Drug Delivery Principles and Engineering
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Lecture – 57
Cancer Vaccines

Hello everyone. Welcome to another lecture of Drug Delivery Engineering and Principles. We are going to continue our discussion that we are having on this particular module which is on vaccines.

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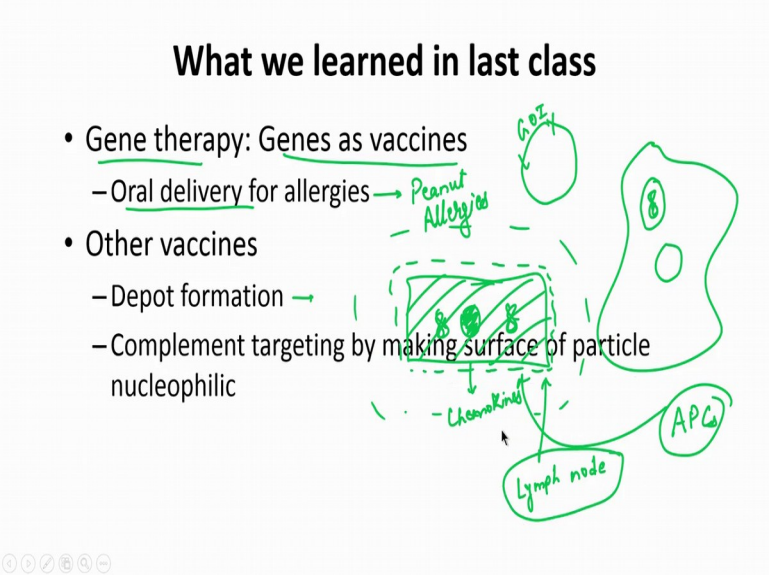

So, just a quick recap of what we learnt in the last class. So, in the last class, we were looking at gene therapy and using genes as vaccines; so, delivering the whole plasmid containing your gene of interest into the cells itself. So, what will happen is because now that you have delivered them, let us say, to a cell, these cells will produce the protein in the cytoplasm and then it will signal as something that is being produced by a viral or a cytoplasmic - more a class 1 response.

So, that was the whole idea there we learned few things in the last class in regards to that. One was how we can use this for oral deliveries to take care of some of the food allergies. In this case, we looked at peanut allergy, then we also talked about other vaccines. In the other vaccines, we had several different formulations one was depot

formation. So, what we were doing is basically injecting something or implanting something which act as a depot.

So, the antigens are here - rather than letting the antigen flow around and find the immune cell, this particular one was asking the immune cells to come to its site only. So, and the way this was doing it, it was releasing chemokines and because chemokines are being released here right at this point, the highest concentration of chemokine is there. And as it moves further away the concentration of chemokine was decreasing and this gradient caused all these APCs to move in and survey the site. And once they come in, there are also antigens that are loaded. This could be either in the same gel, the same as a chemokines are loaded or they could be particles encapsulating your antigens and then these APCs are going to come and take up your antigens which will then cause then enhancement or signal as well as whatever response they need to activate.

So, it is acting as kind of a artificial lymph node and obviously, there are several papers out there which actually do this lymph node mimicking and do a much much more comprehensive sort of setup to get complete mimicking of that. But this is a preliminary paper showing that you can get that and basically ask the APCs to come to the site, get activated, and generate the immune response rather than actually letting the antigen find the APCs. And then one other different type of vaccine we will talked about was using particles and complement system activation.

So, in this particular example we saw that how the authors first of all were using size to target lymphatics.

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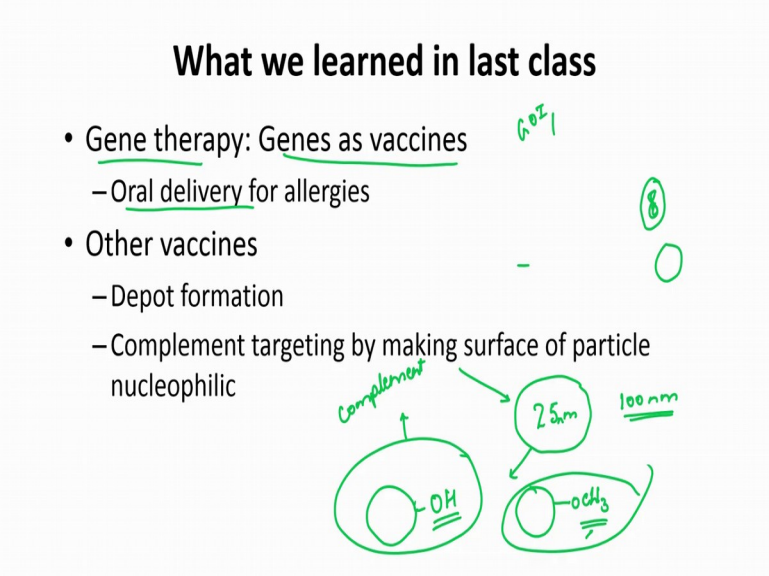

So, what they did, is they used 25 nanometer and 100 nanometer particles and they of course, found the 25 nanometer is easier for diffusion in the lymphatic just because of the size it can go into the lymphatics much easily than the 100 nanometer. And then what they did is of this 25 nanometer, they prepared these particles from two different conjugation chemistries; one that resulted in a hydroxyl present on the surface and one that resulted in methoxy group present on the surface. And obviously, this is more nucleophilic then the methoxy group which is fairly inert and what they found is this did a much much better job in up regulating the complement system.

So, the complement system started to get activated and that act as a innate immune response. So, the secondary response cause a lot more activation of the APCs and much better vaccines, than the one that only use the methyl group. So, this is what we had learnt- there are several other examples it is I mean, I can probably make a whole course out of just these examples of various types of vaccines which are different from the conventional vaccines as I have given you some flavor of it.

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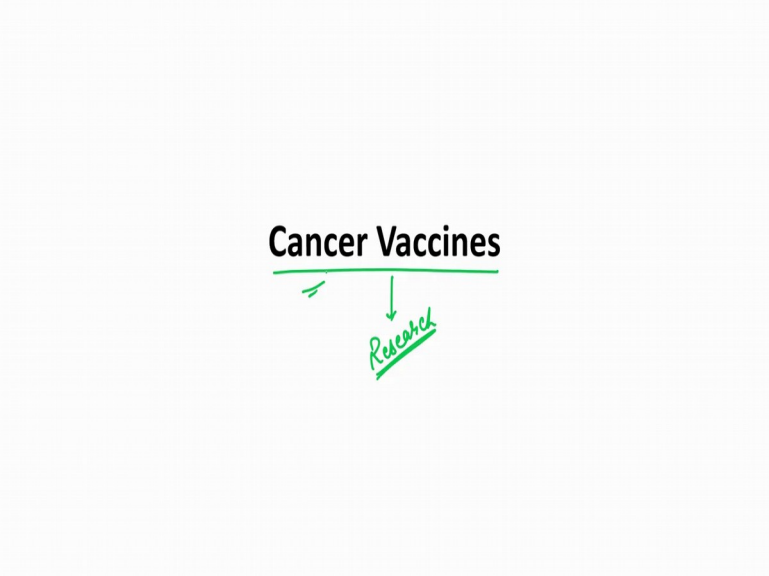

Now, we are going to talk about another type of vaccine that is particular for a disease and these are cancer vaccines. And cancer vaccines are fairly widely used in research phase; not so much in the clinical phase although there are some examples and then we will talk about that. But there is a lot and lot of enthusiasm as well as hope that these cancer vaccines in the research phase will succeed and ultimately translate to the clinics because as we all know cancer is a big challenge that faces humanity at this point.

Pretty much all of us probably knows somebody or the other who is either suffering from cancer or who has died because of cancer and the way our society is going the cancer incident rate are increasing every year. And so, it is very important that we are able to tackle this problem as quickly as possible.

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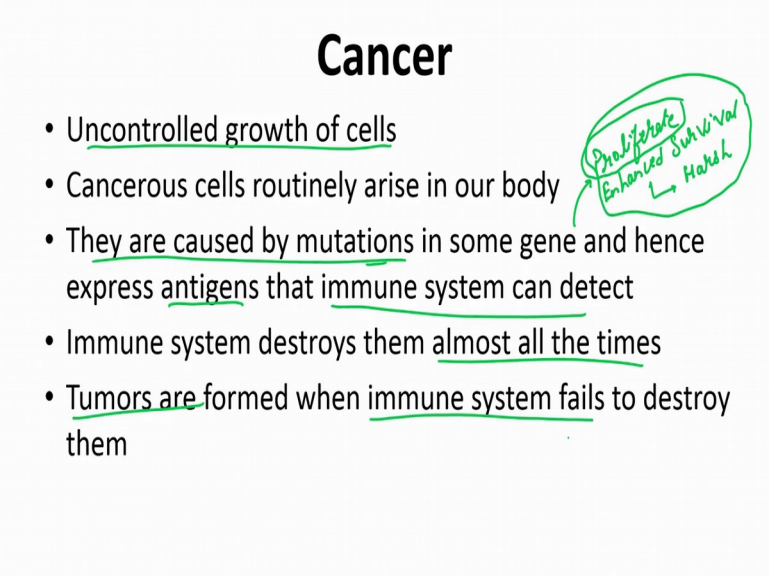

So, first of all before we describe cancer vaccines, I will just give you brief overview what cancer is I am again sure most of you know about this – it is nothing, but this uncontrolled growth of cells which were not supposed to do that. At least not the way the body was designed. These cancer cells actually routinely arise in our body. So, right from your childhood all the way to the old age we have these sporadic cancer cells coming up again and again. And the reason they are coming up because they caused by mutations in some gene.

So, during the replication maybe one gene gets mutated. Most of the time those genes are at some places that are benign so, they do not cause anything. Sometimes those gene lead to a dysfunctional cell and the cell just dies. But then in some cases these mutations either confers the ability to proliferate at a much higher pace or gives it enhance survival capabilities. So, that can grow even in harsh environments and that these little things lead to eventually development of cancerous cells.

Now, because there are mutations so, the proteins that are being produced are not the sequence that they are supposed to be. So, let us say there is a mutation in some of the gene that causes up regulation in proliferation. So, because of that mutation now your original gene that the body knows is no longer exist there is a mutation maybe few amino acids has changed, maybe few amino acids are replaced, the structure is different. So, there is some antigens because now we can call them antigens because these are not

something that the body knows about. So, this is not natural; these are something that have been mutated.

So, that is there are some antigens and these antigens are what the immune system can detect. So, it is fairly feasible for the immune system now to be able to detect such abbreviated or such mutated proteins. And then again this happens as I said much at a much more rate than we know the immune system does continuously survey for these cancerous cell that are being generated. And again almost all of the times the immune system is very good and adept in destroying these cancer cells.

So, that is why we do not really see cancer being developed as often as it could. But then there are few cases in which the immune system then fails to destroy them, maybe they are not as immunogenic. These antigens may be the immune system is not able to survey the entirety of the body and then once that happens these cancer cells can proliferate can form tumors and that is what lead to tumor formation.

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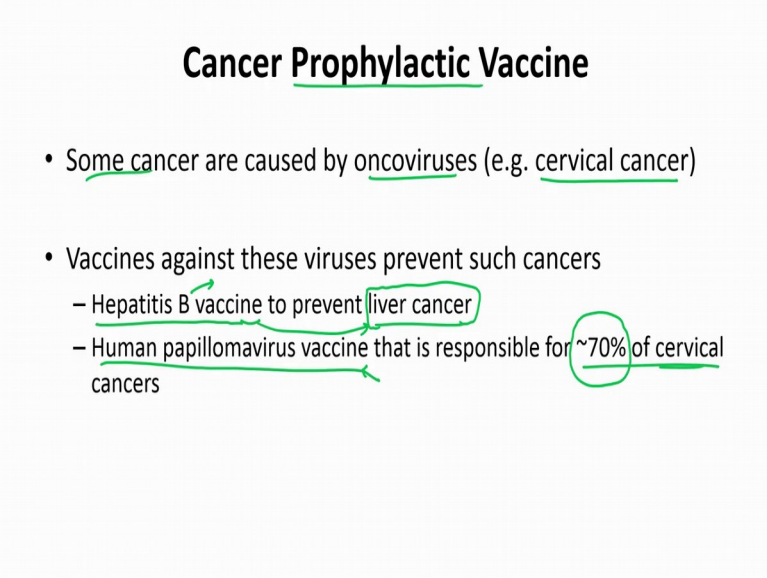

So, again and that is what we are trying to tackle here. We are saying that what if we help the body in tackling those and again like your conventional vaccines cancer vaccines can also be divided into two different types: One is prophylactic vaccine, which as the name suggest and as we discussed in the past is given before the cancer has happened. So, let us see how this how does that work. So, and then this really simple reason is some cancers are actually caused by some oncoviruses.

So, we have previously talked about how I did know virus or retroviruses can actually go into your genome and put in their genome and introducing very strong promoters in front of genes. These promoters were not supposed to be there, sometimes they can go and put these promoters in front of; let us say, a protein that helps in survival, a protein that helps in proliferation, and that may cause the cancer to develop. So, one of the bigger example of this is a cervical cancer. In cervical cancer, most of the time it is actually caused by a virus and at a very high frequency actually and so, this virus goes in and because of it's process it develops these cervical cancer in our cells.

So, very traditionally what is being done is if you vaccinate against these viruses, these pathogens that are leading to oncogenes being expressed, you basically get rid of cancer. So, if these viruses do not get chance to actually go in and inject their genetic material, then it will not be able to activate any oncogenes and our body will be protected from such viruses.

So, again few examples here is a Hepatitis B vaccine is used to prevent liver cancer. So, if you get a Hepatitis B infection, you have a higher tendency to get liver cancer. However, if you get Hepatitis B vaccine, you will not get Hepatitis B disease and because of that the liver cancer incidence will also go down. So, this is given as a prophylactic treatment.

So, even before you are getting cancer, even before you getting Hepatitis B disease, you have been given a vaccine that directly or indirectly leads to prevention of liver cancer. Similarly, this cervical cancer we talked about, this is through the HPV virus which is a Human Papilloma Virus and this virus alone is actually responsible for almost 70 percent of cervical cancers. So, as I said the incidence of cancer infected with HPV is very very high and almost 70 percent of the cancer of cervix is observed because of this infection. So, if we somehow give a vaccine before this virus has chance to infect us, we not only protect against this disease, but we will prevent the cervical cancer incidences by quite a big margin.

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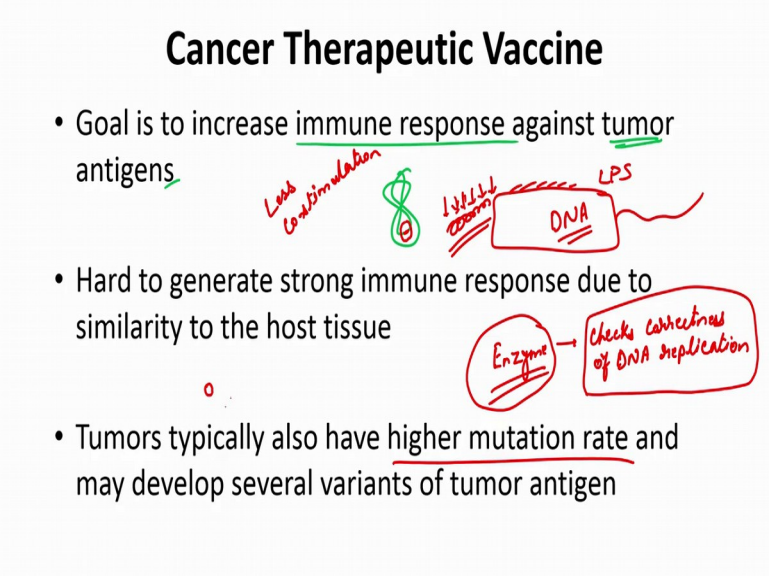

So, that was on the prophylactic vaccines not much as I said this is mostly an indirect way. At least at this point of time people have not been vaccinated directly for a cancer cell which is not caused by any other pathogen in a prophylactic manner. So, that is where the therapeutic vaccines then become very important because these are what will be more clinically relevant at least in most of the cases, where a patient is coming in with a disease already, with cancer already and then we want the body's immune system to be able to fight that cancer. And so, the goal overall here is to increase the immune response against tumor antigens.

So, those tumor antigens that are caused by the mutations in your normal cell initially. If we can get those immune responses against these antigens to go up, then the immune system can come and kill your cancerous cell. So, at this point the major problem is that the immune system is not detecting that these are cancer cells or not able to do much against these because it does not think that these are cancerous or there are too many of them or could be several reasons. So, somehow if we can strengthen the immune response against these particular antigens, we will be able to hopefully cure cancer through our own immune system in itself.

The problem is, it is actually very hard to generate immune response because these antigens are very similar to our own antigens. So, now, we are talking about let us say if this is a protein that the body already has, all that has changed is maybe one or two

amino acids here are mutated. So, the body finds it very difficult first of all to identify that there is a change here and then secondly, there is really no other signal the body gets to get activated for your immune response. Because when you are talking about a bacterial cell coming in, we had talked about there is so, many different innate immune pathways going up - the innate cells are recognizing the patterns, maybe the flagella, maybe the LPS, maybe the DNA, the CpG islands and all.

So, all of this was a very good activator of the immune response and not only that the proteins that these bacterial cells are secreting. Let us say if they are secreting a protein, this protein was completely foreign. So, each and every peptide sequence that makes this protein is new to the body. So, the body can generate immune response against all of these regions and so, there is very high chance that you will get antibodies, you will get cytotoxic T cells to be able to recognize, this kill whatever niche this particular bacteria or other pathogens are surviving.

Now, but in this case first of all you do not have much much co-stimulation. Then the antigen it itself is very very small as well this maybe only one antigen present in one protein. So, all of those result in quite a difficult task for the immune system to be able to mount a good response against it. And then not only that what then complicates a problem is because these cells are now rapidly dividing sometimes the mutation could actually be in enzymes that are supposed to take care of the fidelity of the DNA replication. So, what I mean by that is let us say; there is an enzyme that checks whether the DNA replication has happened correctly or not (checks correctness of DNA replication).

So, now if this enzyme got mutated itself, then what will happen not only there is one mutation here, but every subsequent time the cell is going to divide. There is a chance that there is going to be more mutations or the mutations getting accumulated at a much higher rate and so, that is what it means. You can also have a higher mutation rate that is being developed and then; that means, that let us just say that here is one cell that was cancerous,

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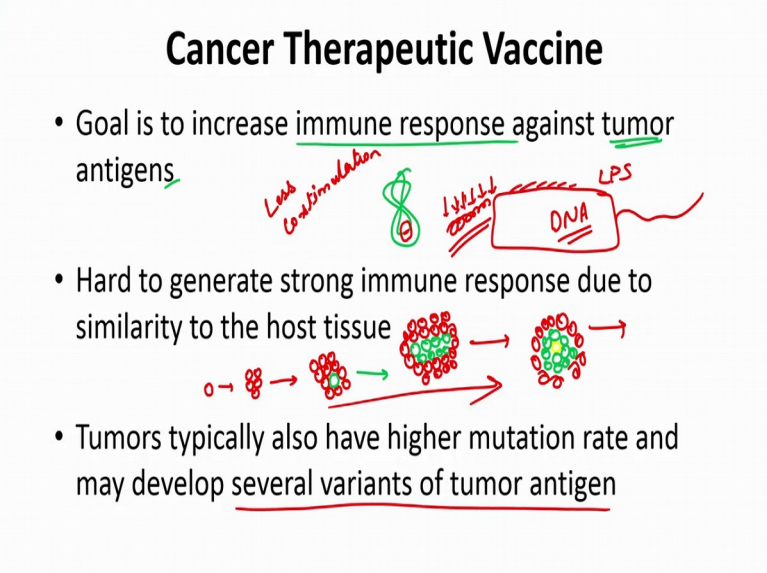

So, it goes ahead and starts dividing and makes several of these. Now because this enzyme was not working that is responsible for making sure that there is a correct DNA replication that is happening. What will happen is as it divides further, maybe one of the protein gets mutated and it is a being another type of cancerous cell. So, now, what will happen is this will divide further and obviously, the red the original cancer cells will continue to divide. And then it could again happen that another cell gets mutated through these green line as well.

So, let us say another yellow cell gets invaded and then this is going to be surrounded by the red original cells. So, this can actually continue to go on. And so, now, what you are doing is not only you have a tumor because there is a mutation, but now as the time is progressing as the tumors are becoming larger and larger, you are actually accumulating more mutations and more different types of cells.

And so, then it becomes very hard for the body to be able to control for all this because let us say if the immune system does come in as able to recognize one of these types. It will still not be able to recognize all three, all ten types that might be there in that large tumor. And so, that is why there are several variants of tumor antigens that comes up and eventually, it becomes a harder job for the immune system to be able to handle that.

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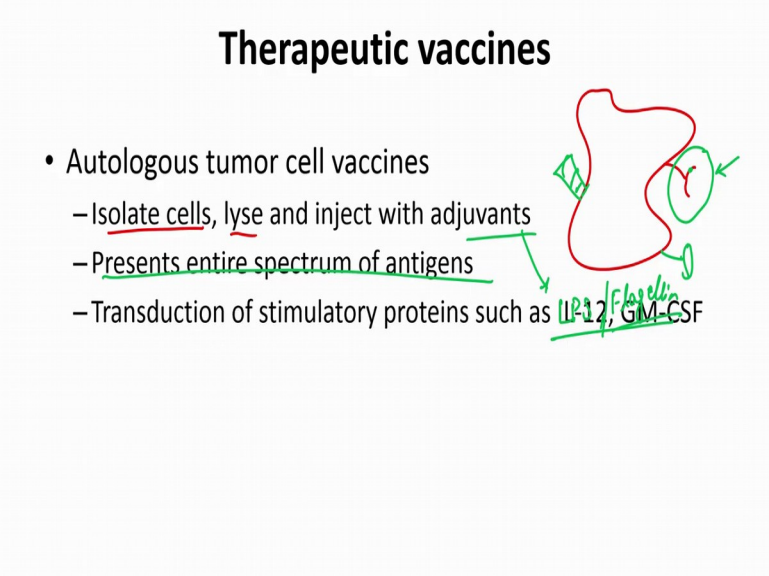

But nonetheless there are some vaccines like the other vaccines they have been classified into different categories. So, one is the autologous tumor cell vaccine and as the name suggest, that means, that this is from your own tumor cells. So, let us say a patient comes in or a animal is induced a tumor, you isolate the cells from the tumor regions maybe at the time of biopsy when it is being confirmed whether tumor is there we isolate some part of the tumor anyways. So, we isolate some cells, we lyse them. So, when I lyse these cells so, an initially let us say this was a cell and maybe one of the protein had this mutation here.

So, let us say when I lyse these cell all of the proteins come out, I then mix it with some adjuvants. So, what I can do is now I mix it with some LPS or I mix it with some flagella; flagellin protein and then put it back in the body. So, now, what is happening is earlier as I said that these tumor cells are not activating the immune system as much as let us say bacterial cell would. So, now, what I have done is because I have given these co-stimulatory signals, this is going to lead to a much enhanced response against any mutated protein that is present in your lysate.

So, at this point you are basically not really testing as to what is the mutation, you are just taking the whole cells, lysing them up, you hope that that cell lysate is now containing several proteins that are mutated and you inject it back in the body along with some adjuvants. And then let the immune system figure out what is wrong, which is the

protein that needs to target and one of the advantage here is you are actually presenting the entire spectrum of your antigen.

So, all the antigens that may be present maybe 1, maybe 5 are being presented to the immune system, which the immune system can then sort through and mount a response. So, maybe let us say this one had two or three proteins that were mutated and even though maybe only one protein is more immunogenic than the other because of the mutations. So, then it becomes a higher chance for the immune system to at least detect one of these different mutations and be able to kill the cell. As I said, this also causes transduction of stimulated proteins such as IL-12 and GM-CSF. So, because of all that you will have much better response. So, the innate immunity has kicked in a much better way.

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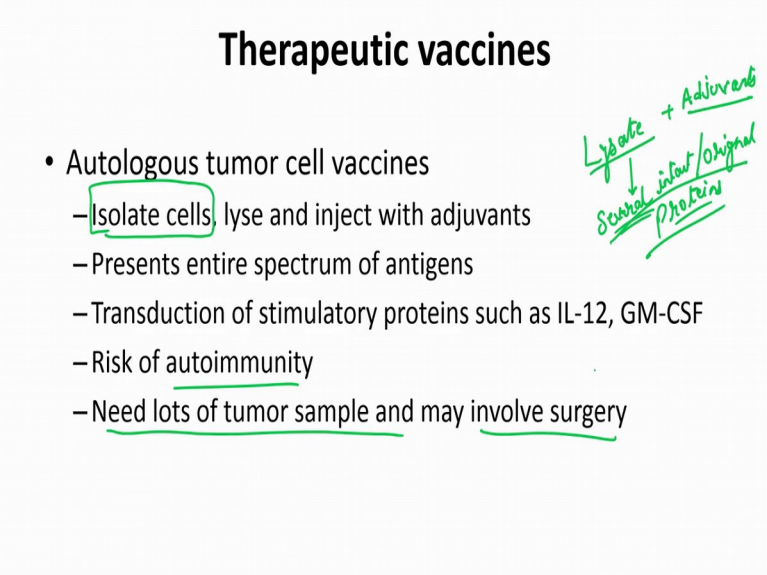

However, there is some risk of autoimmunity now, because as I said you are taking the whole lysate and adding it with adjuvants and putting it back in the body. So, now, this lysate even though it contains some of the antigens which are mutated from the normal cell. This also contains several functioning and intact proteins that you are now supplying with adjuvants.

So, there is a little bit of chance that if the adjuvant has worked very well or the immune system got activated to such a high amount that some of these original proteins, some of these original self-antigens may be recognized by the immune system and immune

system may mount the response against them. So, if that happens, then what will happen? Then your immune system is going to go even to healthy cells, mount a response against them because these healthy cells are producing these proteins, and by that logic though self-antigen and that may cause even more destruction, than what you wanted. And not to mention as I just said how do you get this tumor sample? It may involve some surgical procedure needed to be done to get this initial isolation of the cell. And so, it is invasive you need quite a bit of tumor sample again if you are going to go ahead and inject it; you want to give as much antigen as possible. So, you want substantial amount of tumor sample to be present as well.

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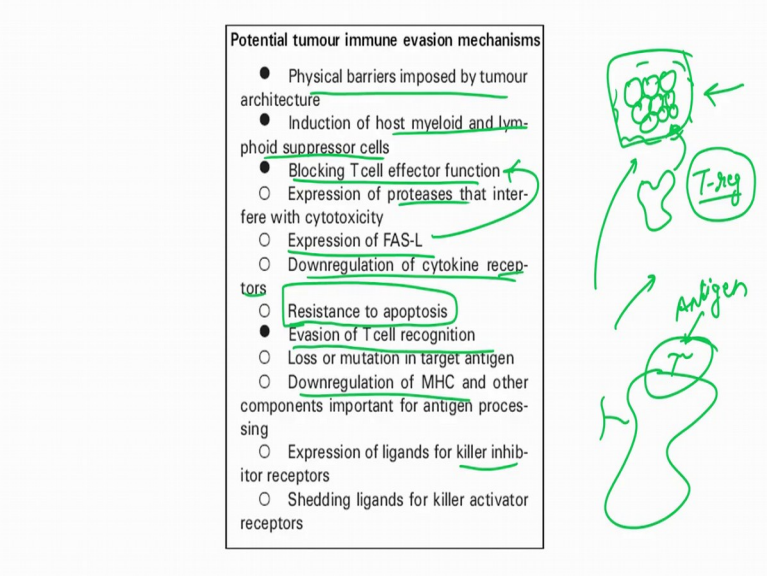

So, here is just list of some of the tumor evasion mechanisms that have been known in the literature. So, first of all is a physical barrier itself. So, as I said that the tumor is growing fairly rapidly and it is filling of whatever space it can find so; obviously, this space never existed before. This was supposed to be only a single cell, now it is converted into many cells. So, it is just pushing around on the body, it is a very tight network; its mechanical properties are much higher than the surrounding tissue in terms of stiffness and all.

So, it is harder for now the immune cell. Let us say, this is an APC which is coming to survey the area. It is harder for this cell to be able to penetrate deep inside this because it will have to navigate through all kinds of cells and to be able to find all different antigens

that are present in this tumor microenvironment. So, there is a physical barrier just imposed by the tumor architecture. Then you have induction of host myeloid and lymphoid suppressor cells.

So, not only that, the tumour environment is very complex, it actually signals in such a way that it causes suppression of your myeloid and lymphoid cells. So, they do not really work very well. Sometimes, it causes T-regulatory cells which we discussed in the past which are T cells that will actually regulate and tell the immune system to calm down to be present in much higher quantity in the tumor region than the surrounding. So that means, that even if the activated immune cell has coming, it is not going to be able to do much because when it sees these it thinks that this is a site that it does not need to get activated on and the tumor then can protect it itself from that.

There are direct mechanisms through which tumor binds to these T cells that are actually immunogenic and actually blocks their function, so, they cannot really do their job. There are expression of proteases that interfere with the cytotoxicity. So, obviously, let us say if I try to deliver a protein that kills the cell, but there are lots of proteases in the surrounding, then that protein that I am delivering it gets degraded in that environment. So, it is hard for those proteins to survive.

There are certain expression of FAS ligand. So, it goes back to what I was saying earlier- So, it expresses some of these proteins on these surfaces which causes the immune system do not really go 100 percent against these particular tumor cells. There is a down regulation of cytokine receptors. So, the cytokines do not really signal it very well on these. So, one of the way the cells can dies lots of cytokines come in bind to it and the cell functioning gets disturbed, but the tumor cells can have down regulation of such receptors and that may not happen. I already mentioned, sometimes there are also resistance to apoptosis.

So, maybe the mutation in such a gene that it does not die and continues to proliferate. And so, they are much more hardy and much more adept to be able to survive in harsh environment that immune system can present. The T cells can actually not be able to even recognize it and that we already talked about that it is not very immunogenic as well as the environment it itself is very tolerogenic environment, not letting the immune system get activated in that area.

Even let us say if the body does able to mount the response; if the immune system is able to recognize a particular antigen, as I said the mutation rate is very high. So, there will be parts of regions of this tumor which would even mutate that and have some other mutation that is causing now tumor. So, because of all that the body may not be able to clear out the whole of the tumor cell even, then some of the tumor cells may survive because they are slightly different and they then continue to proliferate and grow the tumor.

It also down regulates some of the MHC molecules. So, it does not really process the antigen. So, every cell has these molecules; every cell has these MHC molecules that are presenting the antigen. But if the number of these receptors are lower so, let us say, there is a certain density of these receptors on most healthy cells. What tumor does? It then decreases this number. So, that not a whole lot of them are being presented. So, the immune system can really recognize it. Then the expression of ligands for killer cells are inhibited as well as it also shed these ligands out. So, that the immune cells are not able to recognize this

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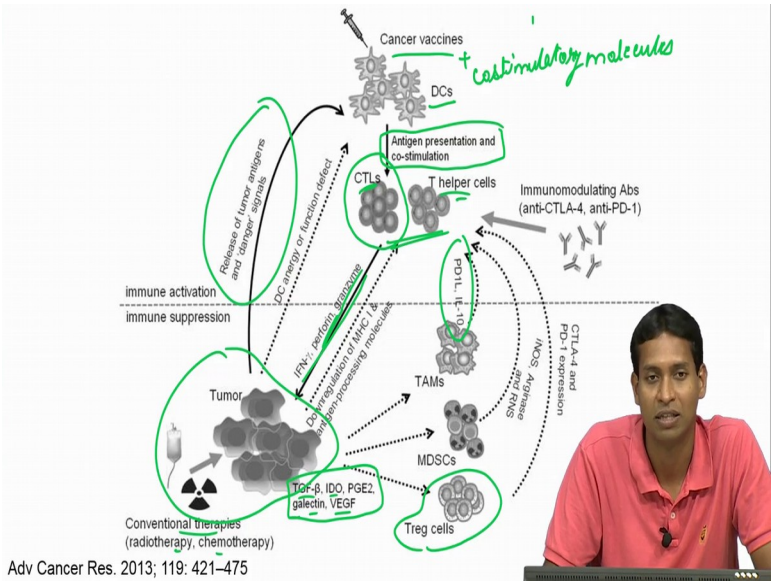

So, here is what ideally we would like to have in cancer vaccines. So, you give these cancer vaccines to dendritic cells or macrophages, which are antigen presenting cells, they get activated against the antigen. So, this probably is along with some co-stimulatory molecule and this is going to go ahead and activate the CTLs (the cytotoxic

T cells) because in this case the antibody can be useful, but it really will not do much stuff; you want to kill the cells itself.

So, cytotoxic T cells and T helper cells are the major players and so, this is the immune activation part of it. This is what ideally we would like and then these should go to the tumor cells and start killing it. Using various mechanisms either by secreting granzyme and perforin or IFN-gamma to further boost this response. However, what actually happens is, as I said, these tumors are secreting all these several proteins that are causing immune tolerance. So, TGF-beta, IDO, VEGF, galectin; it is causing activation of T-regs this is also PD1, PDL1, that is also present, and is causing the tumor associated macrophages and the different types of immune cells to not get activated. And all of this result in modulation of this immune response and in that, suppresses the immune response and be able to then protect this tumor cell.

Obviously, at the same time most patients also get conventional therapies such as radiotherapy and chemotherapy, but then they suffer from various drawbacks that they were not able to penetrate inside a dense tumor tissue. They are not able to kill all tumor cells because of mutations. These tumor cells anyways are fairly strong in resisting cell death and eventually, they are able to survive and obviously, there are tumor antigens in the surrounding that you can again use for cancer vaccines. So, this is kind of how this whole process is taking place in our body.

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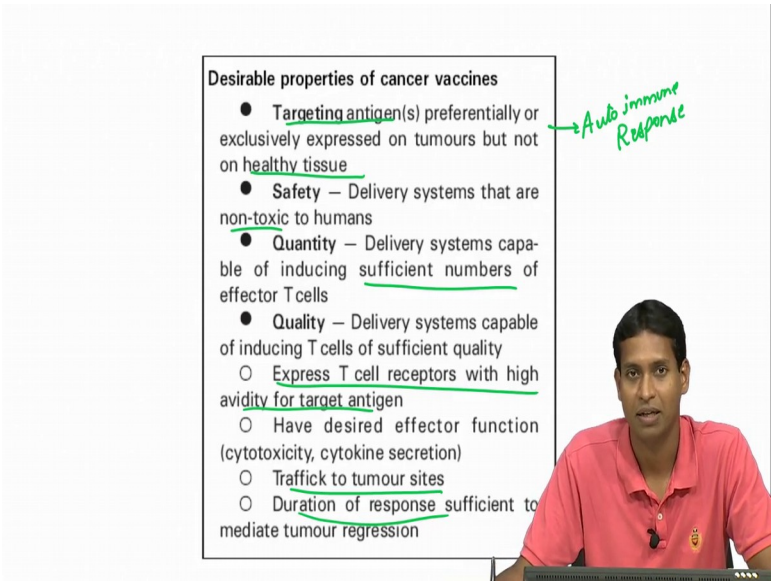

So, what are some of the desirable properties of cancer vaccines? So, of course, one is it is targeting antigens preferentially that are exclusively on tumors, but not on healthy tissue. Again if you use antigens that are present on healthy tissues, then you have autoimmune response being generated. It should be fairly safe and non-toxic to human cells - of course and that is true with any kind of treatment you. What about the quantity?

So, can you deliver enough to get sufficient number of your immune cells to be activated What about the quality? the ones that are activating is being able to go and kill the cells. You can express T cell whether they express T cell receptors with high ability to the target antigen.

So, whether they will bind to it, give the desired function traffic to the tumor sites. So, be able to go and find where it is and then further navigate inside the tumor as well and then how much of the duration of response is given by these cells whether these cells are active. So, for enough duration to kill all of the tumor cells in a large tumor. So, we will stop here and we will continue rest in the next class.

Thank you.