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

Week - 06
Lecture - 28
Mechanobiology of Diseases: Cancer II

Hello and welcome to today's lecture of Introduction to Mechanobiology.

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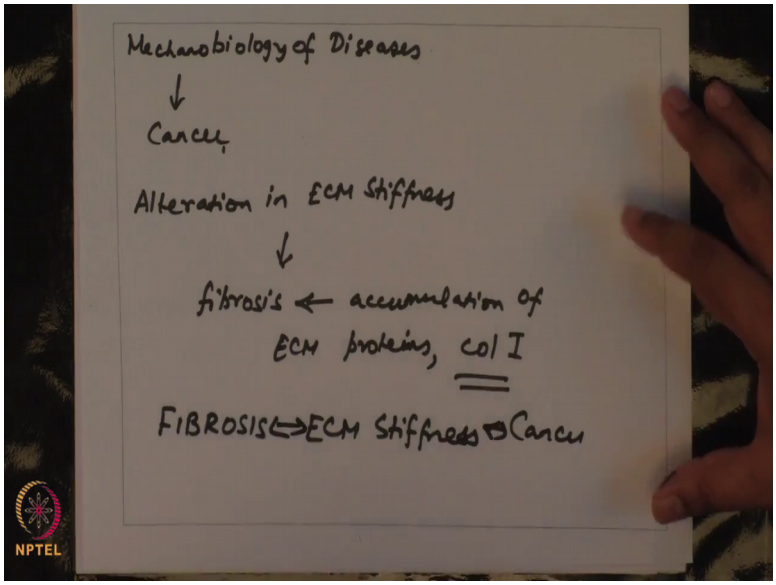

So, in the last class, I had started discussing mechanobiology of diseases so started discussing mechanobiology of diseases and under this started discussing about cancer. So, one of the things which I said in terms of mechanobiology is the alteration in ECM properties namely stiffness. So, this is driven in a process called fibrosis triggered by accumulation of ECM proteins and specially collagen 1. So, these fibrosis happens not only in case of cancer, but even in cardiovascular diseases. So, the question I framed was what is the relationship between fibrosis, ECM stiffness and cancer, what is the nature of the relationship.

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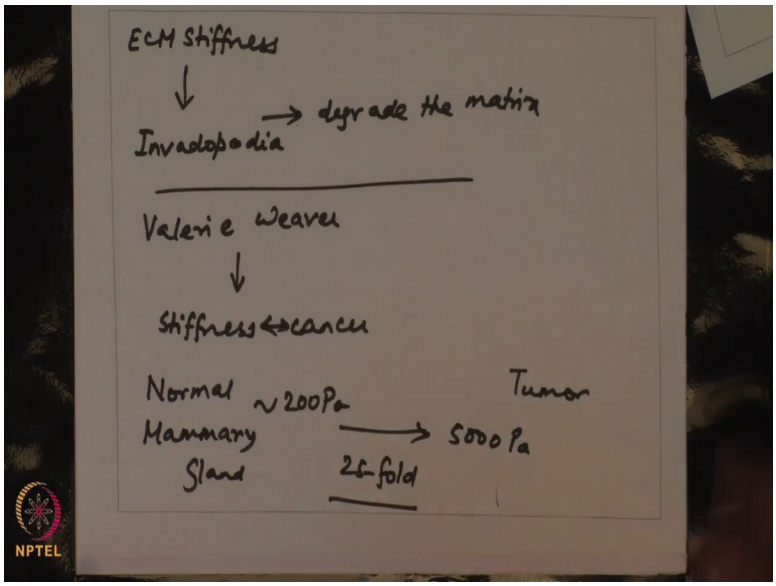

And I began discussing this paper by Alissa Weaver, who showed that ECM stiffness is correlated with invadopodia activity. So, these invadopodia are those invasive structures which locally degrade the matrix and then we started discussing after invadopodia using gels work by Valerie Weaver about what is the effect of stiffness on cancer invasion relationship between stiffness and cancer. So, what she showed was from normal mammary gland in case of normal mammary gland which is ordered 200 pascals in stiffness; in case of tumor this 200 pascal increases to nearly 5000 pascals. So, you have a 25 fold increase in stiffness. And to ask the question that what is the effect of these altered stiffness on the organization of mammary epithelial cells.

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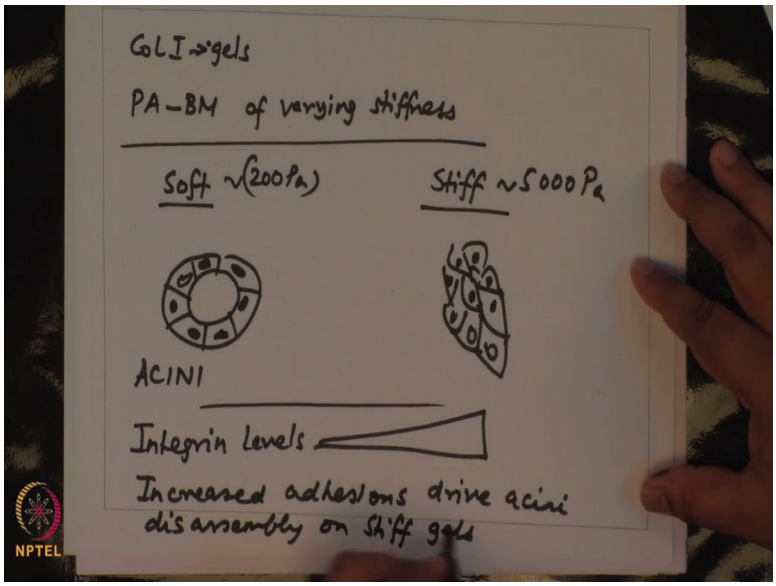

So, she used two different systems collagen 1 gels and polyacrylamide gels which are functionalized with basement membrane of varying stiffness. On both these substrates, what she found was on substrates which were soft order 200 pascals mimicking the native stiffness of normal mammary gland. The cells epithelial cells form these acini structures, they form this acini structures, but in case of stiff surfaces order 5,000 pascals what she found was you have these aggregate of cells which exhibit a very invasive phenotype, these are also to the acini structure falls. And what she showed was this if when she quantified the integrins levels this was increased as we increase the stiffness. So, this suggests that increased adhesion drive acini disassembly on stiff gels.

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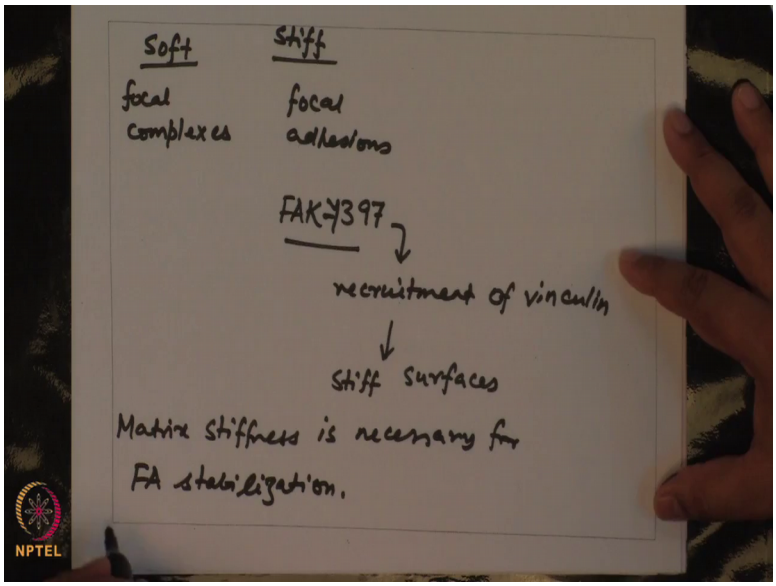

So, what she then showed, so in comparison to soft and stiff surfaces. On soft surfaces the cells the mammary epithelial cells from focal complexes. So, if you recall from earlier discussions focal complexes where small dot like structures. While on stiff surfaces, they found focal adhesions and vinculin or phosphorylation of focal adhesion kinase at 397, tyrosine phosphorylation at of focal adhesion kinase at 397, which is known to lead to recruitment of vinculin was only observed on stiff surfaces. So, this suggests that matrix stiffness is necessary for focal adhesion stabilization.

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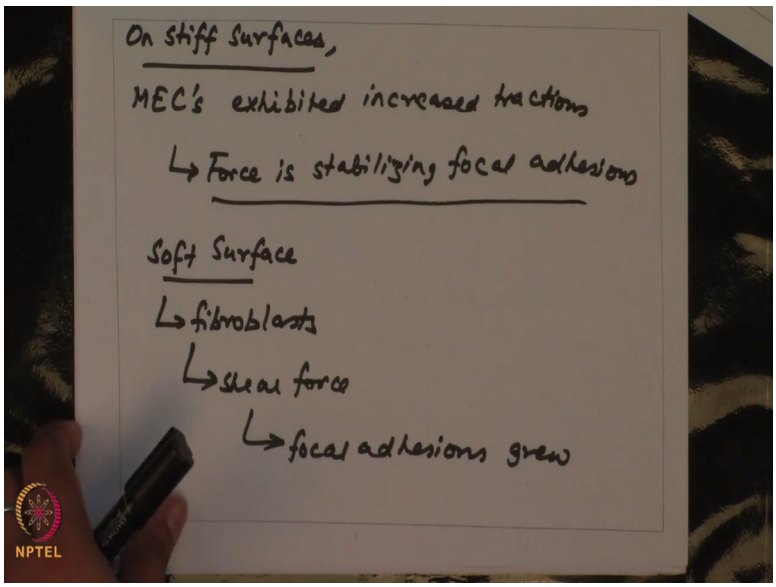

What they also found was on stiff surfaces these MEC's - memory epithelial cells exhibited increased tractions. So, all these kind of suggests that force actomyosin contractile force is stabilizing the focal adhesions. So, they did some other experiments in which on a soft surface, they took fibroblasts and exerted shear force and showed that on soft surface also focal adhesion grew; whenever you exert force, there was a growth of focal adhesion even on a soft surface. So, all of this is that again what is clear is force promotes focal adhesion assembly and leads to integrin aggregation. So, increased contractility is generally driven by rho rock signaling.

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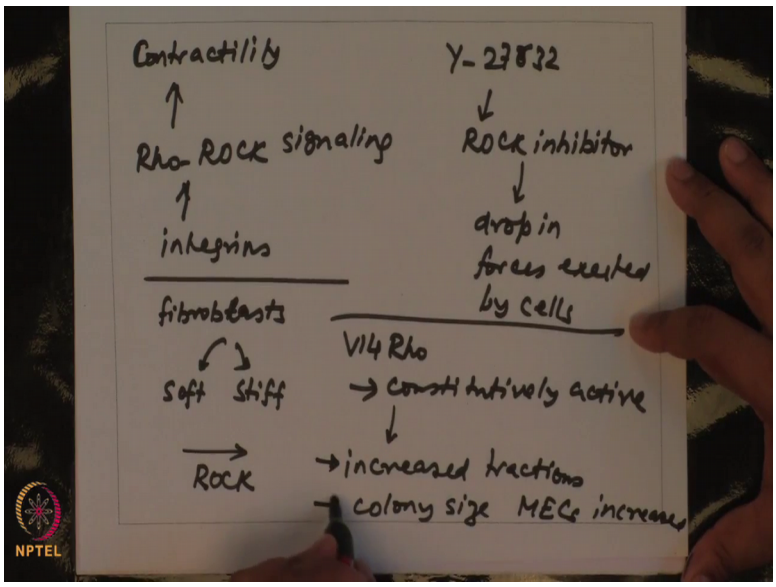

So, you have increased contractility. So, higher traction means higher contractility and this is driven by the rho rock signaling as axis. So, rho rock signaling is actually activated by integrins. So, integrins drive the contractility also. To see how rho rock signaling was perturbed between soft and stiff surfaces. So, what they did was they took fibroblasts and showed that on stiffer surfaces. So, you have increase in rock the self exerted more force. So, higher force if the self treated was if the cells were treated these y 27632 this is a rock inhibitor, this led to drop in forces exerted by cells. So, they also did this experiment where they transmitted cells with v 14 rho, so it is a constitutively active construct. So, rho is always activated. And they showed that in the presence of this, there was an increase this led to increased tractions increased adhesion. So, your colony size of the memory epithelial cells also increased, both these increased and all of these could be inhibited.

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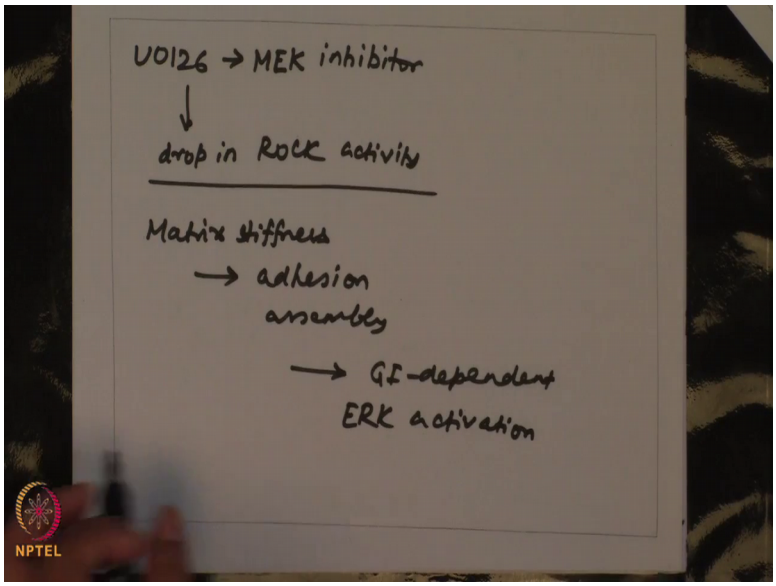

So, when they treated with this drug called U0126 this is a MEK inhibitor U206 led to drop, drop in rock activity. So, rock levels decreased. So, these suggest that you have matrix compliance or stiffness leads to adhesion assembly, and this leads to growth factor dependent, ERK activation.

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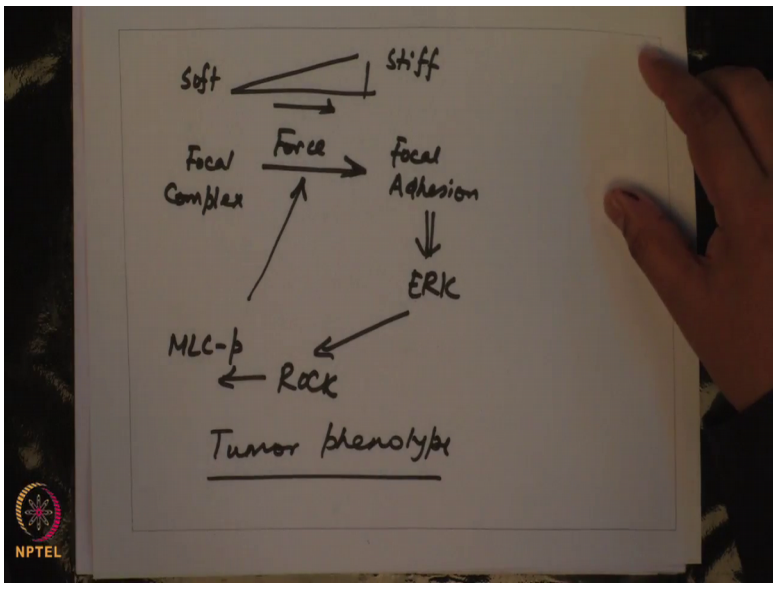

So, to summarize, so what they found was with increase in stiffness, if you have soft and stiff surfaces you have increase in stiffness in this direction. So, on soft surface, you have focal complexes; on stiff surface, you have focal adhesion. So, force converts the focal

complexes to the focal adhesions these focal adhesions drive ERK signaling this activates rock and this rock activates myosin light chain phosphorylation which again feeds back to strengthening focal adhesions. So, this entire cycle is essentially leading to this a tumor phenotype, it drives a tumor phenotype. So, this was the first demonstration in vitro that increase in stiffness can induce cancer invasion. The authors did not stop here; they extended it to in view and ask that can they observed similar observations in vivo using a mouse model.

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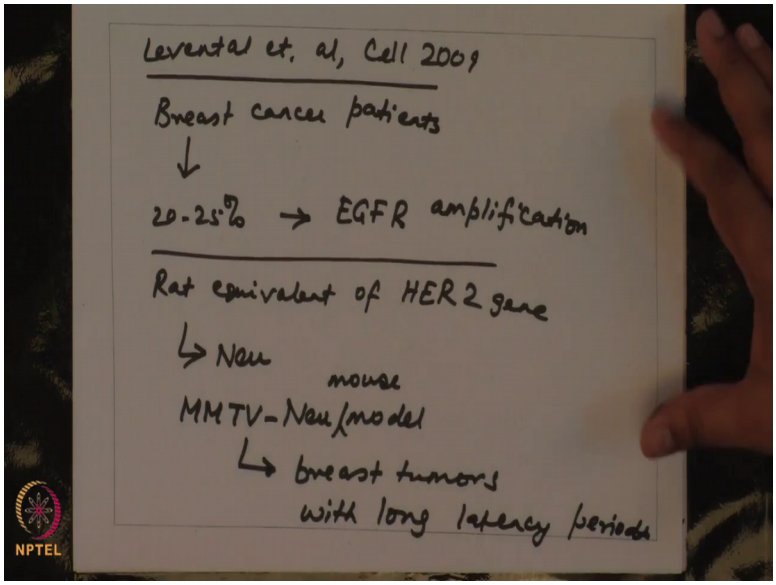

So, this is the paper that I am going to talk about, this was published in cell in 2009. So, what they did was, so in generally in breast cancer patients in 20 to 25 percent of these cases you have EGFR or epidermal growth factor factor receptor is amplified amplification. So, what they did was they took the rat equivalent of it of HER 2 gene which is amplified in humans this is called NEU, and they used a model using the MMTV promoter.

So, in this mouse model, you generate breast tumors, this is a mouse model with long latency periods. So, you have this mouse model in which you can induce expression of NEU which is a rat equivalent of HER 2 and track how tumors progress a form and progress. So, what they did first demonstrate. So, just like in vitro they used polyacrylamide gels or collagen gels to vary the stiffness, they used rheology and compression measurements.

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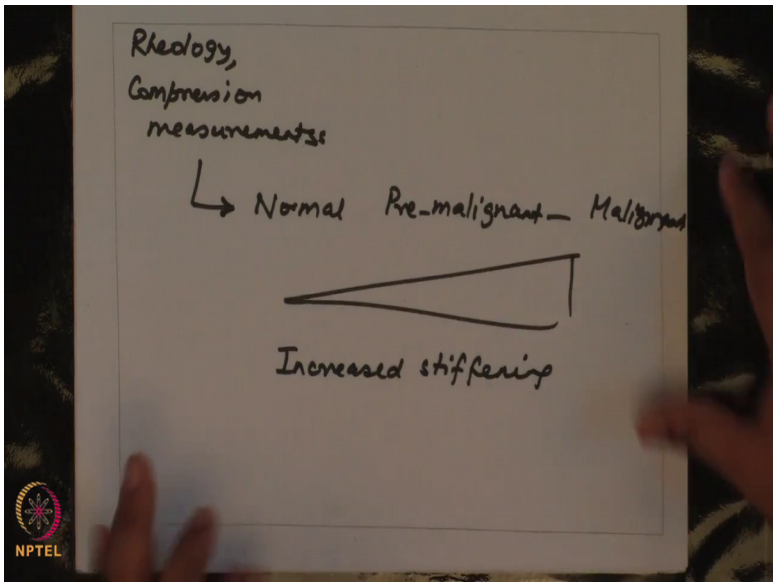

If they use rheology and compression measurements, so these demonstrated that as tumor progresses. So, normal mammary gland, so you have normal pre-malignant and malignant individual stages. So, you saw that she demonstrated these results demonstrated increase in stiffening. So, there is increase stiffening.

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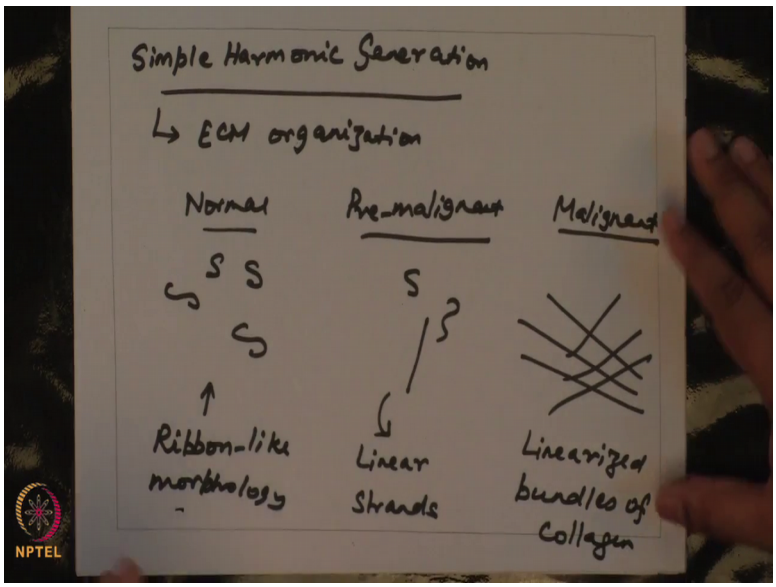

And they went further. So, what they did was they looked the use of imaging my modality called simple harmonic generation. So, this allows to look at ECM organization. So, what they found was in case of normal the image to the collagen fibrils.

So, what you found was this collagen fibrils exhibit ribbon like morphology; in pre-malignant, you had these ribbons, but you also had these linear strands; and in malignant, you had this cross linked very linearized bundles, so you had linearized bundles.

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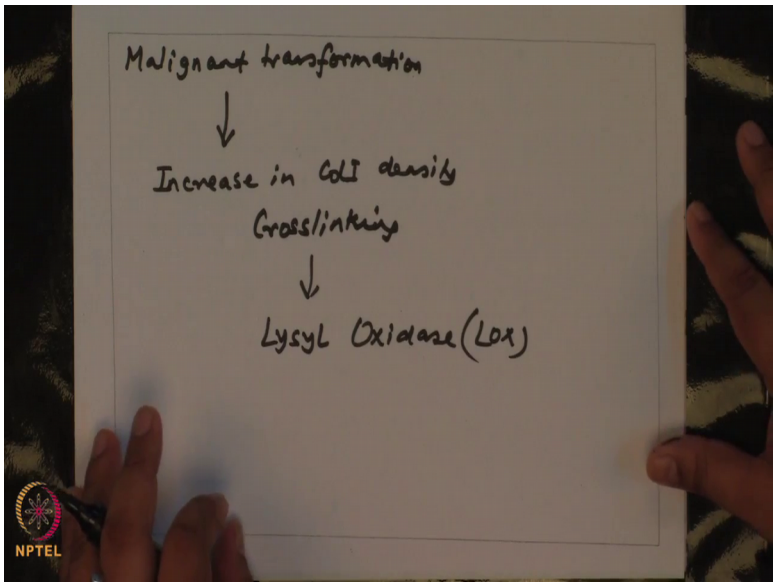

So, and what they showed, so just you have this malignant transformation, it was associated with increase in col one collagen one density and its cross-linking. This cross-linking was mediated by this enzyme col lysyl oxidase or in short lox.

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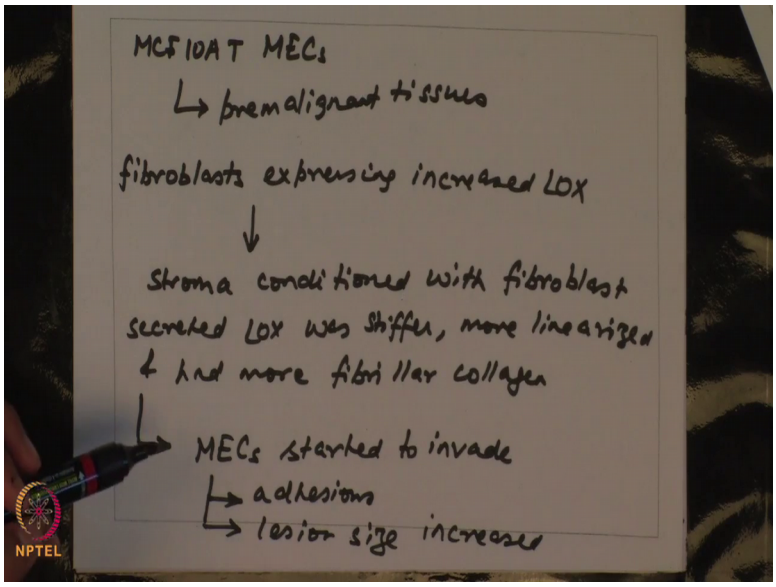

So, in this model, when they injected MCF 10AT mouse embryonic sorry mammary epithelial cells, what they find is these guys form pre malignant tissues. So, to check what is the effect of this increased cross-linking what they did was they did an experiment in which they took fibroblasts which were over expressing, these were expressing increased LOX. So, the idea was the fibroblasts in the stroma would secrete lox which will cross link the collagen and make it stiffer. So, they confirmed rheologically that these wines, so your stroma conditioned with LOX was stiffer, more linearised and had more fibrillar collagen. So, this change in the stroma properties what they found was you have MEC's started to invade. And this invasion was associated with increased in adhesions and overall the lesion size was in also increased. So, this once again demonstrates that if you make the matrix stiffer then these mammary organized start to invade.

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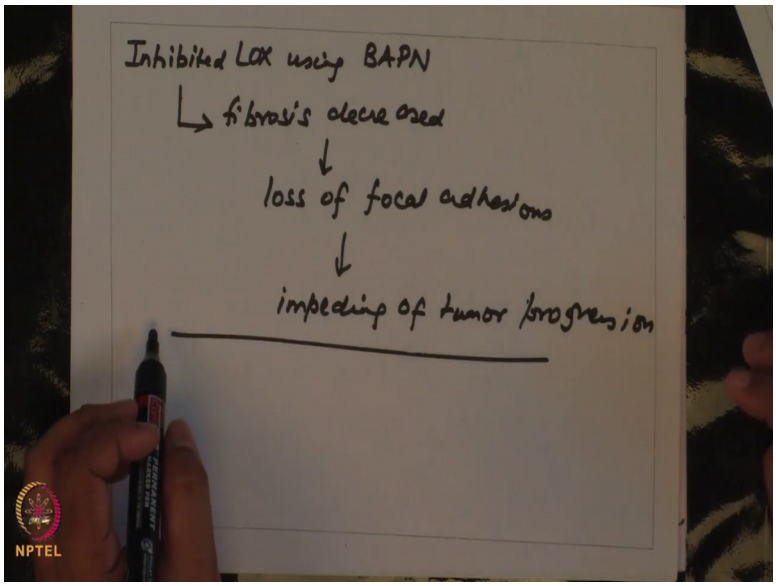

They did the reverse experiment also. So, what they did was, so the inhibited LOX using a drug called BAPN. And then they again showed that this when the inhibited the LOX the fibrosis decreased leading to loss of focal adhesions and impeding or delaying of tumor progression. So, both these experiments kind of demonstrate that if you promote LOX if you increase LOX levels clicks to an invasive phenotype you can reverse it by inhibition of lox activity. So, then they did an experiment that whether the mammary epithelial cells themselves can respond without the need for stromal cells, can they themselves respond or invade in response to increase in stiffness.

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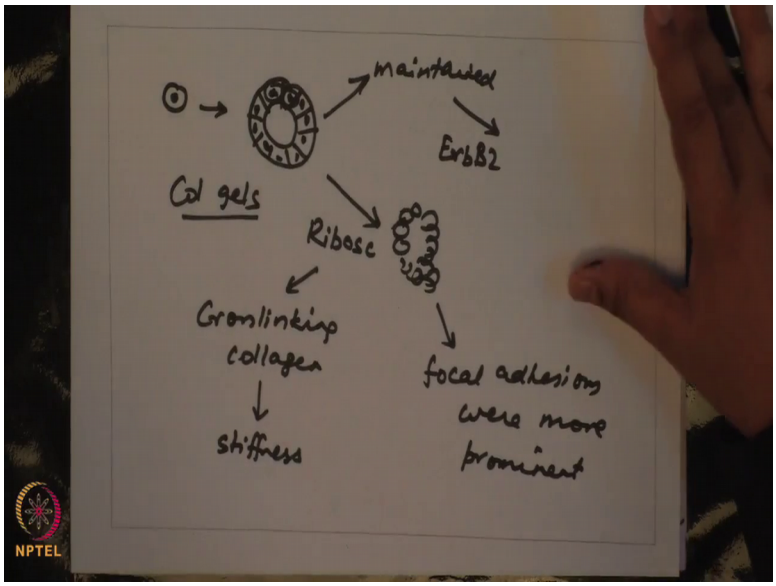

So, what the way this set of the experiment was ok, so they injected the mammary epithelial cells let them form organoids you have this acini structure form. So, this was done using collagen gels, you have the acini structure form. And then there are two experiments in one case they did not do anything, the other case they added ribose for cross-linking the collagen. And low and behold again in this case they began to see this structure fall apart, their organized acini structure was started to fall apart. Either with ribose in this case this was maintained, but if they added ErbB2 activation again the structure started to fall apart. And in all these cases again when they added the ribose, so cross-linking collagen means increase in stiffness. And in these cases focal adhesions were more prominent.

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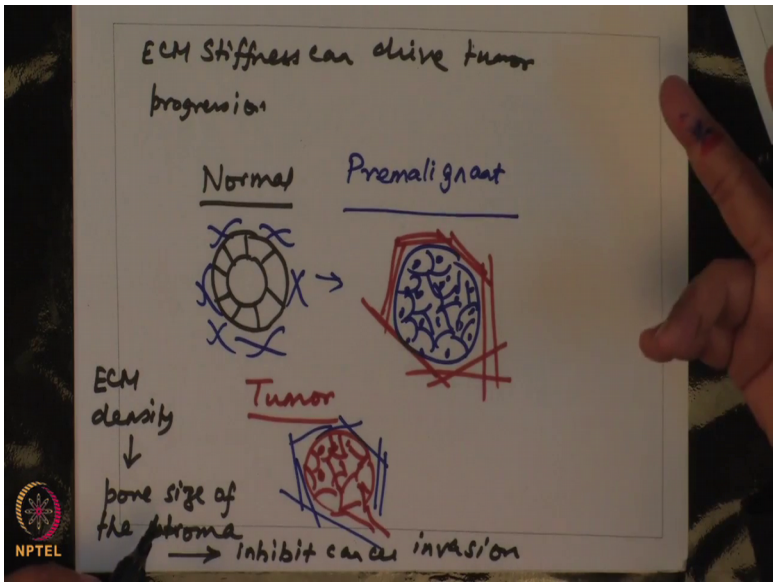

So, taken together what these results suggest is that you can have an effect in which ECM stiffness alone can drive tumor progression. So, in case of normal, you have well defined acini structures surrounded by this ECM which is relaxed. When you go to pre malignant, so what you have, you have these cells fill up this entire space. And in these cases, you begin to see bundles. So, these are your ECM fibers. And in case of tumor, this basement membrane gets breached and you have the entire cells, they start to invade, they breach the basement membrane and they start to invade; so one kind of counterintuitive this that you have increased in ECM stiffness.

So, increase in ECM stiffness or increase in ECM density, this should actually reduce the pore size of the matrix on the stroma. So, if you have increased density then this should actually prevent cells from invading right, your pore size has decreased, they should inhibit cancer invasion this should, but what do you observe is the cells we can more and more invasive how is it possible.

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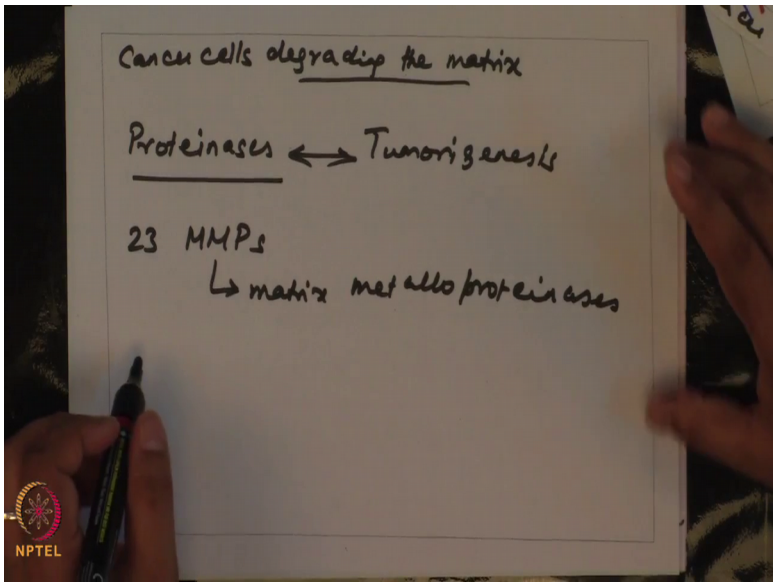

So, the answer lies in cancer cells degrading the matrix. So, not only the stiffness stimulate cells to start to proliferate and start to integrate, but the invasion must be associated with as mechanism which allows cells to secrete proteases to degrade the matrix. So, the degrading these matrixes, some of the most, so you have these degrading matrices are mediated by class of proteins called proteinases. So, this has been linked to tumorigenesis. And there are 23 MMPs in identified in human. So, one of thus classes are called matrix metallo proteinases. So, MMPs stands for matrix metallo proteinases or MMPs in short.

So, I will stop here for today. In the next class, we will discuss how MMP expression is regulated by ECM stiffness. With that I stop here.

Thank you for your attention.