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

Week - 07
Lecture - 35
Mechanical Forces & DNA damage

Hello, and welcome to today's lecture of Introduction to Mechanobiology. So, the last few lectures have been discussing about nucleus what dictates the nuclear properties and how forces get transmitted to the nucleus.

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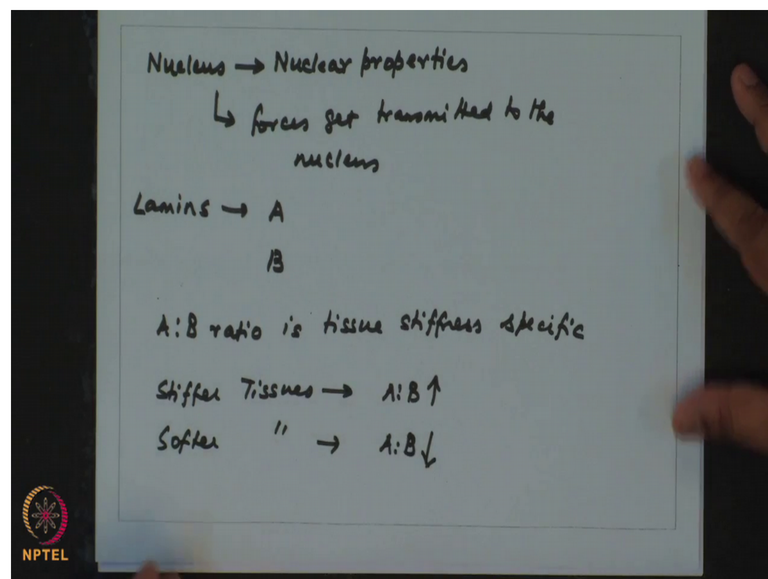

So, in this regard a specifically discussed about lamins, you have A type lamins and B type lamins and what has been observed in the pressure is that A to B ratio is tissue specific or tissue stiffness specific.

So, stiffer tissues have A to B ratio is high and softer tissues A to B ratio is low.

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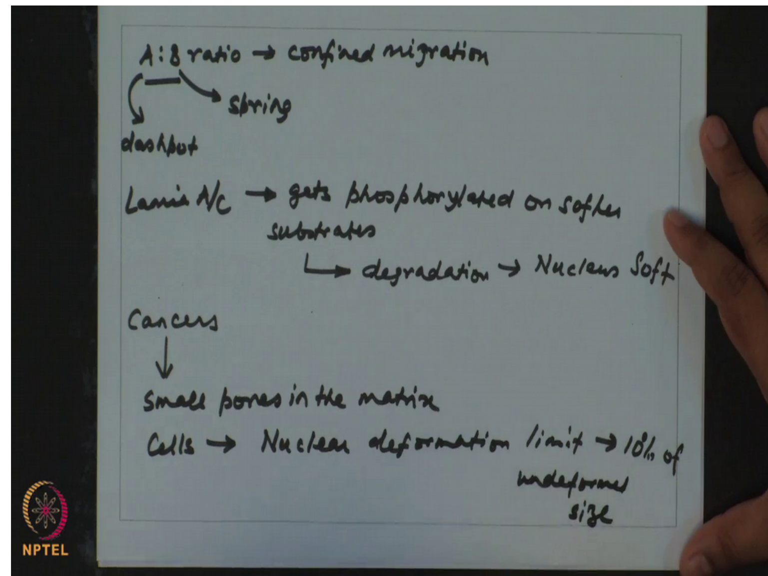

Now this A to B ratio: so I had also discussed that, what is the implication of A to B ratio on motility or confined migration.

So, in this what we observed also A to B ratio saw once again a operates as A dashpot B operates as the spring and lamin A. So, lamin A or lamin AC it is the splice variant gets phosphorylated on software substrates and this phosphorylation leads to degradation of the protein making the nucleus soft. So, in the context of cancers, what has been observed is that for cancers to invade you must have mechanisms by which the cell can pass through small pores in the matrix and cells cannot deform. So, cells we have the nuclear deformation limit which is 10 percent of undeformed size.

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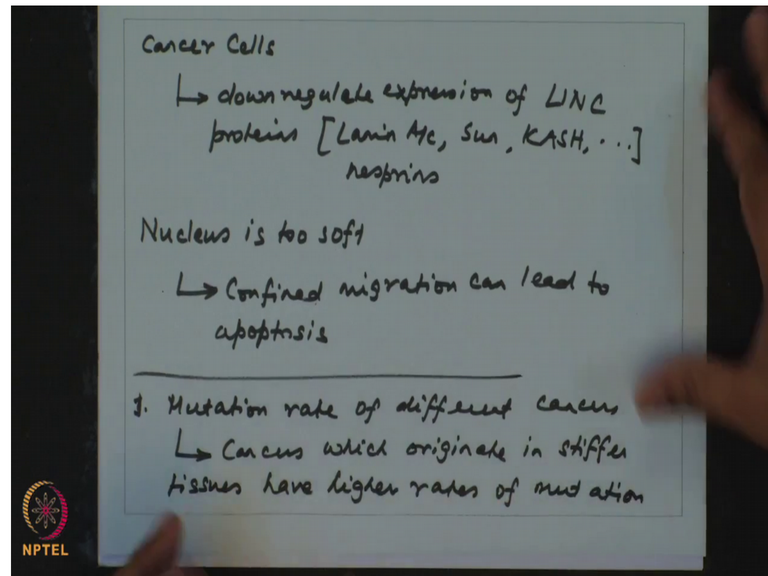

So, the question is how will then cancer cells migrate. So, what has been observed is this cancer cells. So, they down regulate expression of link proteins, including lamin AC, sun proteins cash proteins so on and so forth nesprings.

Ok, but we have also discussed this paper, but if the nucleus is too soft your confined migration can lead to apoptosis. So, there has been another finding that if you compare the mutation rate of different cancers, what has been found that cancers which originate in stiffer tissues like bout have higher rates of mutation.

So, how can we put the pieces of the puzzle together? So, you have mutation rate increases with stiffness.

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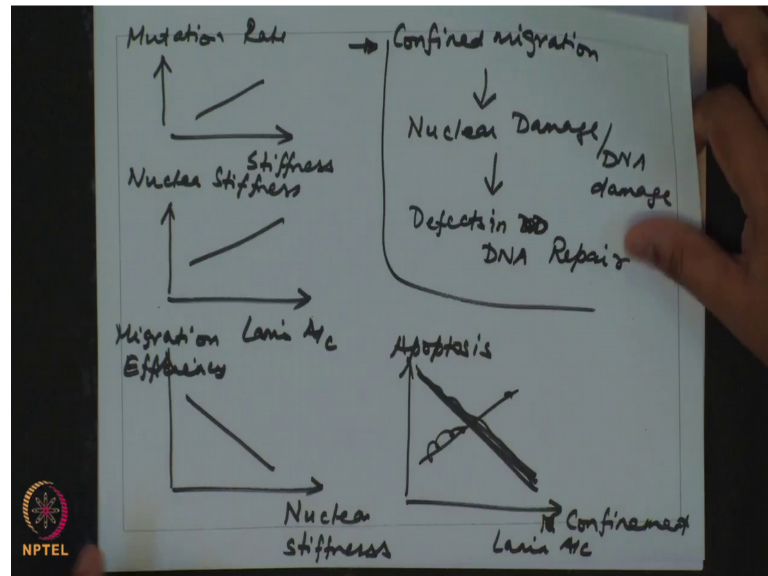

So, your lamin AC a nuclear stiffness also positively related and. So, if you have nuclear stiffness and migration efficiency you have a negative correlation.

So, if stiff if the nucleus is very stiff then this less efficient in migration and not just this connection you also have higher apoptosis, that increased nuclear stiffness increased amount of confined confinement or and when lamin AC levels are low sorry this is the other way round. So, no this is right only.

So, if you have low lamin AC levels you have higher amount of apoptosis. So, instead a link there, is it possible that when you have migration confined migration can this lead to nuclear damage and somehow this translate into defects nuclear damage and DNA damage and can it lead to defects in DNA repair ok.

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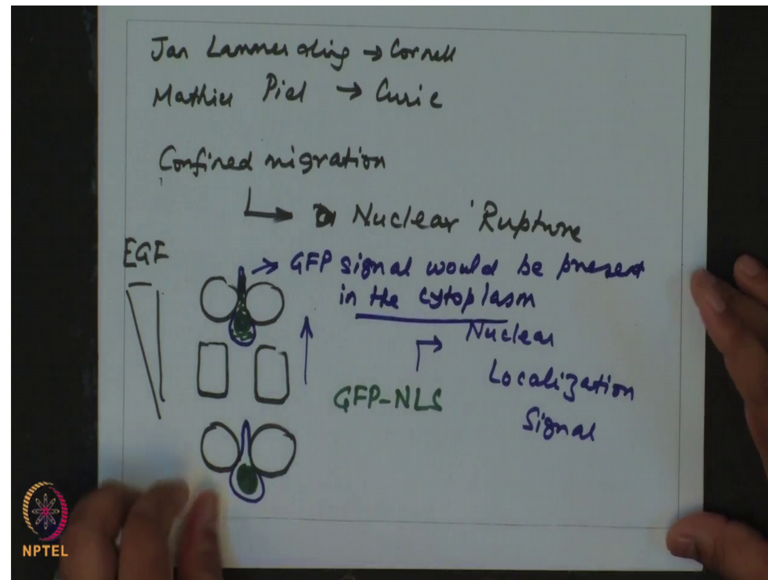

So, this is what I am going to talk about today. So, there are two groups the groups of Jan Lammer and Mathiew Piel at institute curie and he is at Cornell. What they showed that when you have confined migration you can have lot of nuclear rupture events how do they prove it. So, what they did. So, they created these patterns is micro fabricated stamps.

So, imagine you have an EGF gradient in this direction, you have these micro fabricated subscripts and you have a cell let me draw the cell in different way, which is trying to move in the direction of the Kim coin. Since EGF gradient is high these cells will overall try to go in that direction. So, what they did was they transfected these cells with GFP NLS. Now, nuc NLS is nuclear localization signal ok.

So, this should ideally only we presented in the nucleus, but what they found was during the migration process every once in a while they would have a situation somewhat like this, where you have the GFP signal would be present in the cytoplasm.

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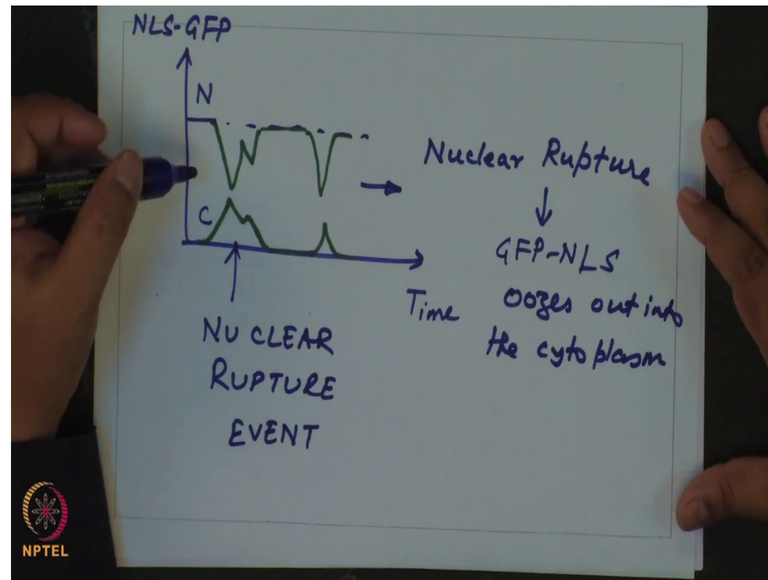

So, it is present in the cytoplasm. So, when the tracked is as a function of time the tracked NLS GFP fluorescence in the nucleus and in the cytoplasm.

So, ideally it should be. So, let us say this is the nuclear signal ideally this should be constant if the nuclear was never ruptured, but what they found was every once in a while they had a dip and then it returns to this value, again a dip and a return to the value. And every time the nuclear signal ended up dipping they found a corresponding increase in the cytoplasm signal which again fell back to 0, again an increase in the cytoplasm signal.

So, this is your nuclear signal and this is the cytoplasm signal suggesting that this every time there is a nuclear rupture. So, since GFP NLS is soluble. So, this oozes out into the cytoplasm. So, this event is a nuclear rupture event. So, this disproved that confine migration can indeed lead two nuclear rapture events, and while this particular geometry was micro fabricated, but they have done in multiple collagen cells.

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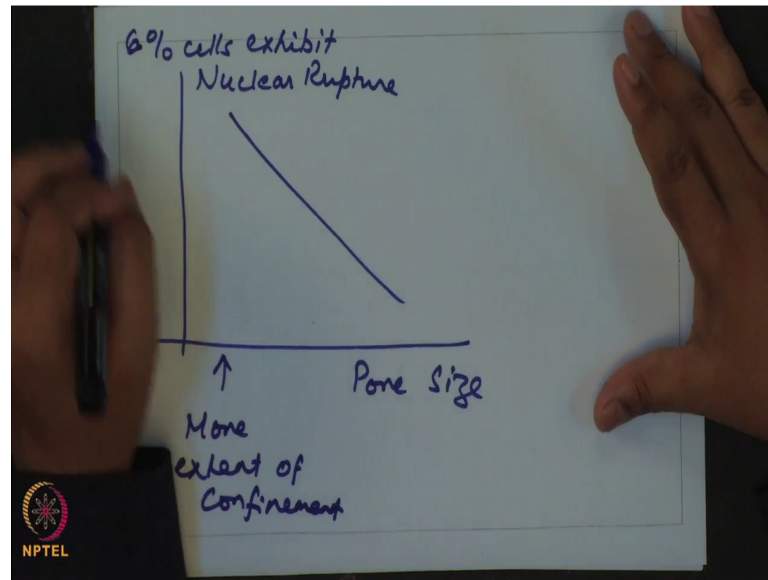

And what they found; if you FA if you find out the average pore size and you find the percentage of cells which exhibit nuclear rupture, they found a correlation ok.

So, increase in pore size there is negligible number of cells with exhibit this nuclear rupture, but if you reduce the pore size. So, this is more amount of confinement more extent of confinement modes of confinement, more amount of nuclear rupture so; however, this rupture does not continue for long ok.

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Cells restore Nuclear envelope integrity using components of the endosomal sorting complexes
[ESCRT-III)
→ Cell Migration incurs substantial physical stress on the NE, require efficient NE repair for cell survival.

So, eventually cells restore nuclear envelope integrity using components of the endosomal sorting complexes. So, you have this family of agents called ESCRT 3. So, we are required for transport. So, ESCRT mediates you know again refilling of the nuclear membrane. So, together what we study show that cell migration incurs substantial physical stress on the nuclear envelope and. So, if this is true then you will do require efficient nuclear envelope repair for cell survival ok.

So, this is the first part, in these shows that you can have nuclear damage and this would lead to cell that if the cell did not have a mechanism of surviving.

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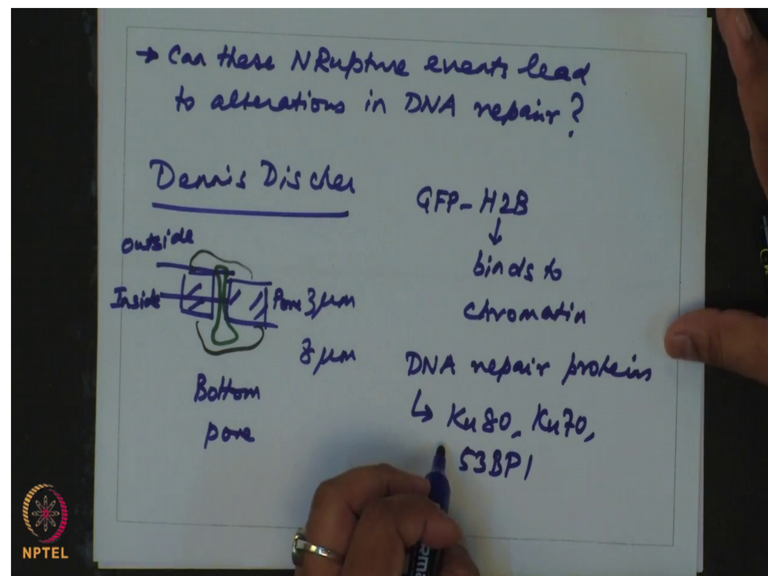

The next part of it is question is can; these nuclear rupture events lead to alterations in DNA repair. So, can it be that DNA repair is impacted negatively. So, the answer this question Dennis Discher at upenn .

So, he used this pore experiment again you have this pores 3 micron or 8 micron pores, from which you transition the cell you pass the cell through these pores and during this process. So, this is the nucleus which is fetched. So, they tracked at multiples points.

So, this is my pore outside the pore, just about inside, the pore and bottom of the pore. So, what they did was the transmitted cells with a multihued of proteins. So, they had GFP H this binds to chromatin. So, cells were transfected with this, but importantly other

DNA repair proteins including Ku 8, Ku 753 bp 1. So, these are all DNA repair proteins. So, what is found, ok?

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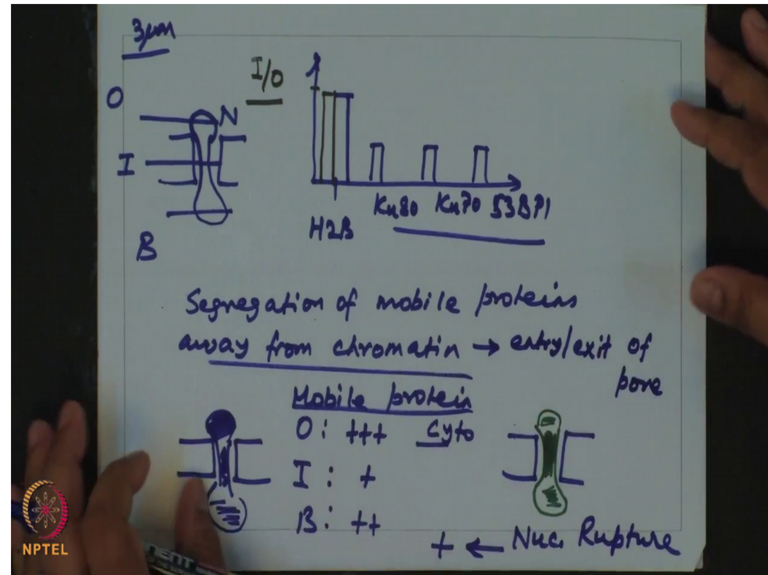

So, again if I were to just draw the nucleus, you see this is my nucleus they were tracking outside inside and bottom intensity of these proteins.

So, what they found if our histode 2B. So, in the three micron pore. So, they could also track the DNA right DNA and the protein. So, let us say the DNA is giving a way a given ratio intensity of inside to outside. So, you are calculating the inside to outside ratio. If this is the profile of the DNA which to be give the exact same profile your pore size is 3 microns. But when you track it when you track it for let see the DNA rate Ku 80 Ku 70 and 53 BP1 for these proteins this is lot less. So, suggesting that there is a segregation of mobile proteins away from chromatin. So, you there is a segregation. So, if I were to draw the mobile protein fraction, outside inside and bottom. In terms of intensity what they found if the outside is plus plus plus the inside is plus and the bottom is plus plus. So, you have in the nucleus you have a lot here, miscue will here, and somewhat more here ok.

So, versus if you do the same thing for the DNA, the DNA exhibited the reversing DNA exhibits maximum intensity inside the pore and every once in a while like the previous study which showed that there is ruptured if you track to the concentration in the cytoplasm, in the bottom there was some signal in the cytoplasm also suggesting. So, this

was the nuclear rupture as demonstrated with the other study. So, this suggests. So, there is a gradation of mobile proteins away from protein and this segregation happens at entry and exit of pore at entry to pore and exit of pore. So, they then did micropipette aspiration ok.

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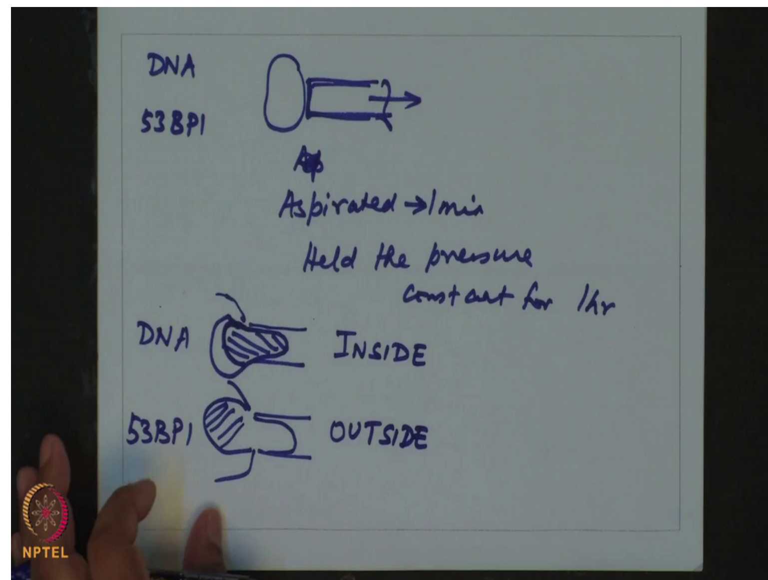

Once again they had, the DNA labelled and 53 BP1 they labelled and what they did was if you have the nucleus they put the micropipette and they aspirated the nuclear. And after aspiration the aspirate So, they aspirate aspirated for one minute, but then they held the pressure constant for one hour, and what they found was after one hour if this was the nucleus outline .

So, most of the DNA was inside, but most of the 53BP 1 was outside. So, DNA was inside the micropipette, but 53 BP 1 was outside. So, thus this again proves that there is segregation. So, they then did these routinely using pipettes of variant diameters.

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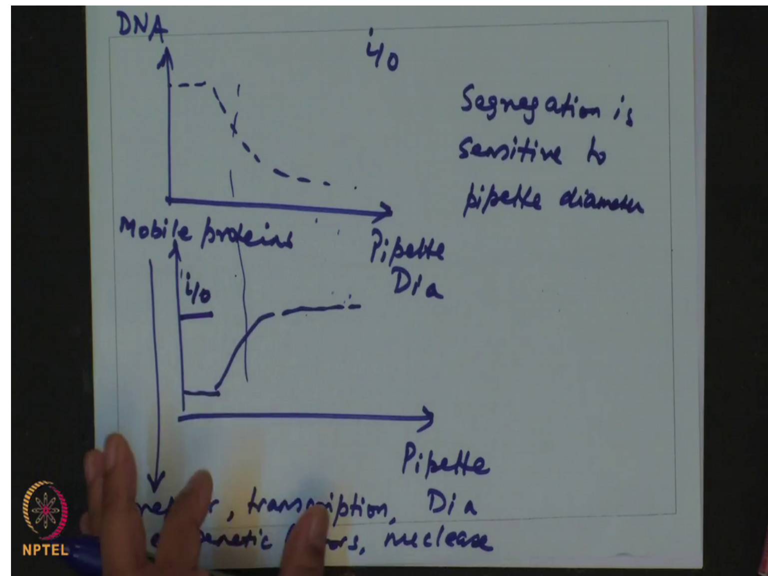

And what they found. So, our x axis is the pipette diameter and then what they are tracking here. So, what they tracked here was the ratio inside to the ratio outside. So, they are tracking the ratio I by o. So, they are tracking the ratio i by o. So, what they found was with increase in diameter. So, this is the DNA intensity ratio and this is your other all the mobile proteins, they exert completely opposite effects ok.

So, when they consider the mobile proteins they had repair proteins repair factors, transcription associated proteins transcription factors, and epigenetic factors as well as nucleus. For all this they find that there is segregation and this segregation is sensitive to pipette diameter n segregation is sensitive to pipette diameter. In this zone when you are drawing reducing the pipette diameter then you see that the mobile intensity int inside to outside drops significantly. So, this is a i by o ratio. So, this suggests that this segregation can be a cause of DNA damage long term. So, to answer this question what they did say they went one step further and what they did was they tracked.

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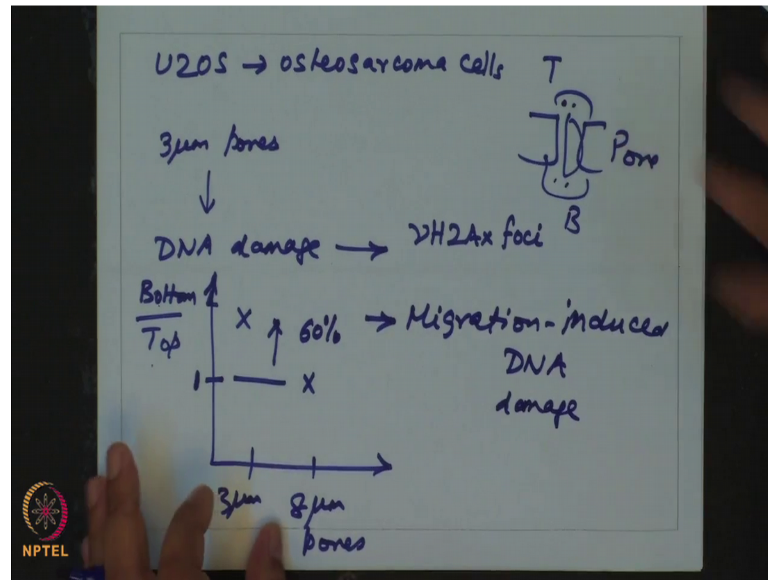

So, they took u 2 0 s. So, these are osteosarcoma cells they passed them through the3 micron pores and then they tracked what is the extent of DNA damage.

So, what they found. So, they track the extent of DNA damage by the number of gamma H2 foci what they found was in 3 micron pores whatever with the gamma is with they had a solid ratio of gamma H2 ax foci and what they did was the tract that they found the ratio at the top versus and over the bottom. So, they tracked bottom versus top. So, you have this is the pore the cell is passing through it. So, this is your top this is your bottom and then they take the number of gamma H2 ax foci in top cells which are at the top and cells which are at the bottom and they find that.

This ratio in a 8 micron pores it should be one right because in eight micron pores the nuclear does not need to be squeezed, but in three micron pores there is a nearly 60 percent increased. So, this is completely migration induced DNA damage. So, and this they also observed in other cells like mesenchymal stem cells, what they then demonstrated that the DNA repair, they did experiments with DNA repair factors and then they showed that repair factors mislocalise with DNA damage levels ok.

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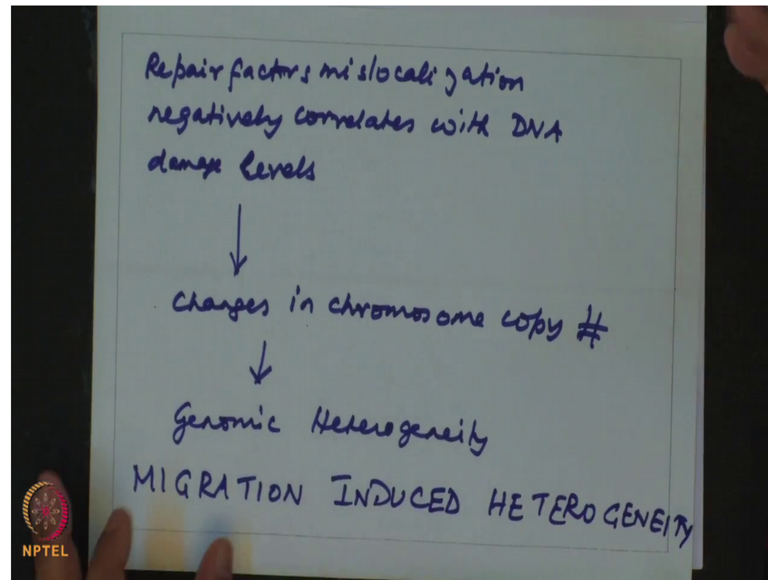

So, more amount of mislocalization then you would find that the number foci I will increase, and these changes led to changes in chromosome copy number and eventually led to genomic heterogeneity. So, this is a clear demonstration that physical damage to the nucleus can induce heterogeneity. So, this is an example of migration induced heterogeneity. So, you know for example, in most cancer cells there are main multiple subpopulations which are present. So, these cells are innately heterogeneous. It is possible that physical factors like pressure, can induce compressive stresses can induce heterogeneity through nuclear damage.

And, so this I would wrap up by saying that in the nucleus.

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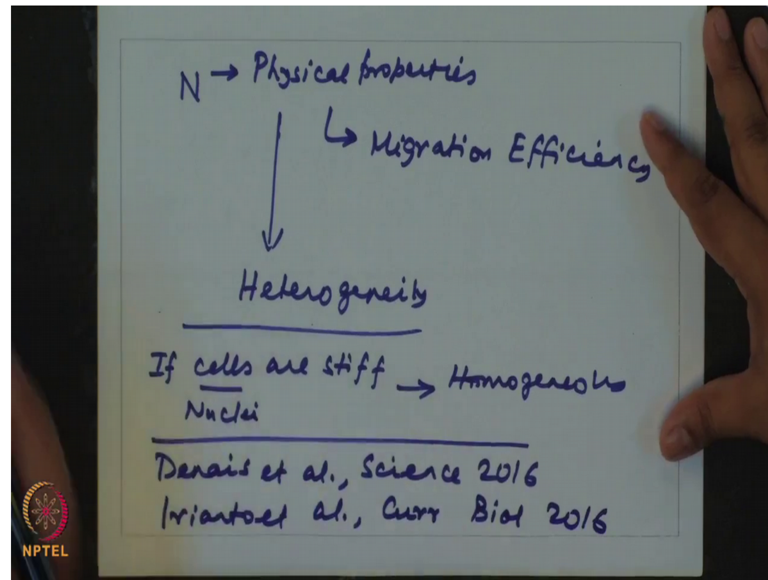

You have its physical properties, which dictate the migration efficiency and also indirectly if they are implicated in heterogeneity. So, a stiffer nuclei if cells are stiff cells a nuclei are stiff, then this population should be more homogeneous, let us likely to migrate and will be more homogeneous, because this kind of defects will be less likely.

With that I stop here as reading assignment, I suggest you to read the following to papers 16, ok.

Thank you for your attention.