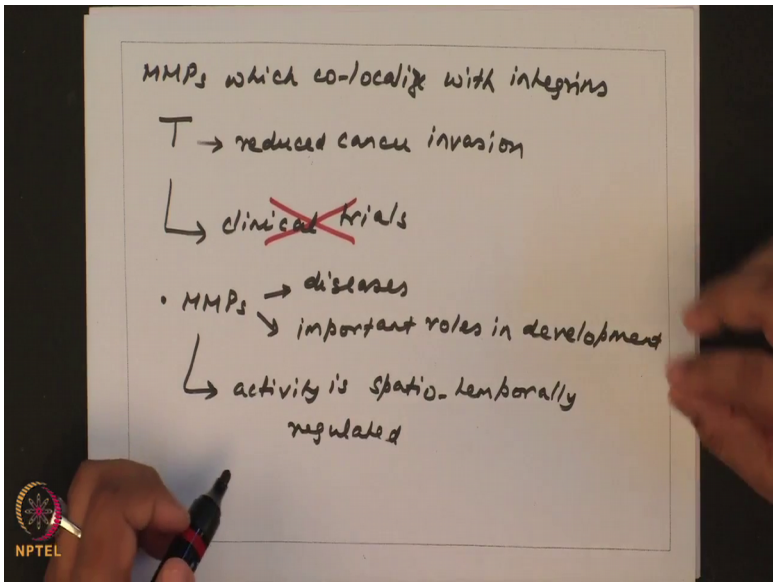

So, one interesting bit of information is that there are many MMPs which is co-localized with integrins suggesting there might be some cross talk between MMP activities with focal adhesion signaling.

This the finding that MMPs are over expressed in the context of cancer would mean that if you inhibit MMPs then it should lead to decrease or reduced invasion and this idea led to multiple clinical trials; multiple clinical trials would try to target MMP activity. Unfortunately all these clinical trials failed and of course, there are multiple reasons for it one of the most important reasons being MMPs are important not only for diseases, but they have multiple physiological roles also they have important roles in development and their activity is Spatio-temporally regulated.

They act in a very context sensitive manner, but there is one more reason which was demonstrated by Peter Friedl.

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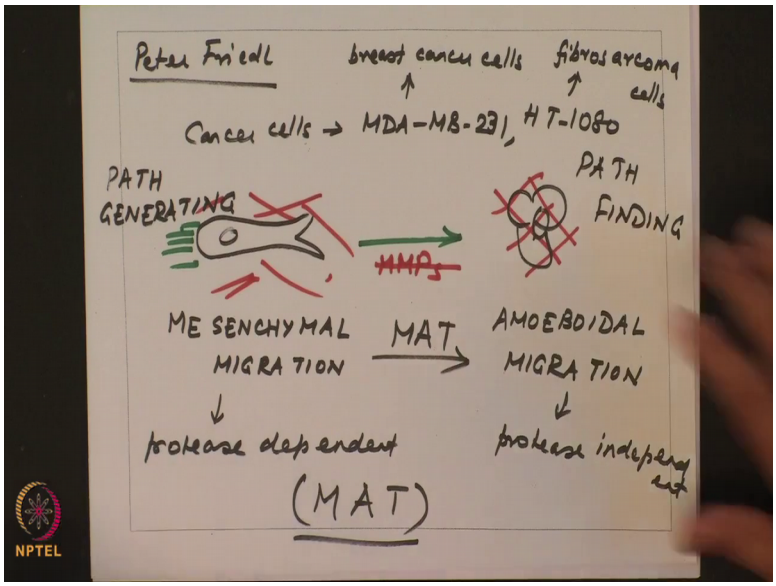

So, the experiment that he did was he to cancer cells namely MDA-MB-231, and HT-1080. These are breast cancer cells and these are fibrosarcoma cells. Both these cells were highly invasive and what friedl demonstrated was when you inhibit proteases.

Generally you have cells which are migrating through these matrices by creating degraded zones. Let us say this is the degraded zone. So, when you inhibit MMPs. So, when you inhibit MMPs; inhibit MMPs what he showed was these cells transition into more rounded cells where your matrix remains intact, but they squeeze.

This is Mesenchymal migration and they migrated in an Amoe boidal manner. So, you remember from our previous lectures that we showed that. So, this is protease dependent mesenchymal is also called protease dependent migration, to protease independent type of migration. And this transition is called the mesenchymal to amoe boidal transition.

Here you have MAT, when you inhibit MMPs the cells transition from adhesion dependent, protease dependent, to adhesion independent, protease independent migration. This is instead of path generating because proteases create path you have transition from past generating kind of migration, to path finding kind of migration.

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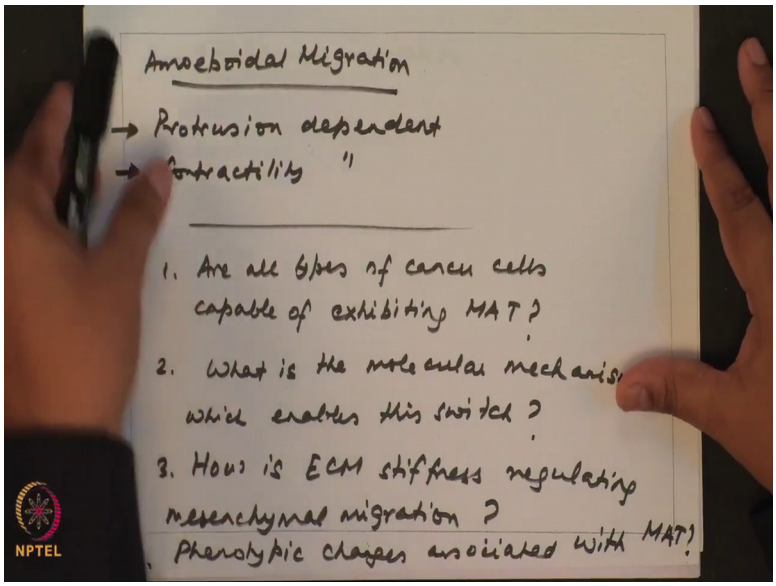

I had previously discussed that in Amoe boidal Migration you can have multiple modes of migration in 3D right using more Protrusion dependent or Contractility dependent. So, the question which arose from all of these studies are all types of cells capable of exhibiting MAT and second is what is the molecular mechanism which enables this switch.

So, one way of seeing this cross talk. So, you have cross talk between MMPs and migration.

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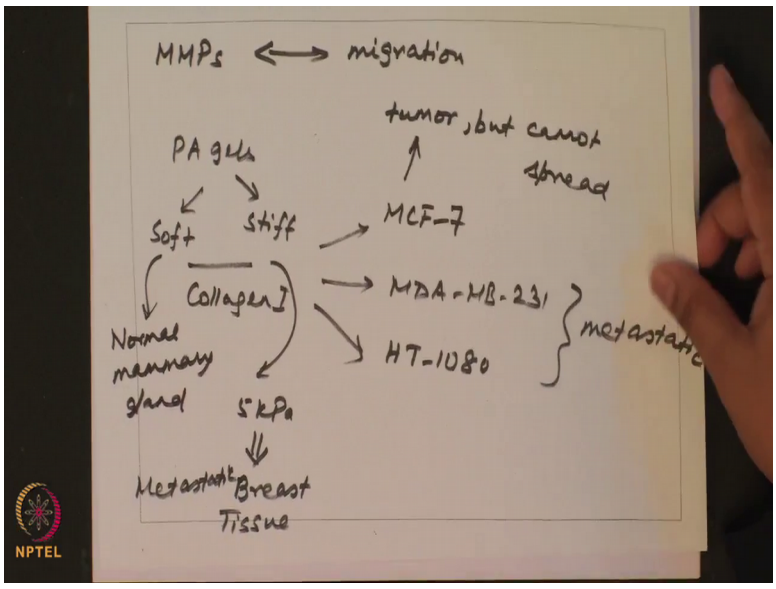

So, what was also not clear is how is ECM stiffness regulating mesenchymal migration? And what are the phenotypic changes; what are the phenotypic changes associated with MAT.

So, to do this one can do simple experiment, you take 2 surfaces. So, to polyacrylamide gels one is soft and one is stiff, let these gels be coated with collagen 1 and imagine you plate 3 different cell lines, MDA-MB-231 HT-1080, both these cells are highly metastatic.

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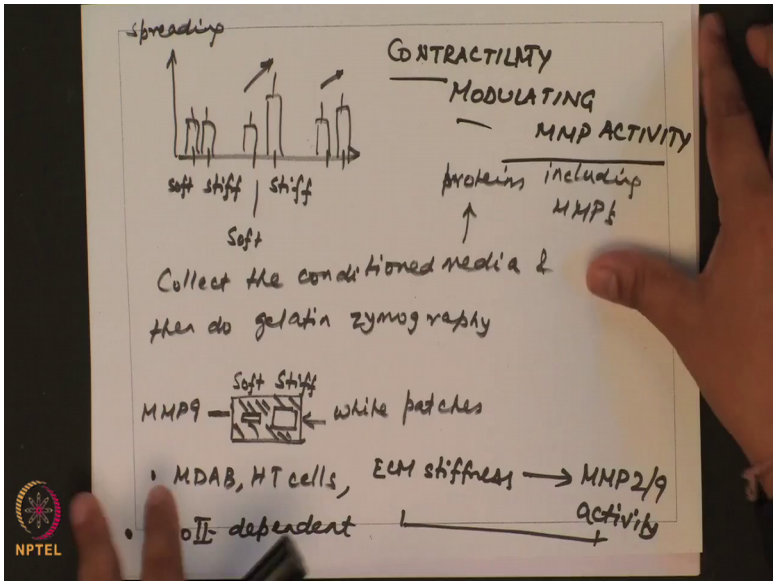

So, imagine you take another cell line MCF-7, this also a breast cancer cell line MCF-7 cells have been shown to be capable of forming a tumor, but they cannot metastasize or cannot spread. So, the experiment is you culture these 3 cells on soft and stiff pas which are coated with collagen the stiff gel you can choose let us say 5 kpa which mimics breast metastatic breast samples breast issue and this one is normal breast issue mammary gland.

So, if you do this experiment. What you can probe is first of all do cells exhibit a stiffness dependent spreading that we observe. What you would realize in terms of soft and stiff if you take MDA MB, this is soft, this is stiff; again this is, soft this is stiff. And this is your spreading axis. So, for MDA MB and HT what you observe is a dramatic increase in spreading between soft and stiff surfaces.

There is an increase, but in MCF 7 cells these guys do not exhibit this stiffness dependence or much weak. What you can also do is you can assay the conditioned media. So, you can collect the conditioned media and then do zymography or gelatin zymography. So, gelatin zymography is a technique in which you have a regular page which has gelatin in it.

So, you run the protein. So, you collect this conditioned media will have multiple proteins including MMPs which are secreted MMPs which are secreted by the cells. So, what this experiment revealed was first of all, from soft to stiff. When you run this zymography what you see is if this entire let us say you have 2 conditions soft and stiff. You have these white patches corresponding to molecular weight of certain proteins let us say MMP 2 or MMP 9.

This would mean that in the gel this is where this protein migrated and when you activated the zymo the zymo gram these guys degraded the gelatin which was there inside these cells so, causing a white zone. And this by comparing the intensity of these white zones you can compare the activity of this particular protein. So, what this would what they observed was that in MDA MB and HT cells in MDA MB and HT cells

So, these cells ECM stiffness led to increased MMP 2 and 9 activities. In other words these cells secrete more amount of proteases on a stiffer environment. This might explain why in a more dense network cancer cells are getting more invasive because the increase in ECM density is struggling these cells to secrete more amount of proteases, which are then degrading the matrix thereby creating paths for escape.

This is the reason why on a stiff matrix or in a stiffer environment the cells are more invasive because they secrete more proteases. Now interestingly this protease secretion is again Myosin 2 dependent. If you inhibit myosin activity using blebbistatin, activity of the protease activities significantly reduce suggesting that contractility. So, you have contractility modulating MMP activity.

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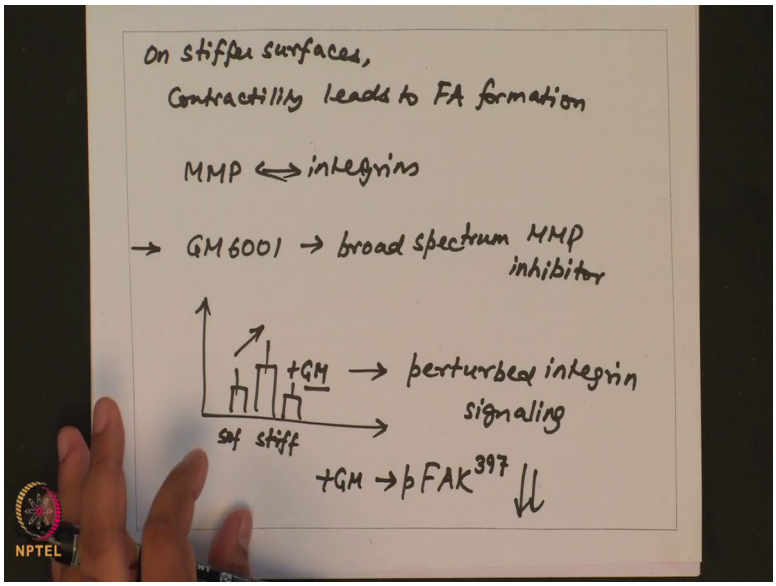

Why must you know what might be the possible reason in which this process is happening. Now you know that on a stiffer surfaces contractility leads to focal adhesion formation forces are required to stabilize focal complexes. So, given that MMPs have a cross talk with focal adhesion is it possible that there is some interaction between MMPs and integrins which is regulating this process.

For these experiments can be done in which you treat cells; you treat cells with protease inhibitors. So, GM 6001 is a broad spectrum MMP inhibitor. So, when you look at the spreading profile of MDA MB on a stiff surface, soft to stiff there is an increase this is soft this is stiff, but when you inhibit GM you are spreading drops. So, this is plus GM condition.

Your spreading increase you add GM it inhibits spreading and what has been observed is when you treat cells with GM this leads to perturbed integrin signaling, in other words your focal adhesion phosphorylation levels of focal adhesion kine is 397 residue is dropping. So, when you had plus GM this leads to inhibition of integrin signaling. So, how is this possible?

So, to address it you can actually look at you can look at integrin signaling experiments integrin recycling experiments. So, imagine you have a cell.

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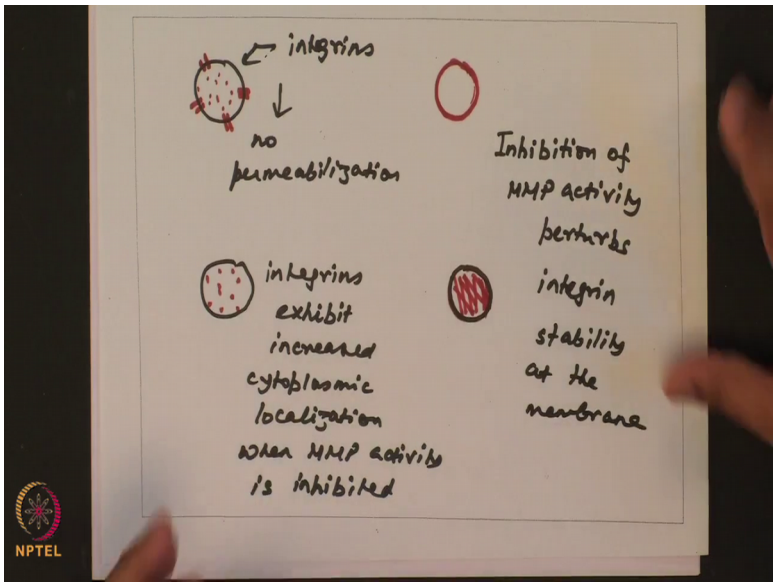

And you look at without permeabilizing the cells you probe for integrins. So, no permeabilization and imagine my there are integrins which are sticking out like this and of course, there are other of these inside the cell.

If you inhibit if you treat cells with GM what is observed is these integrins become recycled. In other words all these integrin molecules, integrins exhibit increase cytoplasmic localization when MMP activity is inhibited. So, if you take an antibody which binds to the extracellular domain. So, what you would see.

If you take an antibody which binds to the extracellular domains under control conditions you would see a very clean red staining along the periphery of the cell, but when you treat cells with GM you would see that in the periphery there is no clear localization instead the entire protein gets localized inside the cell suggesting. So, this suggests that Inhibition of MMP activity perturbs integrin stability at the membrane.

So, what you have is a situation in which if you think of focal adhesions, you know that contractility stabilizes focal adhesions, but also what you have is a situation.

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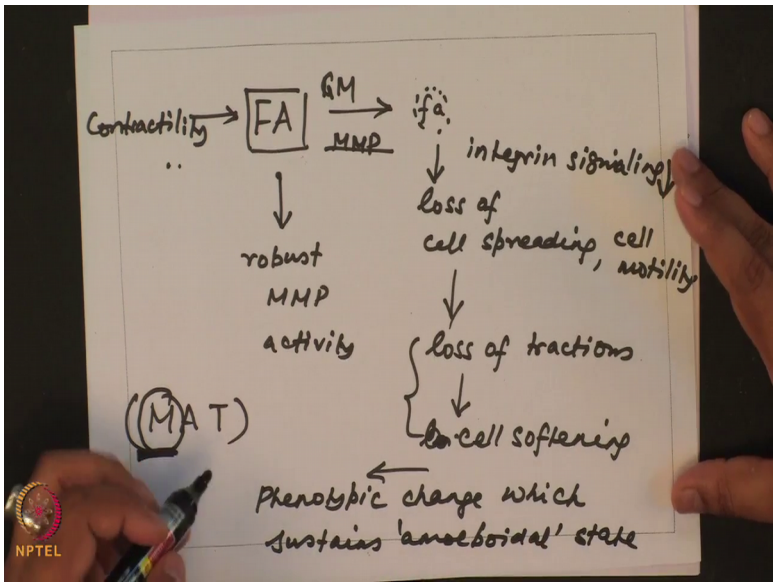

When you inhibit with GM you add GM your focal adhesions disassembled, your focal adhesions there is a drop in focal adhesions this leads to loss of cells spreading.

So, suggesting that you have a 2 way cross talk; on one hand contractility form stable focal adhesions and this leads to robust MMP activity, but on the other hand when you inhibit. So, this is your inhibiting MMP activity using GM your focal adhesions fall apart this is loss of cell spreading and what this leads to is also loss of tractions.

So, you have these results suggest that there is a robust cross talk between contractility and MMP activity, where perturbation of contractility leads to loss of focal adhesions thereby leading to loss of MMP activity on the other hand if you inhibit a MMP activity there is loss of focal adhesions, that leads us to loss of tractions, and also leads to cell softening.

So, could this be a mechanism whereby MAT is enabled. In one direction contractility is stimulating focal adhesions, focal adhesions are reason to robust MMP activity. So, you have stable mesenchymal state. When you inhibit MMP activity using GM focal adhesions are gone, integrin signaling is gone, integrin signaling is also gone this perturbed loss of cells spreading also loss of cell motility. So, cells cannot move that leads to loss of tractions and loss of and cell softening.

Now, these 2 things if the cell becomes soft can this be a phenotype change, which drives or which sustains the amoe boidal phenotype state. Because on the amoe boidal migration case your cell has to squeeze through pores. So, maybe this is the mechanism which an enable cells to switch from a mesenchymal to an amoe boidal state.

With that I stop here we stop our discussion about cancer from in the next 2 lectures I will discuss little bit about one example of atherosclerosis and one example from in mustard dystrophy and we discussed how, again what are the mechano biological changes which have occurred as a consequence of the disease or are associated closely with the disease then sometimes drive the disease.

Thank you for your attention.