Introduction to Mechanobiology
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Week – 07
Lecture – 33
Nuclear Mechanotransduction: LINC complex in cell migration

Hello, and welcome to today's lecture of Introduction to Mechanobiology, in the last case we had started discussing about the nucleus ok.

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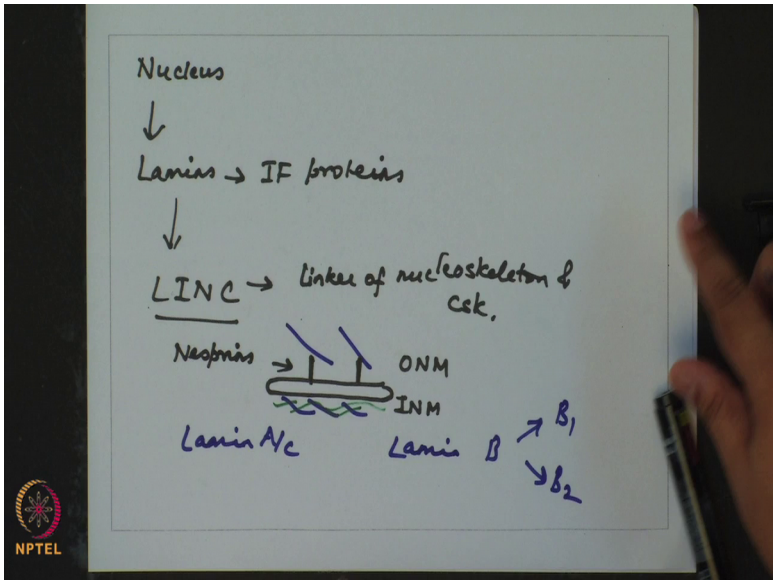

Which is the centerpiece of the cell and which serves as the signaling point where gene expression is turned on during processes like differentiation? So, we have introduce started discussing about the structure of the nucleus and this, in this context we had introduced you have proteins or lamins which are intermediate filament proteins, which serve and this lamins are part of the LINC complex, LINC stands for linker of nuclear skeleton and cytoskeleton ok.

So, in the nucleus, nucleus has two membranes inner nuclear membrane and outer nuclear membrane underneath the inner nuclear membrane you have these networks of two lamins, of two lamin networks lamin AC and lamin B, B can be either B1 or B2 and AC is because of spice variant of the lamin in g and outside. So, outside you have the cytoskeletal network through which you have connections through the nesprins? So, this

establishes the direct connection between outside the nucleus and inside the nucleus. So, lamins not only bind to the heterochromatin ok.

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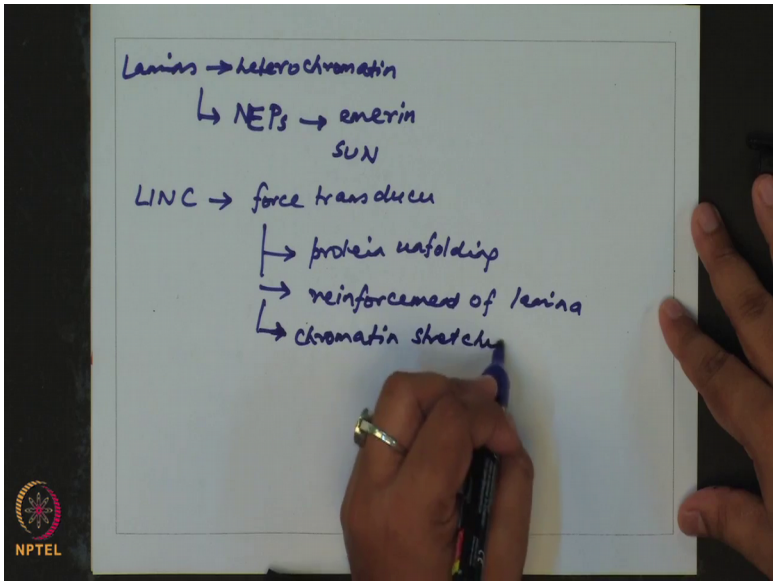

There are various nuclear envelope proteins or NEPS for example, emerin sun proteins etcetera these participate in this cross signaling and the LINC complex, serves as a force transducer ok.

So, force might lead to protein unfolding of various proteins within this network, might lead to reinforcement of lamina at lead to chromatin stretching. Now also discuss this paper we last class there we showed that lamin A to B ratio varies, depending on tissue elasticity ok.

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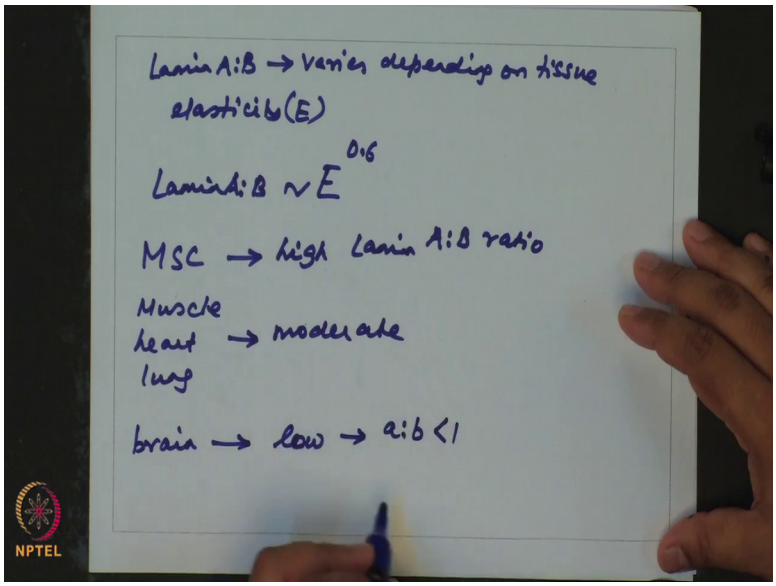

So, this lamin A to B ratio roughly has been shown to scale as e to the power 0.6, where e is the elasticity of the tissue. So, in for example, in mesenchymal stem cells they have high lamin A to B ratio, in muscle cells muscle or heart or lung you have moderate levels of lamin A to B ratio, well in brain cells neuronal cells you have low and here.

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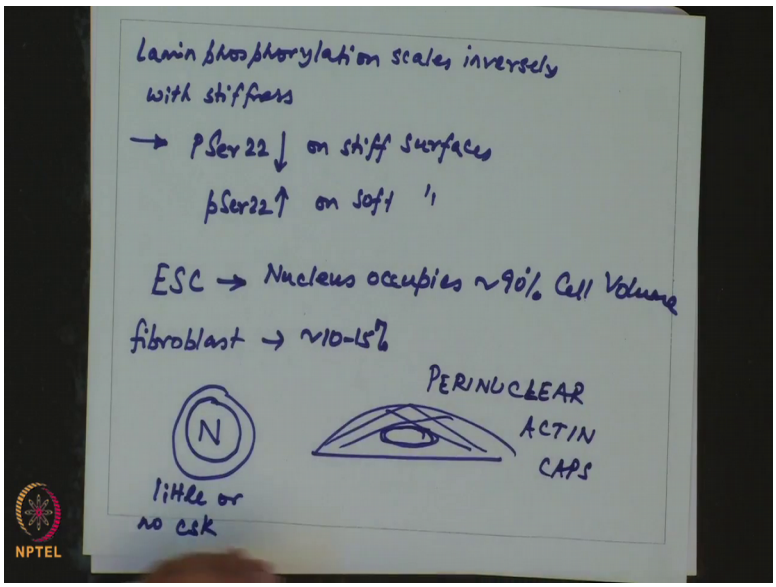

When I say low there is A to B ratio is less than 1, the other aspect which I introduced in last class was that lamin phosphorylation. So, scales inversely with stiffness, in other

words on cells cultured on stiff surfaces phosphorylation is less. So, phosphorylation of serine 22 is less on stiff surfaces, but is high on soft matrices ok.

So, if you look at the nuclei sizes inside the cell, we think of an embryonic stem cell the nucleus occupies nearly 90 percent of the whole volume, while in a differentiated cell like a fibroblast, this is ordered 10 to 15 percent you can clearly see. So, of a embryonic stem cell this is what the nucleus will look like, there is very little or no cytoskeletal network. So, you just have the nucleus occupying the gamete of the space versus for a fibroblast the nucleus is right at the center and it takes a much less amount of space, and it is also more elongated under a lot more stress because you have cytoskeletal fibers, which actually push down on the nucleus in this regard it has been shown that there are PERINUCLEAR, ACTIN, CAPS, which push the nucleus down ok.

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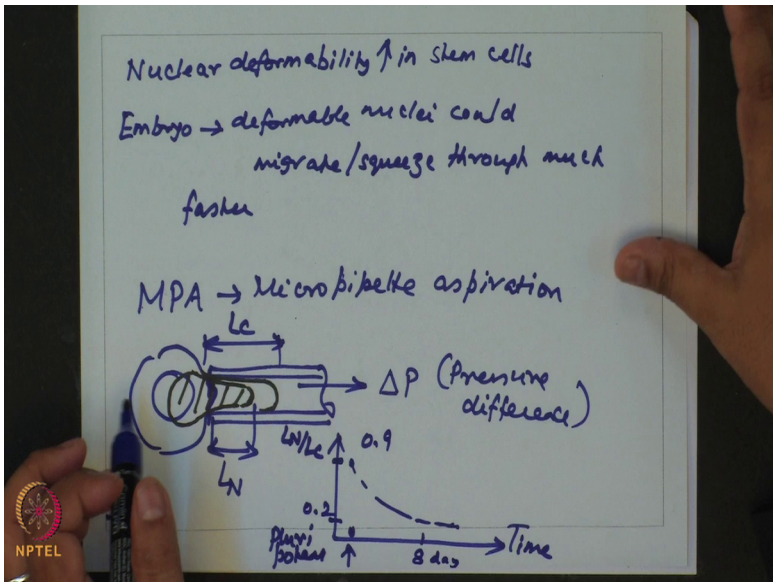

So, in terms of deformability of the nucleus, one would imagine that deformability nuclear deformability will be high in stem cells and why is that. So, for in an embryo we talk about an embryo deformable nuclei could contribute, could actually migrate much faster or squeeze through much faster. So, to do this to as a how nuclear deformity varies between stem cells and differentiated cells a simple experimental setup that has been used is the experimental setup of micropipette aspiration. So, what you do is you have a cell, if this is your cell with the nucleus put a pipette a micropipette and you expose this cell go step jump in pressure, this is a pressure difference.

So, this is equivalent of sacking on a cell. So, what you will have is as time goes on the cell and the nucleus. So, there cell will have a shape like this and the nucleus will also deform in this kind of configuration. So, what you can track is this length of the cell and this length that the nucleus has entered into the micropipette. So, this is ideally if the nucleus was more deformable. So, what you should have is when you sack just like the cytoplasm will move in the nucleus will also move in now what people the group has tracked this deformability as a function. So, you can track the ratio of LN by LC as a function of differentiation ok.

So, what has been shown is as a function of differentiation, so initially so LN by and you have plotting LN by LC here, so at early times so when it was in pluripotent state this ratio is as high as 0.9, but this drops rapidly as the cells starts to differentiate and at large time points that is say order 8 days, this ratio drops to as little as 0.2. So, this demonstrates that the nucleus difference as a different shapes. So, if a nucleus defense has different shapes, what is the LINC on cell migration? So, ideally a soft nucleus should mean that the cell is more invasive ok.

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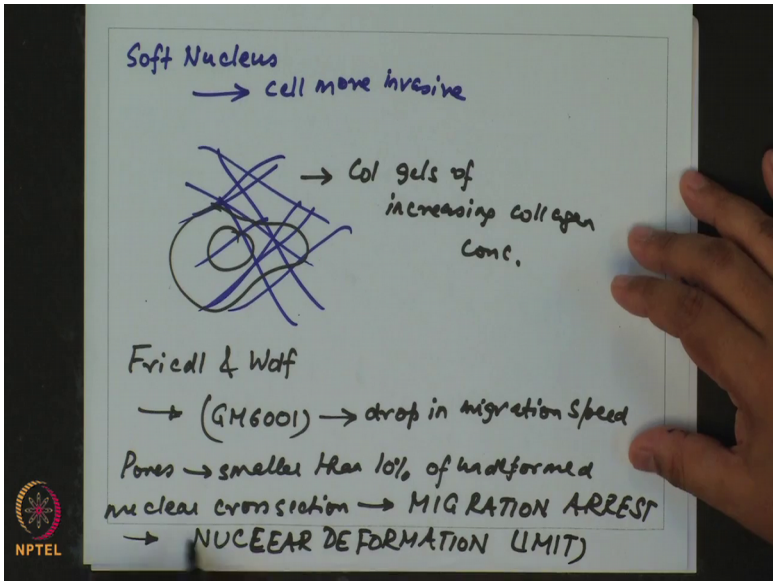

So, why because, in three d matrices when cells are migrating, the cell has to squeeze through the pores in the matrix. So, if the matrices very dense it is very difficult for the nucleus to be squeezed. So, there has been seminal study by Friedl and Wolf and then they showed that when a cells migrate through matrices. So, what they did was they

tracked cell migration through collagen gels of increasing concentration, and what they showed that if the cell is secreting mmps then whatever be the concentration does not matter because the cell will secrete mmps and they will degrade the matrix thereby making holes, which are big enough for the cell to squeeze; however, in the presence of mmp inhibitors. So, GM 6oo1 is broad spectrum mmp inhibition ok.

So, then you have a drop in migration speed, and for pores which are smaller than 10 percent of undeformed nuclear cross section. So, there is complete migration arrest mean that cells are completely stuck. So, this limit the authors named as a nuclear deformation limit. So, in other words and nucleus cannot be deformed to below 10 percent of its original undeformed cross sectional area. So, consistent with this if you think of leukocytes or dendritic cells their nuclei do not express lamin AC ok.

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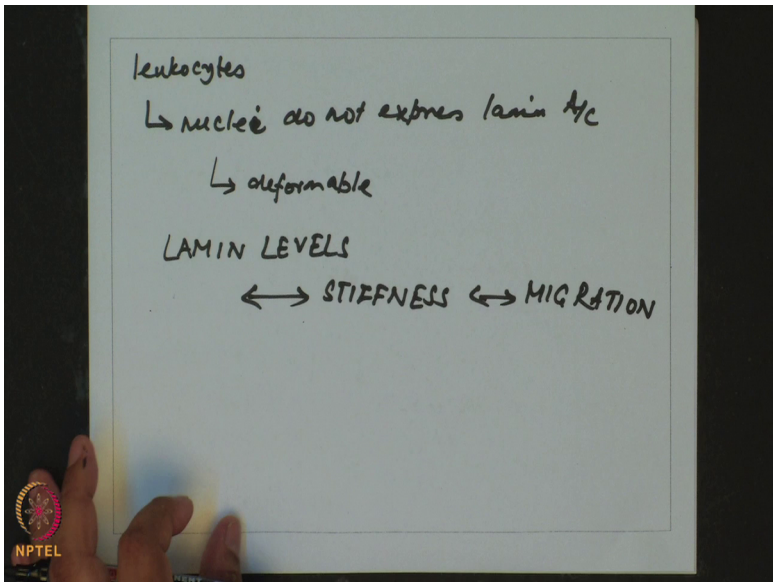

So, this makes the nuclei mode deformable. So, that leukocytes can go all over the place within the tissue, so this suggests that there is a relationship between lamin levels, which dictates this nucleus stiffness and this impacts its migration ability ok.

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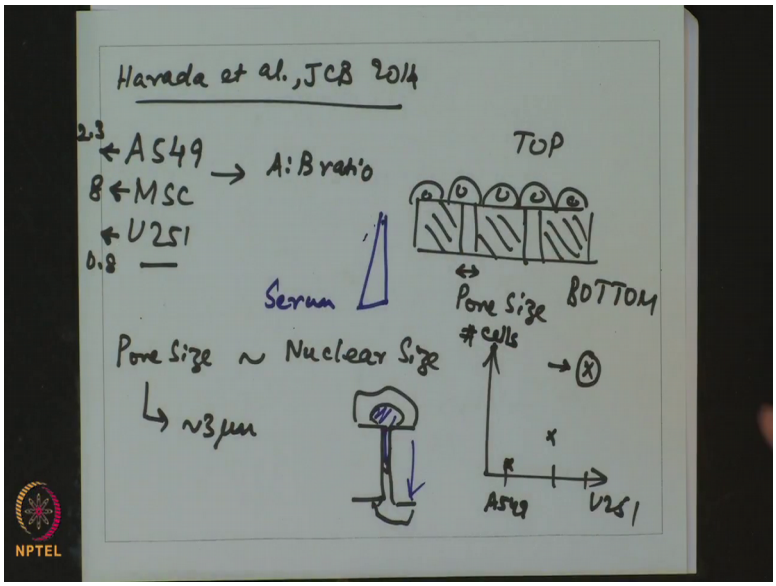

So, to test this, so I will discuss this paper, so what these authors did was took cells with three different types of cells A549 mesenchyme stem cell and U251. So, these have different A to B ratio, so for A549 this is 2.3 MSC have high they have A to B ratio of 8, and U251 have 0.8. So, A to B ratio is varying in these cells while B ratio B1 and B2 are roughly comparable ok.

So, they asked, so they used transfer migration force what are these. So, these are transfer migration inserts in which you plates cells on the top, put a gradient you put a gradient in serum. So, this acts as a cumulate active cube making the cells squeeze, but if so if you reduce so this is your pore size. So, if the pore size is comparable to the nuclear size. So, ideally there should be no difference, but if your pore size is ordered 3 microns then the real picture is something like this this is your cell and this is your nucleus it has to deform significantly to make it through the pore ok.

So, what they counted was they plated the same number of cells on top. So, this is your top of your pores so this is the bottom of your pores and then they asked that how many over the number of cells at the bottom and what they found was so U251 cells, which have the lowest lamin A to B ratio. So, this x maximum number of these cells, but able to transit and which was lowest in case of A549 cells. So, suggesting once again that well lamin A to B ratio is high. So, this is number of cells at the bottom, the bottom of the pores ok.

So, in U251 cells you have the maximum efficiency of migration because the A to B ratio was low and these cells were soft, but what they also observed was when they looked at the nuclei underneath in the bottom pores many of the nuclei.

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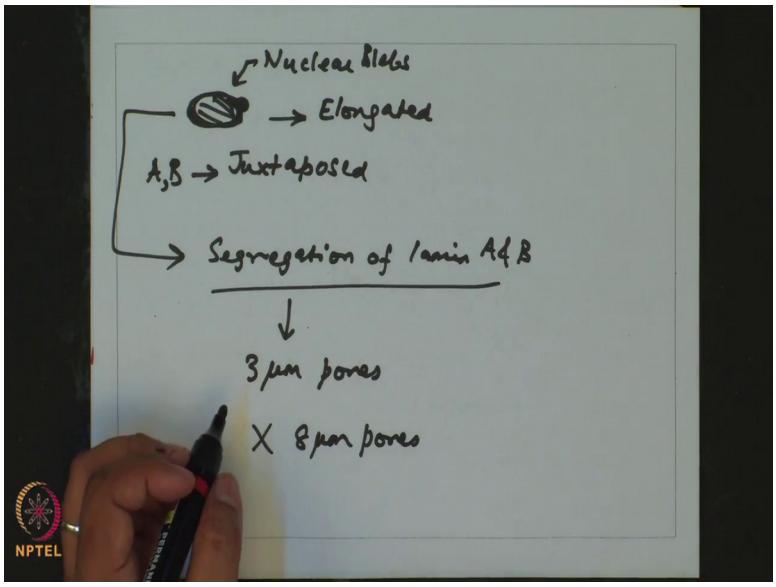

They exhibited these blebs. So, this is a regular shape of nuclei after migrating through the pores they had this kind of shapes. So, these are examples of nuclear blebs and what they found was in general case A and B networks, lamin A and B are juxtaposed, but in these nuclei there was a separation or segregation of lamin A and B ok.

So, first of all not only were these cells nuclei were elongated, but you have this odd shaped nuclei and the segregation of lamin A and B. So, this segregation of lamin A to B an all this was only observed in three micron pores, it was not observed in eight micron pores, but the pores are big enough that the nucleus doesn't need to do for much. So, this shows that having low lamin A to B ratio actually helps the nuclei to migrate and during the migration there is significant amount of change in nuclear shape, which correlates with the migration process ok.

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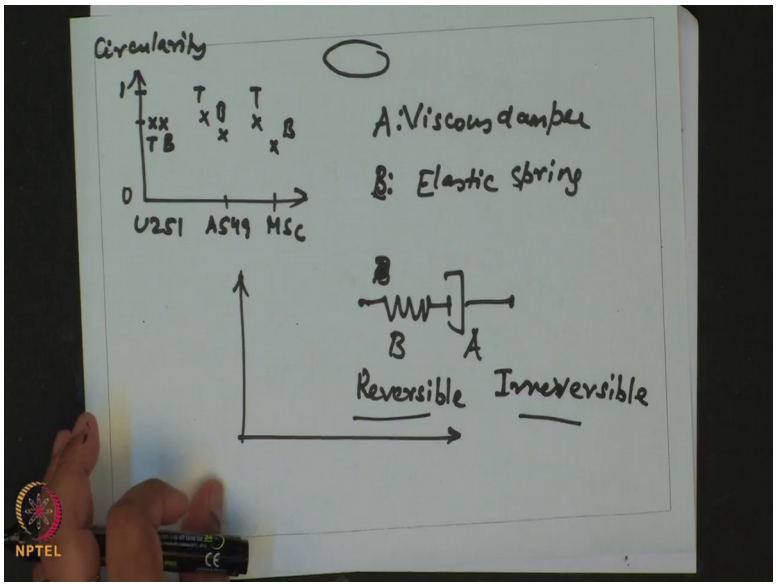

So, what they then did they compared the nuclear. So, if you have a nuclear shape like this you can track the shape by using a measure of circularity, which returns the value of 1. So, it is a value between 0 and 1, 1 means perfect circle. So, in U251 cells which had blown a lamin A to B ratio, there was no change before and after at the top and the bottom the circularity remained unchanged, but in case of mesenchymal stem cells the bottom cells were much more elongated, and what they also formed was you could track this change in stiffness with the lamin A levels ok.

So, what it is also suggested so based on their experiments they suggested that lamin A acts like a viscous damper, and lamin B acts as a elastic spring in U251 cells, as soon as it deformed when you when they let go when they came underneath they immediately regain their shape. So, that is why and they had A to B ratio of 0.8, which is less than 1 which means that lamin B was significantly high in these cells, in these cells there was no change in circularity, but in these cells in MSCs, as well as in A459 cells which had much greater lamin A to B ratios you formed a significant drop in this nuclear shape ok.

So, you have so mechanically you would risk you would represent this network to a lamin B would, sorry lamin B would be the spring and lamin A would be this dashboard. So, the lamin B network when it deforms after deformation is removed, because it is an elastic network. So, this deformation is reversible why in the lamin A network which is a viscous damper, whatever you deform it won't regain you lose that. So, you have

irreversible deformation. So, you have two different deformations what they also observe that given that there was some heterogeneity in the population.

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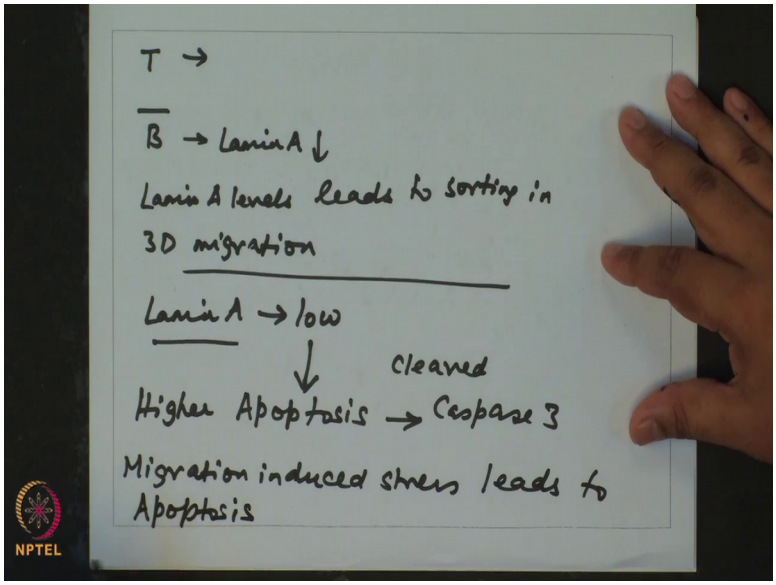

So, the ones at the bottom, so they checked; so you have the top cells and the bottom cells and then the check what is the circularity of the cells at the top and the bottom and they found, but the cells which were at the bottom also expressed lesser amount of lamin A ratio was less compared to those at the top ok.

So, even for the cells which migrate, so you have lamin A levels leads to sorting in 3d migration. So, you would see that there is a greater amount of lamin A in the cells which is remained at the top, were unable to migrate compared to the cells which did migrate to the bottom, but there is one cost of low lamin A less what they formed was in the cells which exhibited less amount of lamin A expression, when there was low lamin A expression, these cells also had a tendency to apoptosis after they migrated and this they did this as a by proving the caspase 3 cleaved caspase 3, the proportion of cells which had positive cleave caspase 3. So, suggesting that while lamin A is needed low lamin A enhances migration, but this migration induced stress, you have migration induced stress leads to apoptosis suggesting that the lamin A not only is needed for lower lamin A is needed for deformability, but lower lamin A makes the cells more susceptible to cell death.

So, lamin A has a protective role it protects the nuclear from damage and what they also observed ok.

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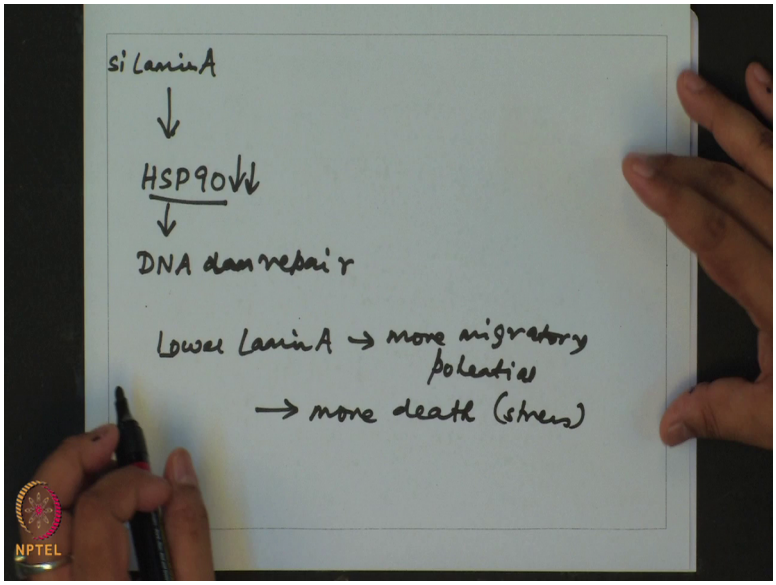

That when they did a deep knockdown of lamin A. So, when they did a deep knockdown of lamin A using si or lamin A what they found was in these cells, you have this HSP 90 heat shock protein is a protein which is associated with DNA damage or DNA repair expression of HSP 90 was significantly dropped. So, si lamin A lead to significant dropping HSP 90 cells. So, these cells can not only will have more amount of DNA damage, but they lose the ability to restive themselves ok.

So, this shows that you have a relationship that higher lower lamin A, leads to more migratory potential, but it also leads to more death due to stress. So, essentially you need to optimize the amount of lamin A, so that you are also deformable and you can also migrate and you don't die. So, consistent with these observations what has been found is in most of the cancer cells

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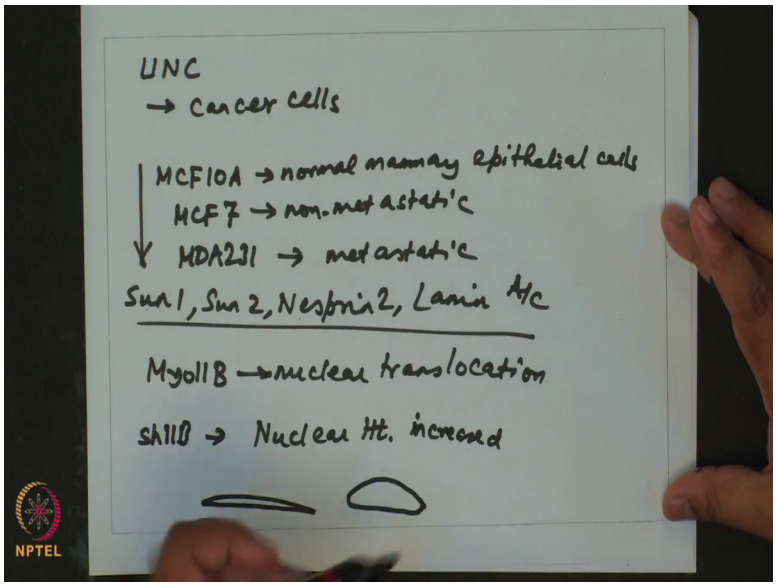

So, there was a study which compared the expression of these proteins. So, LINC proteins in cancer cells, how their expression is altered and what they found was say it compared MCF 10 A, MCF 7 and MDA MD 231 one and what they found us. So, this is normal memory epithelial cells, this is not non metastatic and this is highly metastatic and what they formed was as the cells became more and more metastatic the LINC protein expression was going down, and these included sun 1, sun 2, nesprin 2, and lamin AC. So, expression of all this proteins was going down.

Now, even though this expression might go down just nucleus softening may not be sufficient for having the cells to migrate, so you still required the given that the nucleus is. So, huge you require additional forces on the nucleus to deform. So, there is a study which recently demonstrated that myosin 2 B is required for nuclear translocation, and what they showed was when they knocked down, when they knocked down 2 b the average nuclear height increased. So, this was indirect hint that if as opposed to a nuclei been very thin if you knock down myosin 2 B, if the nucleus becomes big then it will be that much more difficult to squeeze through a small pore and then they subsequently showed, that there is they subsequently showed that there is accumulation off. So, if a cell is migrating

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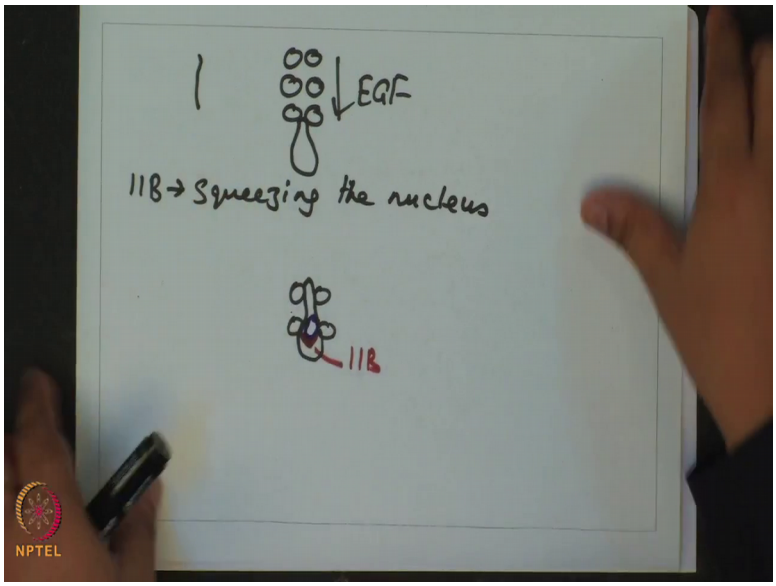

So, they used a variety of pillars, where the spacing was reduced and they track the cell migration against an EGF gradient, and then they showed that whenever the nucleus was trying to pass through these pores you had an accumulation of myosin 2 at the bear. So, you have 2 B at the rear, and what this is doing 2 B is actually squeezing the nucleus ok. So, even the nucleus softening helps you still require myosin generated forces to pull the nucleus forward ok.

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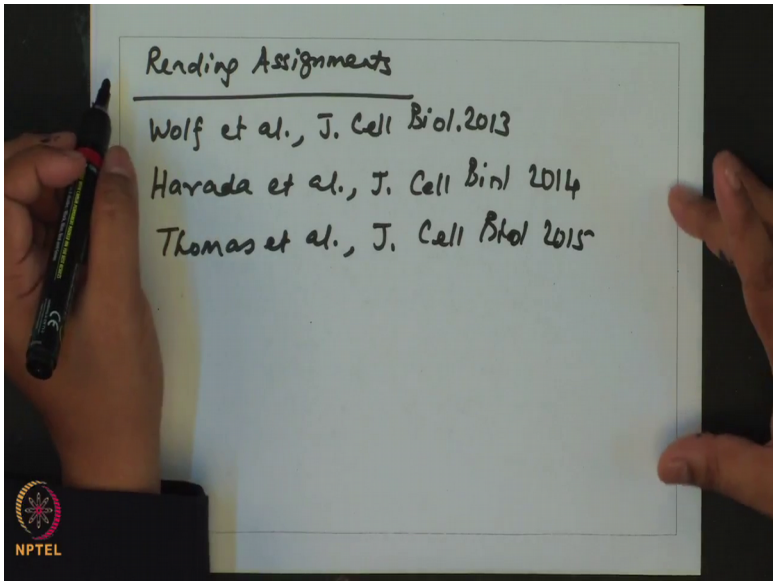

with that I end here today, and there be several reading assignments for this class, this was the physical limits paper which showed that the nuclei cannot be deformed beyond 10 percent, this was the paper which I discussed today just now, and this is a recent paper which showed that myosin 2 B is required for pulling the nucleus forward with that I stopped it today.

Thank you for your attention.