Essentials in Immunology

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Lecture No. # 17

Autoimmunodeficiencies of the B cells

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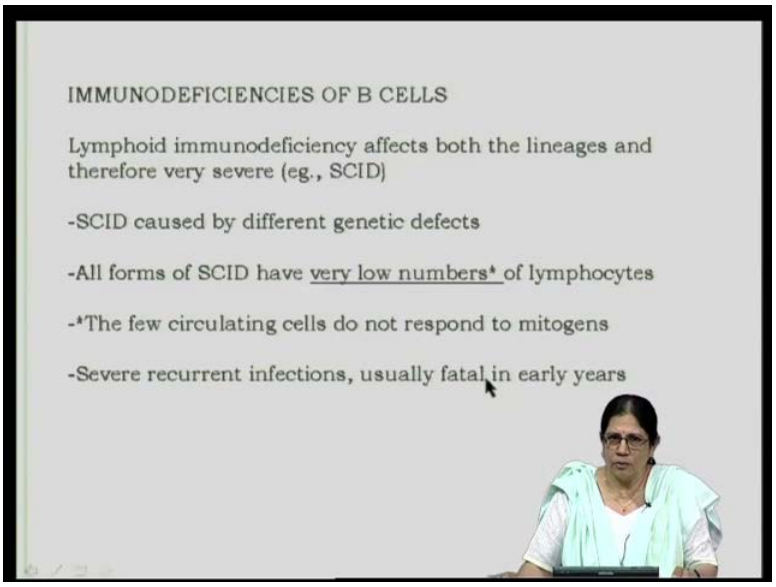

So, I will take on from where I left last time. I was talking about immunodeficiencies and to which I will go over, I had gone over very quickly over a couple of slides, and I will take some time now to go through them a little bit in detail. Now, immunodeficiencies of the immune system are very well known. The immunodeficiencies of T cells, B cells, as well as, complement components. Now, immunodeficiencies of the T cells will be dealt with in another class, I will restrict myself mostly to immunodeficiencies of B cells.

Now, lymphoid immunodeficiencies, which would mean, that any deficiencies, which would led to the development of the lymphoid series would effect, of course, both the lineages T cells, as well as, the B cells and this, therefore would lead to severe combined immunodeficiency.

SCID, in short for severe combined immunodeficiency, this can be caused by different genetic factors. However, all forms of SCID have very low numbers of lymphocytes and even if there are lymphocytes, even in low numbers, it is been seen, that the few circulating cells, which you see, I mean lymphocytes, that you see, they do not respond to antigenic stimulus as has been studied by stimulating with mitogens.

Therefore, now lack of T or B cells would result in severe recurrent infections. Now, these infections are less, such people who have these deformities are kept in isolation, away from the regular atmosphere. This condition would lead to lethality and that too, of course, in early years.

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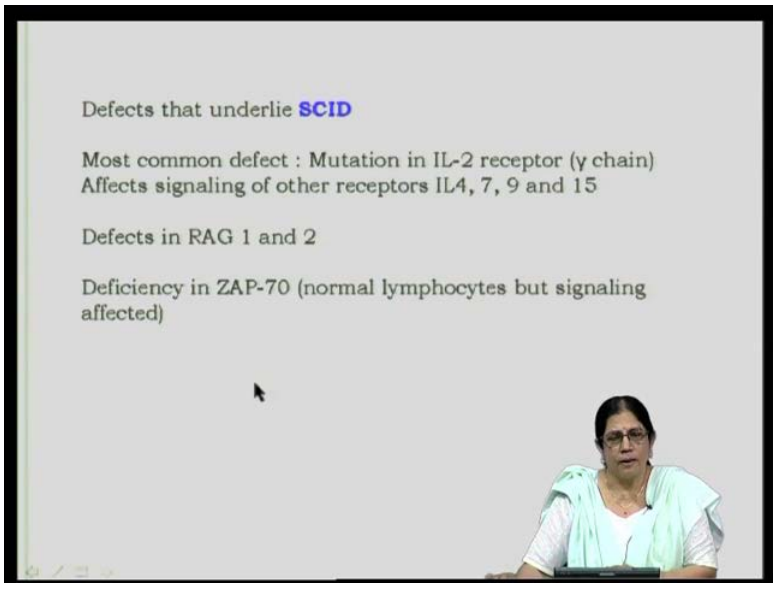

But see the defects that can underlie SCID, severe combined immunodeficiency. The most common defect under SCID based mutation in the interleukin-2 receptor and the gamma chain, that too.

Now, interleukin-2 receptor, I hope this rings the bell, interleukin-2 receptor is absolutely essential for the signaling of T cells. Interleukin-2 receptor, also required for B cells, but you have also interleukin 4 and 5 receptors, that can take over. Now, mutations in this receptor affects also other receptors, such as interleukin 4, 7, 9 and 15 because the same gamma chain is associated, makes a part of these receptors. So, ultimately, therefore one can imagine, that mutations in IL-2 receptor can, in fact, bring about

immunodeficiencies, you know, severe immune deficiencies, because other receptors also get involved.

RAG 1 and 2, the recombination activating genes 1 and 2, the products of which are required for the development of both T and B cells because these enzymes, I mean, the products of RAG 1 and 2 genes, enzymes are required for the T cell receptor organization at the gene level, as well as immunoglobulin gene organization. So, defects in either, RAG 1 and 2 mutations, in either, RAG 1 and 2, is too severe, combined immunodeficiency.

And you might remember, that I said, there are mice, that do not have RAG 1 and 2 and these mice, therefore lack lymphocytes and can be used as animal models for adoptive transfers of lymphocytes from other animals, as well as, one can also test humans. In this, so RAG 1 and 2 deficient mice are very good animal models for studying immune responses restricted to T and B cells. Now, there are also defects, that in mutations of ZAP-70 and I mean, this is one of the common ones and therefore, I am describing these to you. ZAP-70 is the synkinase, which is required for signaling. Now, deficiencies in this particular gene for ZAP-70 would result in lack of signaling of normal lymphocytes. So, there are lymphocytes, which develop normally, but because of lack, lack in ZAP-70, where signaling event is very much curtailed.

Now, there are synkinases of course, but the importance of ZAP-70, in fact, of synkinases become very apparent because of the description of these deficiencies. So, there are normal lymphocytes, but signaling is affected and therefore, of course, mounting immune response to the optimum level is compromised in these individuals.

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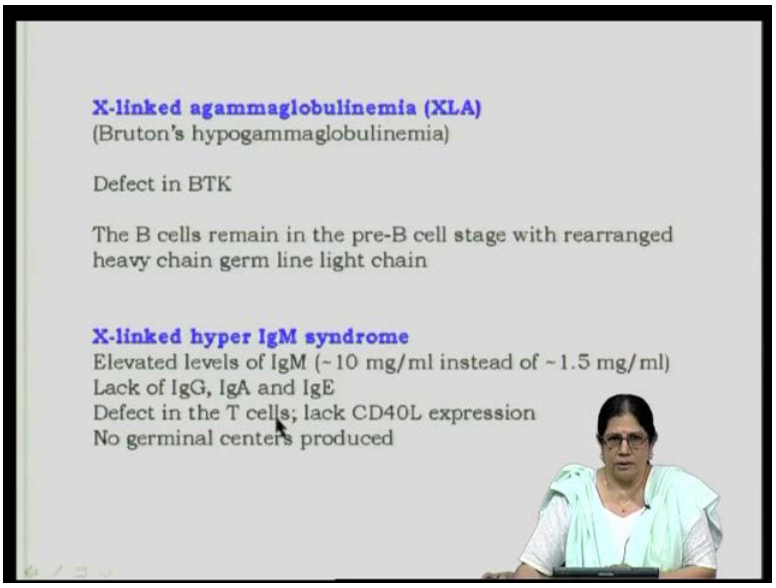

Let us look at now other immunodeficiencies, such as X-linked agammaglobulinemia. Now, this is also known as Bruton's hypogammaglobulinemia hypo, which would mean, that the mutation in the Bruton's tyrosine kinase.

If you remember when I described the signaling events, that go, goes on in B cells Bruton cytosine kinases, one, I mean, activation of which is one of the arms of the downstream processes from the (( )) or the immunoglobulin receptor activation, which activates the (( )) activates, therefore a large number of molecules, which is because of phosphorylation. Now, BTK, Bruton's tyrosine kinase is again another phosphorylating enzyme and defects in this have been shown to have severe effect on the development of the B cells themselves. And it has been seen, that these cells, under these conditions, they remain in the pre-B cell stage though rearrangement of the heavy chain germ line light chain gene has taken place, but these cells remain in the pre-B cell stage and cannot now go on for with further development and therefore, cannot become mature cells, and therefore, there is lack of these cells in circulation, mature cells in circulation. Obviously, such individuals are come down with severe infections.

Another condition is X-linked hyper IgM syndrome. I had briefly mentioned this when I talked about the signaling again, where T and B cells interact and this interaction is necessary for both T cells, as well as B cells. Now, in these individuals you have X-linked hyper IgM syndrome. As the name suggests, they have extremely high levels of

IgM. Now, normally in circulation the amount of IgM is about 1.5 milligrams per ml, but in these individuals it goes as high as 10 milligrams per ml and they as well, they should not be much of a problem with respect to such high levels of IgM, but these individuals do not have IgG, IgA or IgE, any one of the isotype subclass immunoglobulins, that is because there is no class switching scene in this individuals. If you do remember, of course, that IgM is the first antibody that is secreted in the primary immune response and cells, these cells memory cells undergo class switching from IgM to any one of the isotypes, first two secretary proteins, the, I, the class switching would be to IgG.

Now, this requires the interaction between T cells, activated T cells, which express CD40 ligand, which is recognized or which binds to the CD40 receptor on B cells and this is what affects or gives the competence signal to B cells. Now, in, if there is no CD40–CD40 receptor interaction, then there is no isotype switching. In fact, there is no memory cell generation; no isotype switching and therefore, these individuals would make only IgM. Now, what should be the problem with just IgM?

Along with the isotype switching, if you remember, there is also affinity maturation. You might remember, the affinity maturation is because of error prone polymerase and this happens only because of antigen mediated stimulation of memory cells. If there are no memory cells, therefore no isotype switching, therefore only IgM, such a, because there are no memory cells, these individuals also do not have germinal centers. So, they have highly improved, these are highly improved immunocompromised situations.

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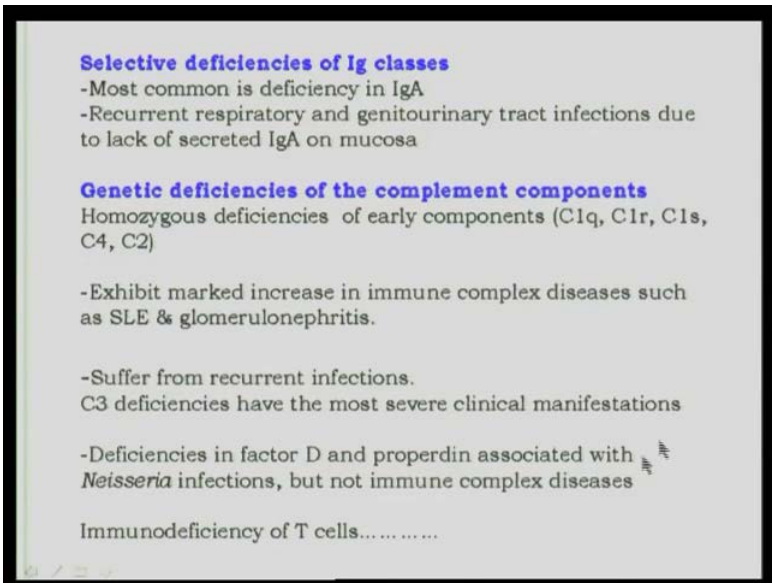

Selective deficiencies of immunoglobulin classes are also, have also been seen. There are individuals who do not make IgA or IgE, well, E or Ig, well, A, E and G, the most common one in, under these deficiencies is lack of IgA. What we talked about earlier is lack of the any of the classes, but you can have selective deficiencies. Now, lack of IgA could be because of mutations in the switch region, which does not allow switching of IgM to IgA; such individuals have recurrent respiratory and genitourinary tract infections. And you know, one can imagine why these type, you know, respiratory and genitourinary tract because you do remember, that IgA is the class of immunoglobulin or the antibody, which is required, which in fact, is present in the lining of the mucosa. So, lack of and that, that is, where IgA is extremely efficient with respect to counteracting pathogens. So, there are recurrent such infections.

Genetic deficiencies of the complement components, I have also mentioned this to you earlier when I talked, when I, we, when I taught you the complement cascade, that there are homozygous deficiencies of early components of the classical path. We known C1q, C1r, C1s, C4, C2, now lack of any one of these or mutations in any one of these definitely would lead to abrogation of the classical complement pathway. Such individuals again would have recurrent, not only recurrent infections, but also the exhibit marked increase in immune complex diseases, that is, because the complement component C3 is extremely efficient in the process of opsonization, also extremely

efficient in removal of complexes by the receptors through the receptors present on phagocytes.

Now, if there are no, there is no triggering of the complement cascade, the split products are not formed and therefore, there would not be any clearing of the immune complexes, so increase in immune complex diseases, such as SLE, as well as glomerulonephritis. Now, these are secondary, as opposed to what we talked about, with respect to autoimmune diseases. In respect of complement components or deficiencies, these individuals who have C3 deficiencies have the most severe clinical manifestation because of the reasons I just mentioned.

Also, apart from the classical components individuals with deficiencies, either in factor D and properdin has also been reported and these are associated with specific infections, Neisseria. But these individuals do not have the immune complex disease, one can understand why because the immune complex diseases, because of lack of C3, which is absolutely required for the clearance of immune complexes. Now, as I said earlier there, of course, equal number of immunodeficiency of the T cells, but that will be dealt with in a separate lecture.

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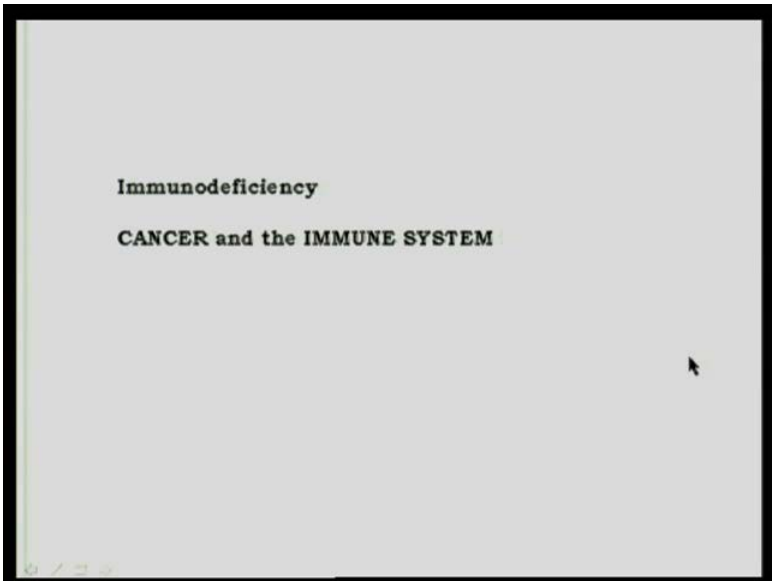

Now, I come to the subject of cancer and the immune system. Why does cancer come under immunodeficiencies? Why do we study this disease cancer under the immune, immune system?

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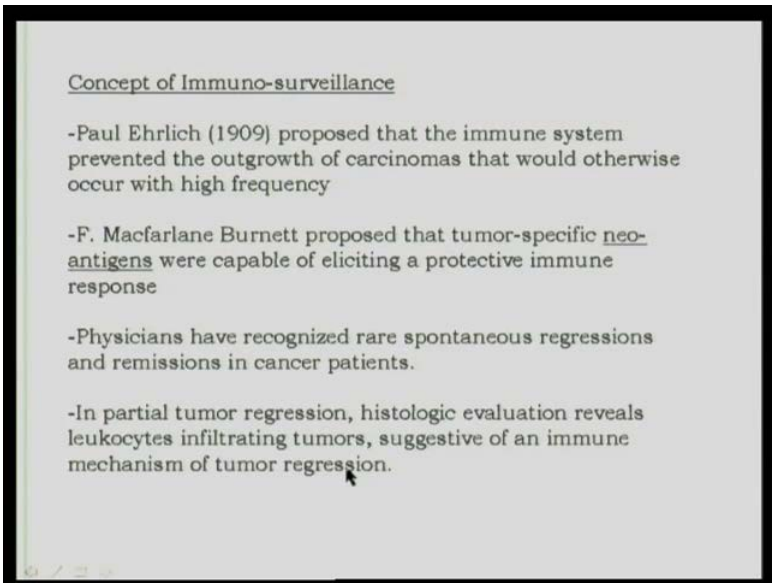

Now, it was, I will just like to tell a little bit about the history of immune, I mean, cancer and the immune system. Now, this concept, there is a concept of immunosurveillance. It was as early as 1909 that Paul Ehrlich proposed, that the immune system prevents the outgrowth of carcinoma that would otherwise occur with high frequency.

And there was another study, that was made not as early as 1909, but somewhere around the 1950s and 60s, where it was seen, that all individuals, individuals would die of old age, die not of cancer, but with cancer. What would that mean, that mutation, now how do cancer, cancers arise? This everybody knows, this is because of accumulation of mutations. Now, it is seen, that all of us are exposed to carcinogens, those chemicals, which can or physical agents that can induce cancers.

Now, how does this induce cancer? It is because of mutations; now, every time there are mutations, that are taking place in our cells, any molecules. We have an extremely immune, you know, sensitive and robust immune system, which looks at these cancers, looks at these cells, which are, which now have modified and therefore, these cells get cleared. In case, you know, it has been seen, that in, you know, the immune system also starts to degenerate and it is related to H, with H. The immune system starts to degenerate and therefore, accumulated mutations slip by, therefore now the cancers would arise.

So, in old individuals there may be cancers, that have established themselves, but before the cancer has taken its tone, the individual dies because of other reasons, maybe because of the circulatory system failure or kidney failure or whatever. So, Paul Ehrlich said, that immune system prevents the outgrowth of carcinoma that would otherwise occur with high frequency. Soon after that Macfarlane Burnett proposed, that tumor specific neo antigens were capable of eliciting a protective immune response. So, these actually go hand in hand. So, Burnett described these neo antigens.

Now, how is the immune system and this immunosurveillance for that matter? Is there any evidence, that the immune system must be in place to reject, you know, neo cancers, which might be developing or the immune system has a role to play in regression of tumors? Now, physicians have, there are a large number of reports and literature, physicians have recognized rare, but spontaneous, rare, but spontaneous regressions and remissions in cancer patients.

One such cancer, (( )) Burkett lymphoma, which is associated with (( )) viruses, in these individuals there are often spontaneous regressions and this can be associated with the, to a great extent by circulating antibodies, but more so by activated T cells against cancers, the cancers, which are Burkett lymphoma. So, therefore, the immune system plays a role in allowing the cancer or not being able to, you know, mounts an active fight against the cancer. Also, evidence for the immune, immune system being responsible for not allowing or allowing cancer to develop is in partial tumor regression. Individuals who have had tumor, now it regresses spontaneously, histologic evaluation of such tumors have been shown to have in large number of infiltrating lymphocytes or leukocytes.

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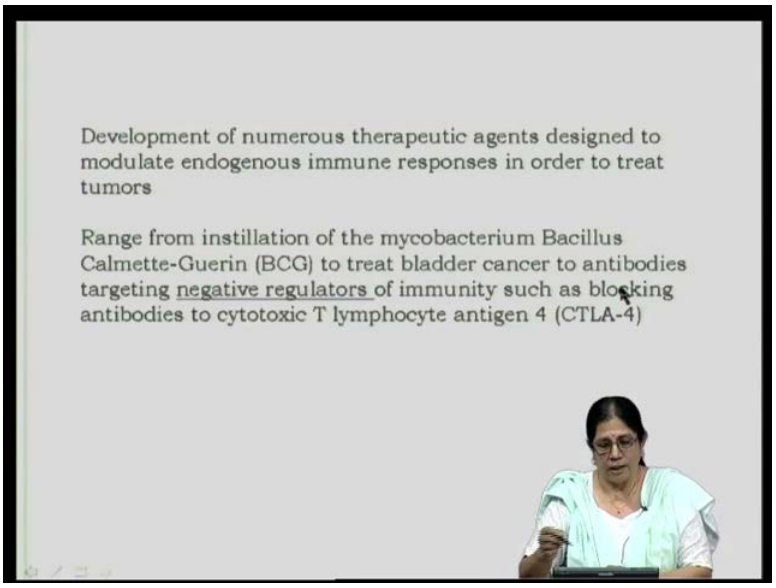

Now, this would mean, that the immune system, in fact, is active in bringing about tumor regression. There are, therefore, now with all this in the background there is now an effort to develop numerous therapeutic agents, which can, are, which are designed to modulate the endogenous immune response, in response, in order to treat tumors. Now, we will come to that in next lecture, but let us look at what has already been established to some extent. Now, this, of course, is not the mode of treatment now.

But the range of these agents to modulate the endogenous immune response is from injecting mycobacterium bacillus, you know, BCG, to treat bladder cancer. Now, what is the reason for, this is, BCG can activate microphages, activate the immune system, activate leucocytes now, bringing therefore, these cells to the sight of the cancer, can be beneficiary to the, to the regression of cancer or treating, treatment of cancer; now, to the other end of the spectrum, this is the simplest.

The other end of the spectrum is to activate the immune system in a manner by inhibiting negative regulators. You may have studied by now the molecules CTLA-4, which is a negative in regulator of cytotoxic T cells. Now, if CTLA-4 is inhibited, then by, you know, let us say, blocking antibodies, that, then there would be no negative regulation and therefore, there would be enhanced cytotoxic T lymphocytes to the cancer cells.

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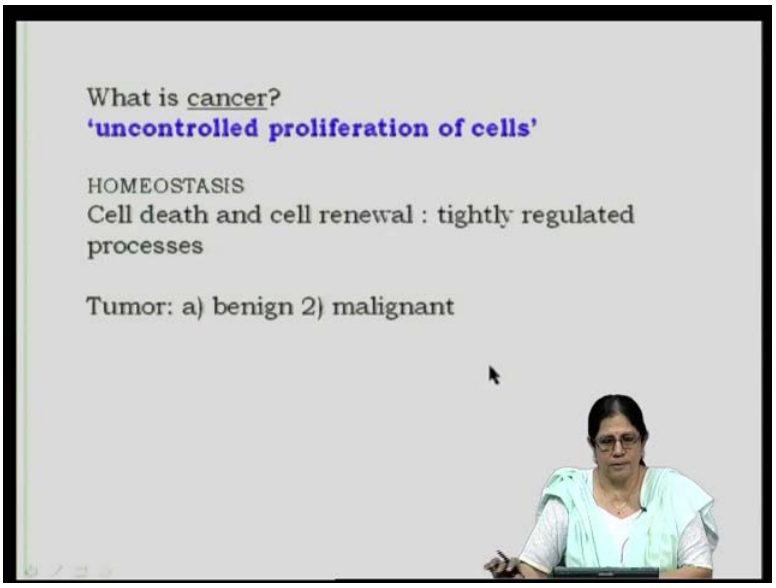

Let us go to the ultimate, you know, to be able to understand the immune system with regards to cancer or cancer and the immune system, but first we need to understand what is cancer and how does it develop.

So, what is cancer? The definition is very simple. It is uncontrolled proliferation of cells, uncontrolled proliferation, which would mean, of course there should be some kind of a regulatory mechanism, which allows cells to renew and die, this system, this process is known as… Homeostasis cells are short lived, of course, depending on the type of lineage. The lineage cells can be, can live for up, you know, 3 to 4 weeks and cells, some cells, like RBCs, would live for a few days, there is a constant need for cell renewal; whenever there is cell death, there is cell renewal and this is an extremely tightly regulated process.

However, in certain conditions you can have uncontrolled proliferation of cells, once they have started to proliferate. So, there is, now you know, one can explain the scene a little bit easier way, but if you take away a small slice of a liver from an animal, now there would be intense proliferation of the cells where the cells have been destroyed and this proliferation goes on at a heightened pace until that, that little portion has regained its structure. This happens, you know, we, we are constantly being infected with small cuts and bruises and you will see, that the skin finally comes to its normal self, there is no outgrowth there.

There is no more cell division after attaining that particular contour, which was there before. However, in case of tumors, there is, this control or regulation is lost. You can have, I mean, this mass of cell is called a tumor. The tumor can be the benign or malignant, as seen. This is something, which is quite well known and the benign tumor, of course, is a mass of cell, which is restricted. It is, in fact, isolated and compartmentalized benign cells, therefore you know, benign tumors, therefore can be treated very easily.

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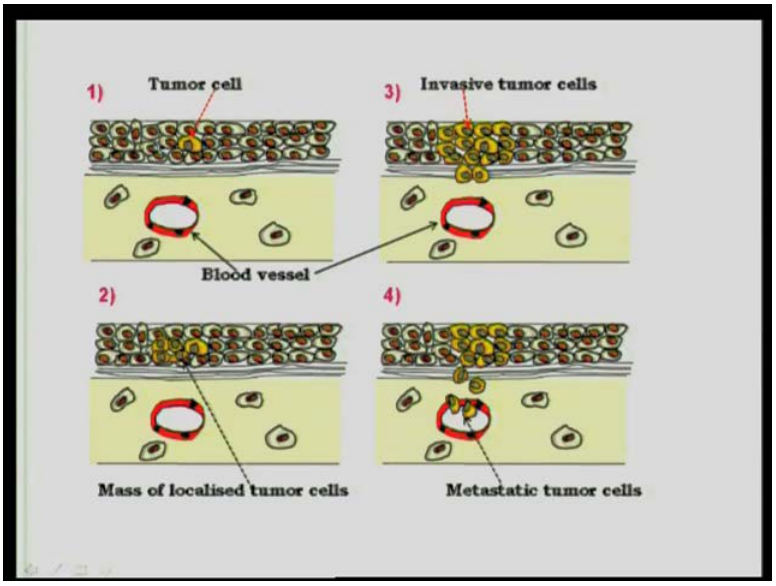

Malignant, on the other part is, when cells now grow out of this mass and go into circulation and can now establish themselves elsewhere. Let us look at how this can happen. I like you to remember, that usually tumor cells arise from one single cell. One can call all cancers monoclonal, all cancers, though later on, might be a huge mass of tumor, I would, arise from a single cell. Of course, by the form, a single cell, when it becomes a huge tumor there would be, of course, large number of processes by which, that single cell has changed. So, its progeny has changed to, well we will talk about this later because that is one of the mechanism by which cancer cells can invade the immune system. But suffice here to say, that a tumor cell arises from one, I mean, the tumor arises from one single cell.

So, this is another, you know, this is an epithelium. You can see, underlying there are blood vessels and this is the stoma. Now, a single cell, which may be because of

development of, you know, accumulation of mutations, now this has established itself as a tumor cell. The tumor cell starts to proliferate and become a mass of cells. It then, if it is a malignant tumor, then it starts to invade, you know, go outside of this epithelial compartment, starts to go (( )) to the stoma. Not only that, these cells can also enter the blood vessel, that is what is shown over here, that the tumor cell now starts to metastasize, enters the blood cells and now, through the blood, it can go elsewhere. Metastatic tumors are cancers and those are the ones that meet, of course, treatment

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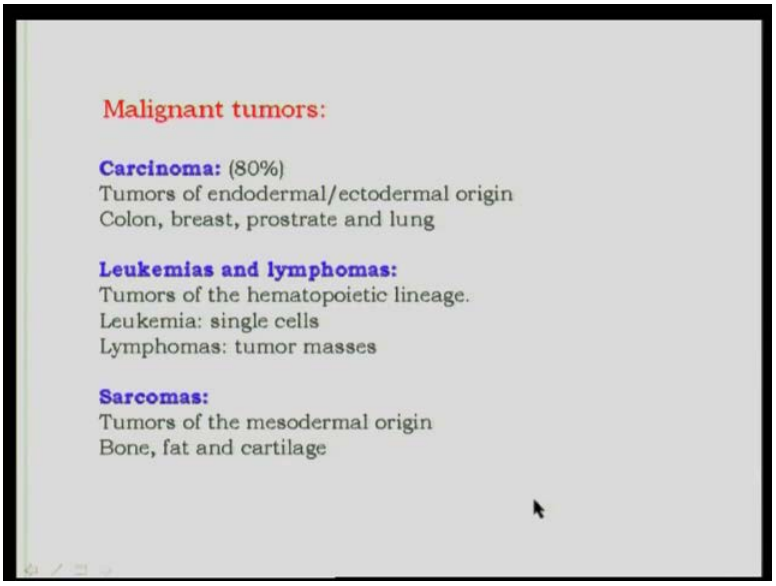

Malignant tumors can be, are classified under three categories: carcinoma, leukemias and lymphomas and sarcomas. Carcinomas, now this is dependent on the, the lineage of the cells, I mean, the lineage from which they arise, for example, carcinomas are tumors of endodermal or ectodermal origin. Leukemias, on the other hand, are tumors of the hematopoietic lineage; sarcomas are tumors of the mesoderm origin.

Now, carcinoma accounts for 80 percent of the cancers known today; examples, colon, breast, prostate and lung cancers. Leukemias and lymphomas - now leukemias are single cell cancers, which, which are formed as single cells, whereas lymphomas as tumor masses. Sarcomas are tumors of the bone, fat and cartilage and they are quite rare. As I said, carcinomas form 80 percent of all tumors, leukemias and lymphomas are about 10 to 12 percent and sarcomas are much, much lower.

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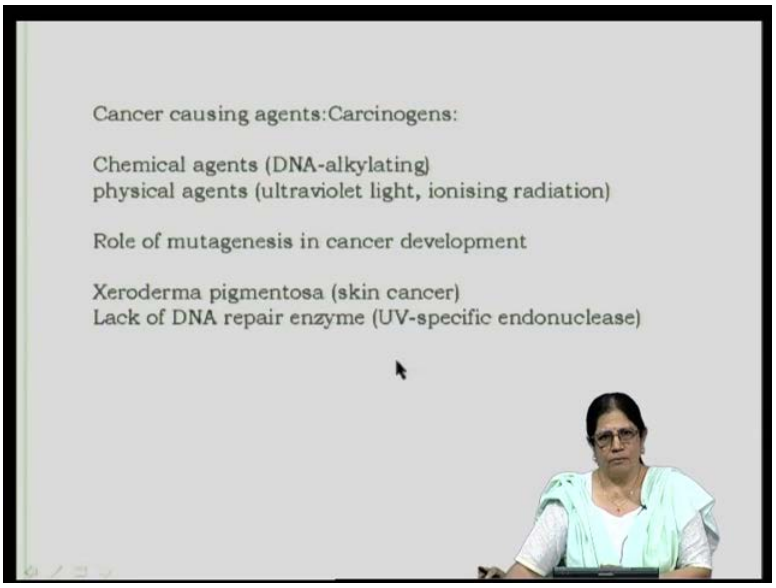

Now, there must be something, some agents, which we know as, we know, that these are carcinogens, cancer causing agents, which actually are responsible for inducing mutations. Chemical agents which could be DNA alkylating, that means, DNA modifying agents or physical agents, such as ultraviolet light, ionizing radiation, now these are carcinogens.

The role of mutagenesis, now I have been saying all this while, that cancer rises from accumulation of mutations. Now, how was that established that mutations, in fact, are what cancers, which leads to cancer development? One very good example, there are several such examples, the one, that I can immediately tell you is a condition known as xeroderma pigmentosa or skin cancer. Now, in individuals, with this particular condition lack a particular DNA repair enzyme. Now, apart from the DNA repair, that I have talked about with respect to immunoglobulin gene organization, I am sure you can appreciate, that DNA repair enzymes, that absolutely required even during the DNA, you know, during, during transcription, transcription stages.

DNA is prone to get damaged by several mechanisms and there are DNA repair enzymes, individuals who love this repair enzyme and specially the one, which is specific with respect to the ultraviolet. Now, UV-specific endonuclease, this repair enzyme is required, individuals who are exposed, I mean, who have this condition, who lack this DNA repair enzyme when they are exposed to UV. In fact, just being in sun for

longer, I mean, long period of time, these individuals develop skin cancer. So, this is direct evidence of mutation and cancer.

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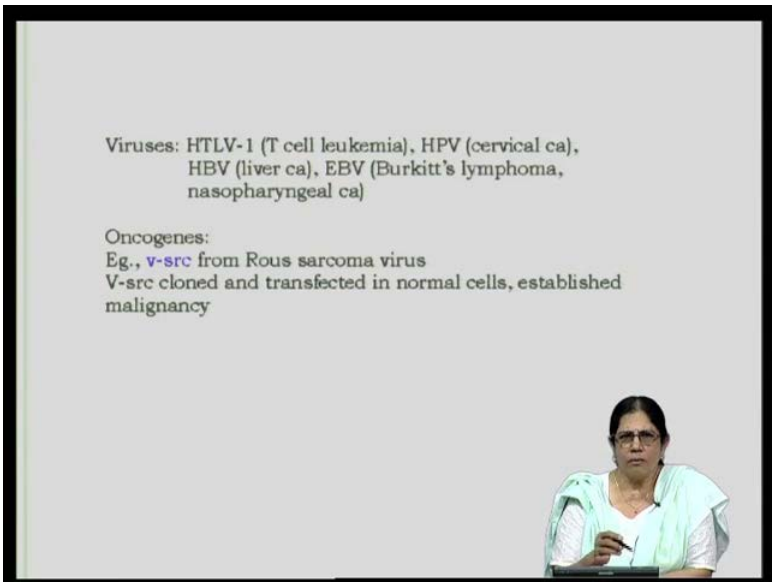

There are viruses, which are also known to cause cancer, they are also cancer causing, you know, they are not chemical reagents, but they are also cancer causing agents, examples are HTLV, human T lymphocyte virus, HPV. Now, HTLV is associated with T cell leukemias, HPV, cervical cancer and HPV, of course, has been in the news in the last couple of years because there are vaccines available now, which can control cervical cancers; HPV liver cancer; EBV, Burkitt's lymphomas, well, as nasopharyngeal carcinoma. So, these are viruses, which are known to be associated with human leukemias and cancers.

Now, how do viruses in any way are, how are they are associated with cancers? Now, viruses are known to, known to have oncogenes; onco is a name for cancer. So, genes, which are associated with cancer, for example, I mean, I will talk about this a little later, but oncogenes, for example, V-src from Rous sarcoma virus and is, of course, Rous sarcoma virus is a virus in mice. The V-src, when this particular gene of the Rous sarcoma cloned and then transferred to, into normal cells, establishes malignancy, so again, directly relevant oncogenes, which have been shown to cause cancer by a simple transfection or introducing this gene in normal cells.

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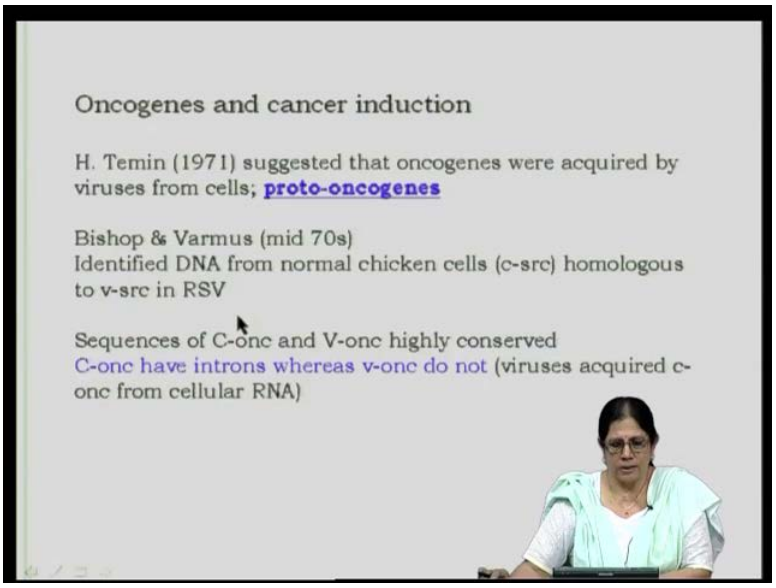

Now, let us talk about oncogenes and cancer induction in respect of other cancers, other than from sarcoma virus. How early, it was 1971, Temin suggested, that oncogenes are acquired by the viruses from cells. So, those oncogenes, that are, you know, their V-src, that I talked about is, should have its partner in normal cells. What, therefore Temin said, that these viruses when they infect, they picked up these genes from normal cells and he called them proto-oncogenes, that means, oncogenes, which are in, in the form where they are not oncogenes, you know, or the initiation of oncogenes, proto-oncogenes.

Bishop and Varmus later identified genes called C-src in chicken cells, normal chicken cells and these, he showed homologous to the V-src in Rous sarcoma virus, RSV, Rous sarcoma virus. Later on, more and more oncogenes were studied and it was recognized, that the cellular oncogenes and the viral oncogenes are highly conserved. The cellular oncogenes, as one can imagine, has introns, whereas viral oncogenes do not. We know, therefore, that viruses have acquired the C-onc from cellular RNA and not from the DNA because the C-onc has introns that means, cellular normal cells have these genes and there are introns.

So, these viruses, where have they picked up? They have picked up, picked up after these genes have been transcribed and therefore, they have taken from cellular RNA, the messenger RNA.

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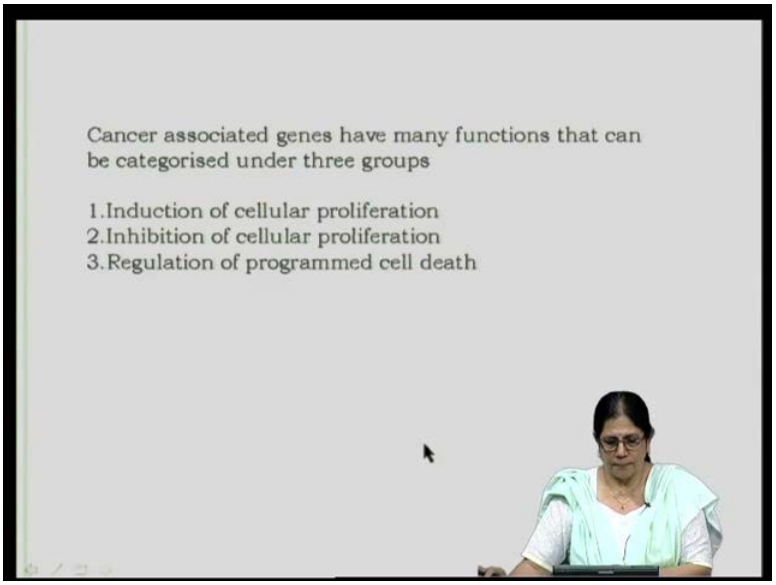

Cancer associated genes, what do they do? Do they have functions? Yes, they do have normal functions in the cells, which are absolutely essential, that can be categorized under three groups: one, induction of cellular proliferation; the inhibition of cellular proliferation. Now, why are we talking about induction and inhibition?

Remember, that I said, homeostasis is an extremely regulated process, you have proliferation taking place as soon as the cell death. Now, this proliferation, cellular proliferation is stopped, as soon as now, adequate numbers are formed. So, therefore, lack of any, either of these can lead to abnormality and we will see how.

Thirdly, regulation of programmed cell death; these are the three cancer associated genes are associated with three normal functions.

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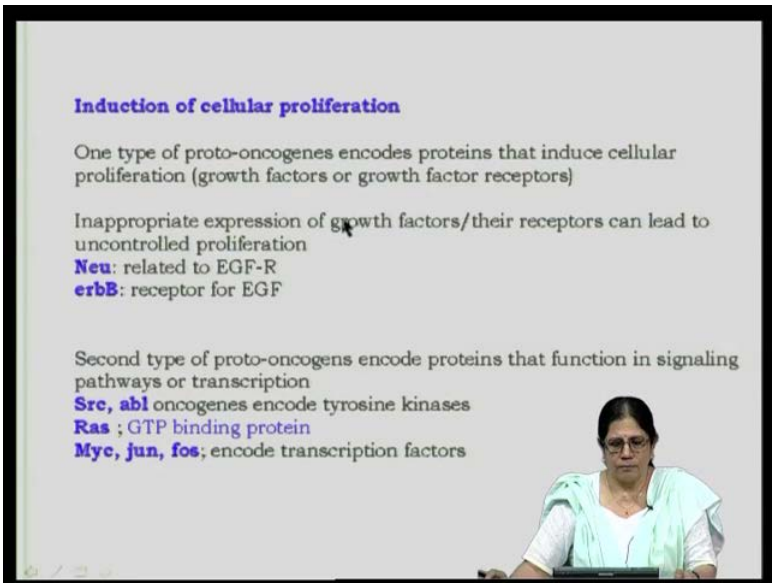

Let us first come to induction of cellular proliferation. Now, one type of oncogenes encodes proteins that induce cellular proliferation, very well known are growth factors or growth factor receptors. Secondly, now, ok, before we come to that, so how do these cancer, you know, how are these proto-oncogenes, how are they associated with cancer? Is, if there is mutations and therefore, when the growth factor should be x numbers and they, now because of these mutations are, you know, the expression is heightened, then either the growth factors or the receptors being expressed in an appropriate way would now lead to uncontrolled proliferation.

The oncogenes, which are associated with this are Neu and erbB and there are several others, I have just taken two examples. Neu is related to EGF-R, epidermal growth factor receptor and erbB is a receptor for epidermal growth factor. 2nd type of proto-oncogenes encode proteins, that function in signaling and, or they could be transcription factors. Src, again, this is something, that you remember are proteins, which are absolutely required for signaling in B cells and T cells. So, src and abl, src and abl, these oncogenes encode tyrosine kinases; the kinases, which are required for signaling.

Ras is a GTP binding protein; myc, jun, fos, these encode, these genes encode transcription factors. So, now, these all are required in a normal function of growth and I mean, signaling and growth.

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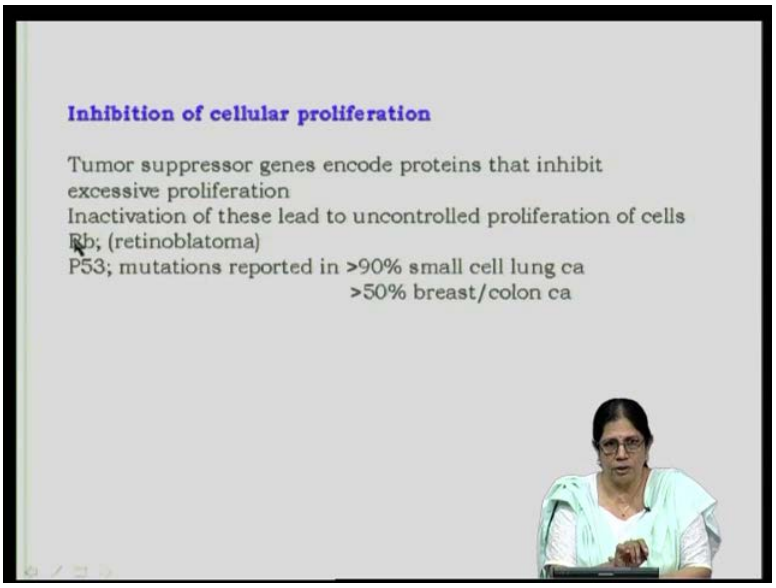

If there are mutations, therefore, in any of these, this can lead to uncontrolled proliferation. Now, these are genes, which are associated with proliferation, either at the level of growth factor themselves, I mean, the receptor and the legend itself, which induces signaling. So, there less signaling molecules, which now are mutated in a manner, that there is no control, can lead to extensive proliferation or uncontrolled proliferation.

There are proteins, which inhibit the tumor suppressor genes. Now, inhibition of cellular proliferation is the next, they are tumor suppressor genes. These suppressor genes encode proteins that inhibit excess of proliferation. So, therefore, what we know is tumor suppressor genes, in fact, are the ones, that are playing a role in the normal process of proliferation. One such gene is retinoblastoma, they are the ones; very well known P53 in activation on mutation. Any one of these would now lose, such cells would lose control on the, control of cellular proliferation and check proliferation. Therefore, mutations of these two genes are involved in, Rb is involved in the establishment of retinoblastoma and therefore, in fact, the gene was named Rb because it was first isolated from retinoblastoma. P53 mutations had been reported more than 90 percent of small cell lung carcinoma and more than 50 percent of breast and colon cancer and of course, in several other carcinomas.

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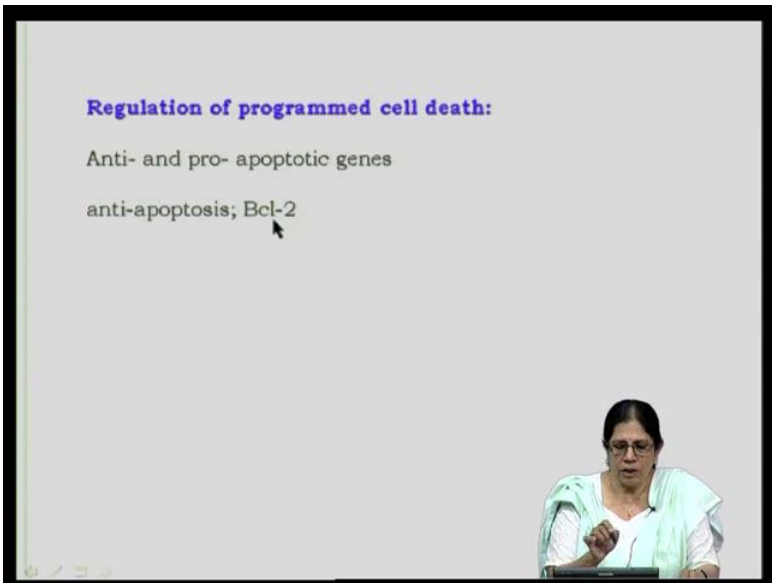

So, we have seen mutations of those genes the products of which induced proliferation, growth factors and those, that are induced are involved in signaling, which ultimately again go on to, you know, cell proliferation or the proteins, that can check proliferation. Mutations of any of these can lead to uncontrolled proliferation.

There, there are proteins, which are also large number of them, in fact, which regulate programmed cell death, a programmed cell death, this is apoptosis. You know, apoptosis is programmed cell death; apoptosis is absolute phenomena, which is absolutely required right from the development, from the embryonic stage, where differentiation is taking place up to, you know, in adult's life. Cells, especially one can think of cells, which are undergoing mutations, there are proteins, which can guard these mutations. In fact, I mean, in a way, that the proteins, when they, they are able to recognize mutations and therefore, stall the cells in a particular cell cycle.

There are pro-survival proteins and there are anti-survival proteins. Those that are pro-survival proteins such as Bcl, anti apoptosis protein, that means, pro-survival something like Bcl-2. Mutations in these, again would not allow a cell to undergo apoptosis and therefore, there would be uncontrolled proliferation.

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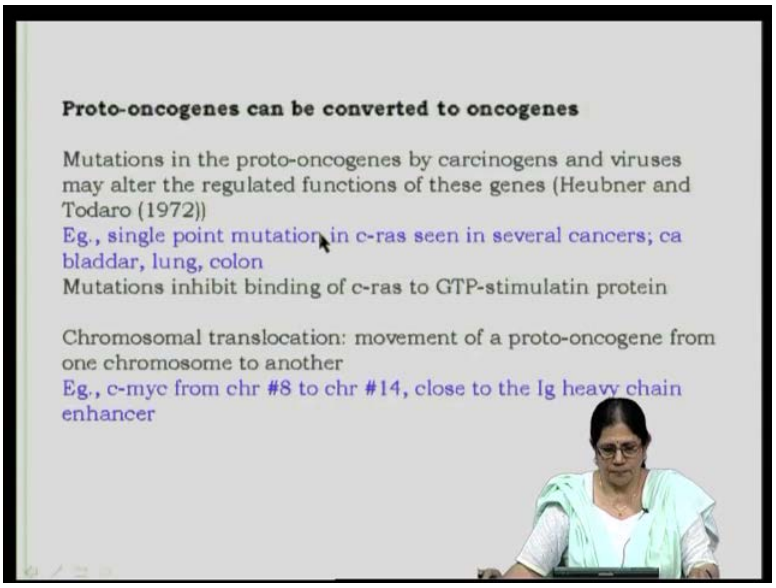

Going back now to the same thing that I said, proto-oncogenes can be converted to oncogenes and mutations in the proto-oncogenes by carcinogens, so this is where we link.

We know that carcinogens are cancer causing agents. They are the ones they think about mutations in the proto-oncogenes, making them, you know, oncogenes. It has been shown, that single point mutation in the c-ras, another oncogene, as seen in several cancers. And chromosomal, then next one is chromosomal translocation movement of a proto-oncogene from one chromosome to another can also lead to cancers.

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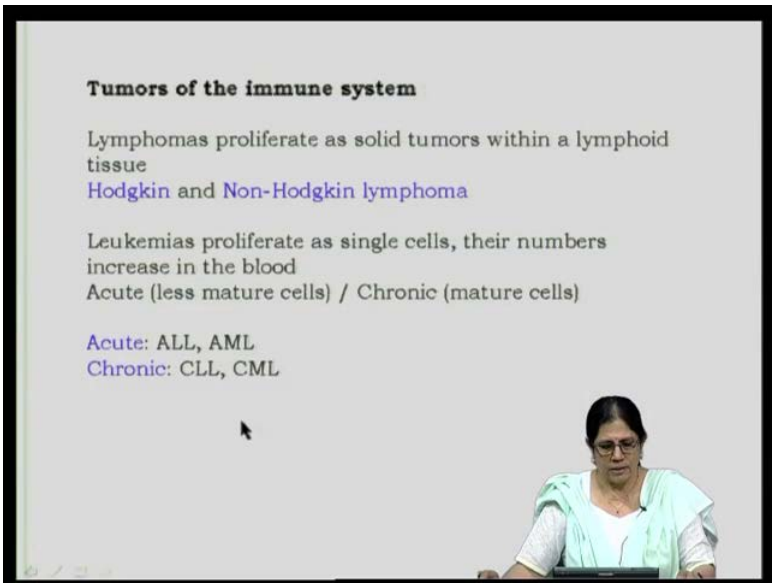

I will talk to you about this translocation in a while before which I have to introduce to you tumors of the immune system.

Tumors of the immune system, lymphomas proliferate as solid tumors, I have already mentioned that to you, with a lymphoid tissue and these are Hodgkin's and Non-Hodgkin's lymphoma. Leukemias proliferate as single cells, their numbers increase in the blood. Whereas, in case of lymphomas, they stay on as solid tumors and lymphoid tissue, well not really solid in the way carcinomas are, but they are enclosed in lymphoid tissue, whereas leukemias, which proliferate as single cells, they circulate in the blood.

Leukemias could be acute or chronic. Acute are cells, which are less mature in the development of T and B cells and are dealt with, you know, you will have acute cells where or chronic, now chronic will be mature cells. If you are talking about B cells, those will be the ones, which already have IgM and IgD on the cell surface; acute would be a step before, you know, pro- or pre- B cell stage. So, acute leukemias are known as acute lymphocytic leukemia or acute myeloid leukemia, depending on which lineage; chronic would be chronic lymphocytic leukemia or chronic myeloid leukemia.

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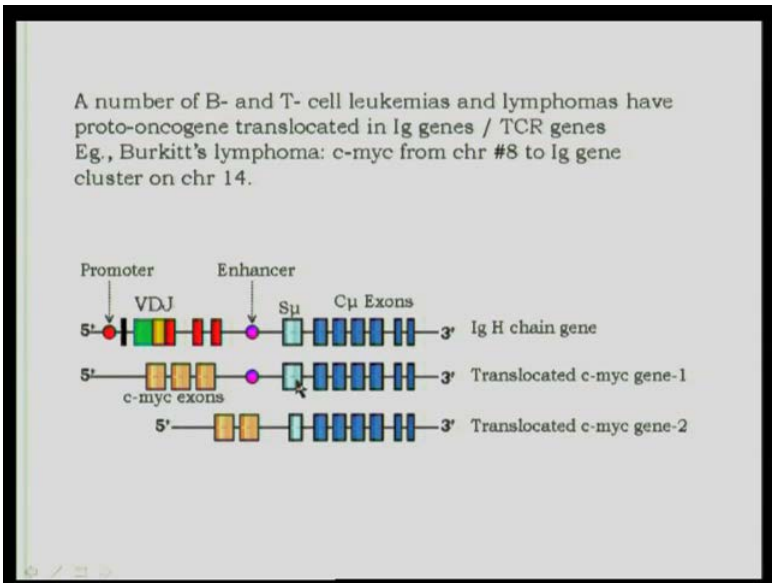

Now, I will come to the translocation. A number of B and T cell leukemias and lymphomas have proto-oncogene translocated in immunoglobulin genes or the TCR genes, very well known is the Burkitt's lymphoma, where the c-myc from chromosome 8 is translocated to the immunoglobulin gene cluster, which is present on chromosome 14.

Now, how does this happen? I mean, how does this lead to cancer? You can see, if you remember the immunoglobulin gene organization, this is the heavy chain and this immunoglobulin heavy chain has already undergone recombination giving VDJ. Now, if you remember the immunoglobulin gene organization, you have the promoter, which is on the 5 prime site of the leader sequence, which is on the 5 prime site of VDJ; you have the enhancer sequence on the 5 prime sites of the switch region genes.

Now, there are different conditions, two of which are being depicted here. You have the c-myc, exons; c-myc, which comes from chromosome 8, which is translocated just before the enhancer gene and therefore, now you have extremely high expression of c-myc. Now, there is, there are of course, these are translocation of c-myc on this with respect to gene 1 and gene 2.

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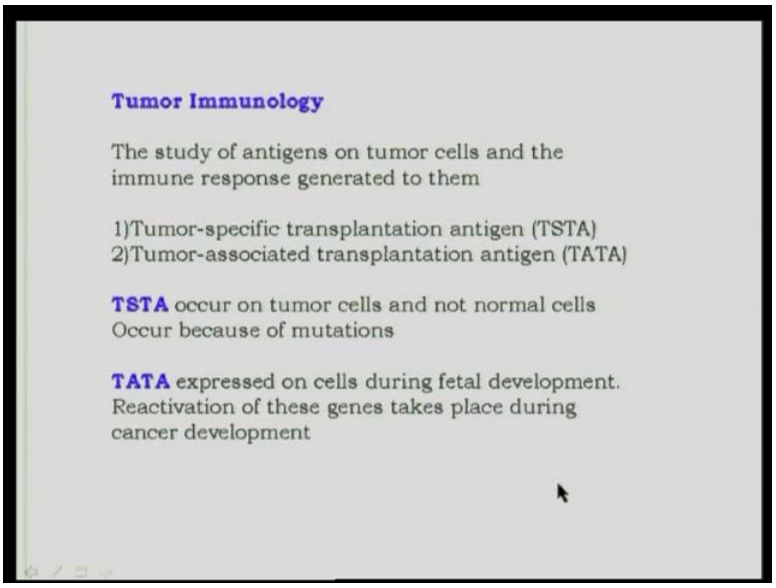

Tumor immunology, what is tumor immunology? The study of antigen on tumor cells and the immune response generated to these tumor antigen. So, are there any tumor antigens? That is why, in fact, we needed to, we need to know why are we studying cancer under immune system is because I said, that any mutation, that takes place in cells may bring about change in the cell surface, expression of certain molecules and they can, they, people, I mean, have seen, that there is either immune response, that can be generated to these antigens.

So, are they tumor specific antigens? Yes, tumor antigens can be studied or tumor specific antigens are known to be either tumor specific transplantation antigens or tumor associated transplantation antigen. Now, I will describe what each one is, but I would like to also tell you, that several years ago when (( )) of mice were not available, you know, cancer biologists were doing experiments where they were, they transplanted tumors from one mouse to another and found, that almost always, tumor regression did take place. It is only later, that they found, that that was not because of tumor specific antigens, but the regression was because of graft versus host reaction because of the expression of, you know, class 1 molecules on nucleated cells.

So, they have described these are transplantation antigens because all the experiments were done from one animal to other after transplanting this tumors. So, tumor transplantation antigens occur on tumor cells and not on normal cells and this is because

I mean, these are of course, tumor specific, so they occur on tumor cells because of mutations. On the other hand, tumor associated transplantation antigens are expressed on cells during fetal development, but these get reactivated during cancer development. So, they are tumor associated; we will deal with this in a little while.

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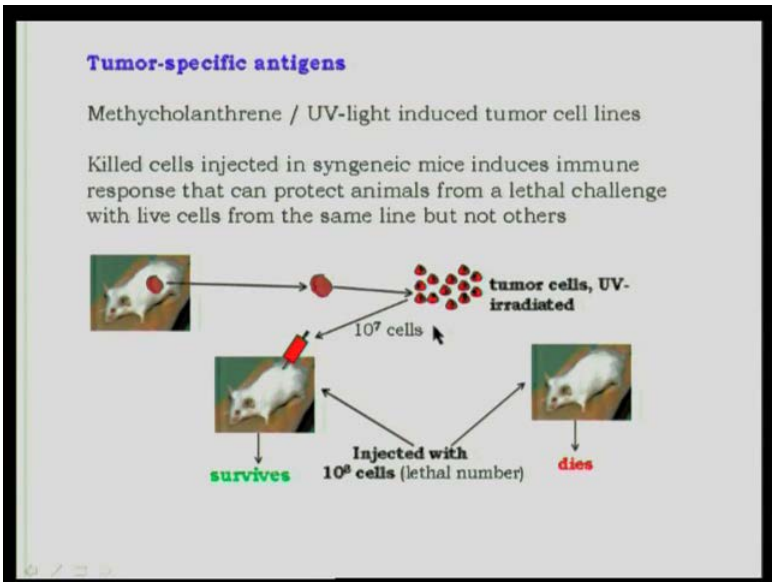

Now, were there any experiments that were carried out to show, that tumor specific antigens, in fact, can induce an immune response and whether this immune response is protective? There are classical experiments and large numbers of experiments; I will just tell you this experiment, which is a prototype experiment. Tumor specific antigens can, you know, have been seemed to be expressed in tumors, which have been established by the carcinogen methycholanthrene. In fact, this is one chemical carcinogen that has been utilized extensively on ultraviolet light induced tumor cells. So, mice that are now methyl, which, which have, which have received methycholanthrene developed tumors. You know, almost 80 percent of the mice, that received this with this reagent have been shown to induce tumors.

Now, if this, these tumors are, I will, I will describe the experiment. The tumors is now (( )) and single cell suspension may and these can grow cell lines. Now, if such cells are irradiated so that they cannot multiply anymore, so cannot establish a tumor and such cells when injected in to a syngeneic mouse, may be the choice of the same haplotype, the same HLA molecules or MHC molecules in case of mice, so that the rejection will

not be because of the MHC class 1 molecule. So, now, these, now the, a healthy mouse when injected with these irradiated tumor cells, a small number anyway, even let us say a 10 million. Subsequently, a few days later when the same mouse, which has received this irradiated cells is injected with a lethal number of a 100 million cells survives, and this survived. When the immune cells were then analyzed, this animal had T cells, which could respond to the cancer cells effectively, the cytotoxic T cells, as well as, had antibodies, that could specifically bind to these cells, also induce or activate the complement.

Now, an animal, a healthy animal, which has not received these irradiated cells earlier, this animal, would die. Now, such, this experiment has been done several times with several different cancers and yes, shows this, showed conclusively, that cancer cells have the capacity to induce an immune response that can be effective and protective.

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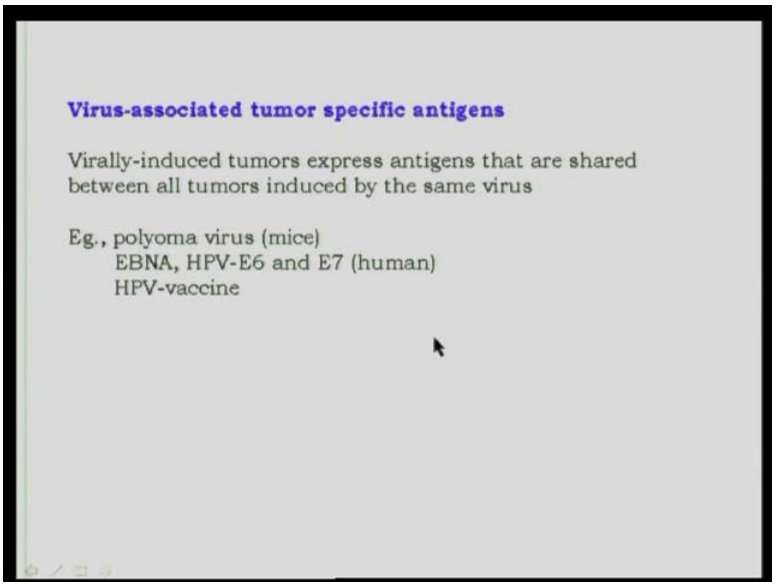

Virus associated tumor specific antigens, what I talked about is the one, that is associated with carcinogens. Now, virus, virally induced tumors express antigens, which are of virus origin, that are shared between all tumors induced by the same virus.

Now, this is different from what I discussed earlier with carcinogens, such as methycholanthrene, where every tumor could be different. In case of viruses associated tumor cells, all the, the one virus, when it induces a particular tumor where most of the antigen expressed on this tumor cells would share antigens from between other tumor

cells, but induced by the same virus. The examples are polyoma virus in case of mice, EBNA or Epstein Barr virus associated nuclear antigen, HPV-E6 and E7 in case of human and I like to just remind you, that HPV vaccine, in fact, has been in the market for controlling cervical cancer, which is associated with HPV.

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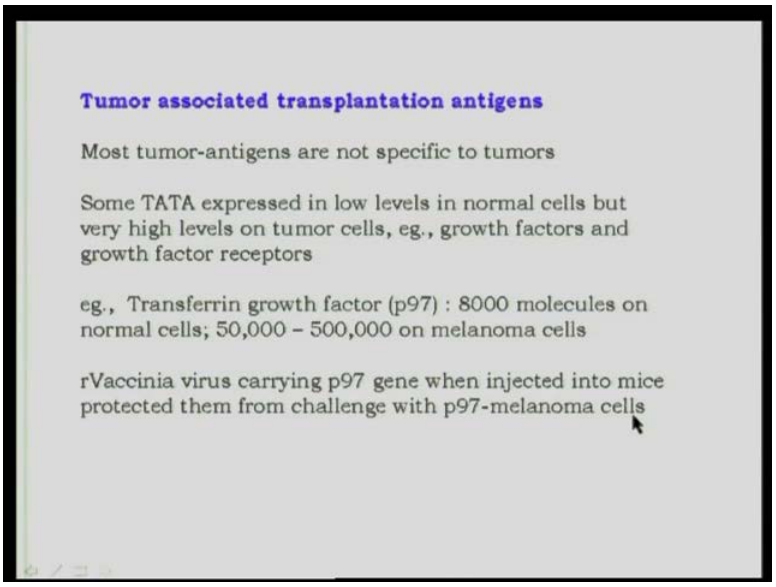

Tumor associated transplantation antigens, we talked about tumor specific antigens so far and we are talking about tumor associated transplantation antigens. These are not specific to tumors; they are present also in animal cells. Some of these antigens are expressed in lower levels in normal cells, but at very higher levels on tumor cells. Examples are growth factors and growth factor receptors. For example, transferring growth factor, which is known, which is known as p97, there are about 8000 molecules on normal cells, but these become 50,000 to 500,000 on melanoma cells. So, you can see, that this means over expression of growth factors.

Now, why are two cancer cells express these growth factors? Now, an example of this transferring growth factor and what does transferring growth factor do? It picks up iron and therefore, increase in the number of receptors would make such cells very efficient in sequestering iron. Similar would be in case of other growth factors, like epidermal growth factors. Increase in the number would now make the cell get the, give the cell an advantage of picking up even small, small number of the ligands. Normal cells would of

course, take, would require minimum molecules to be able to effectively pick up those that are required for the metabolism.

Now, in association with the, you know talking, about this p97 again. Now, recombinant vaccinia virus, which carries p97 gene recombinant virus, when injected into mice protected them from challenge with p97-melanoma cells, again showing, that one can generate an immune response to this p97 and this could be protected.

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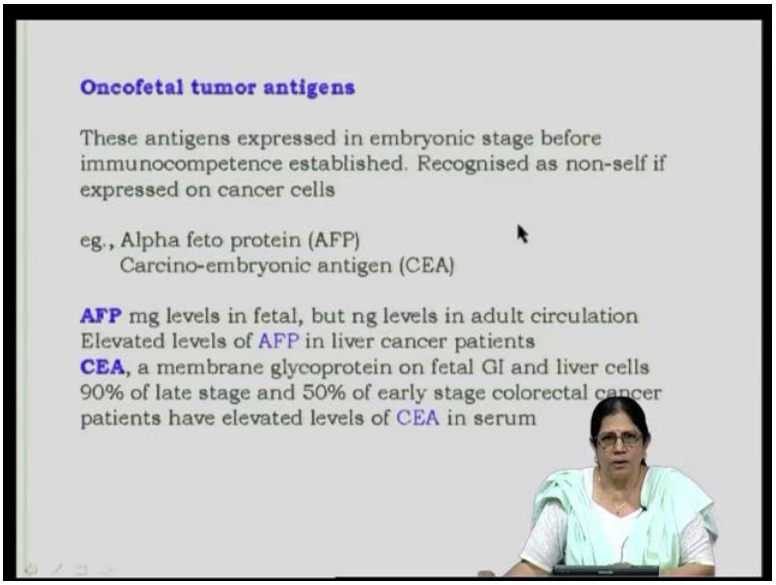

Another set of oncofetal tumor associated antigens are AFP or alpha feto protein and carcino-embryonic antigen. These are, what are called, oncofetal because these antigens, because these antigens are expressed in the embryonic stage before immunocompetence has been established, therefore they are recognized. So, that means, in the uterine, of course, condition when the embryonic is expressed in, then these would be recognized as non-self if they expressed on in adulthood. So, there are some cancers specially.

Now, let us talk about, alpha feto protein is present in, alpha feto protein is present in milligram levels in the fetus. Now, this is very similar to the serum albumin, but the same protein is present only in nanogram levels in adult, adult circulation. So, if this, like a million times lower concentration in adult as compared to the fetus. In certain cancers, in liver cancers, alpha feta protein is highly elevated. Therefore, in fact, alpha feto protein is a marker, a biomarker for liver cancer.

You know, where would it be used, that if the primary cancer is of liver, but metastasis occurs elsewhere and in fact, the primary cancer is very, very small and has not been detected and if I, alpha feta protein is measured or detected in the circulation of these individuals, this points to the person having metastatic liver cancer.

The other protein is, come, carcinoma embryonic antigen. It is membrane glycoprotein on fetal gastrointestinal cells, as well as liver cells. Now, this is CEA, this antigen is present in more than 90 percent late stage and about 50 percent of early stage colorectal cancer patients, so this is also another biomarker. So, these proteins are present in the fetus and are down regulated in adulthood, but certain cancers express these proteins and therefore, can be used as biomarker. So, therefore, these are new antigens though they are, they should, they are present, they are only present in the fetus and therefore, they are antigens of the cancer.

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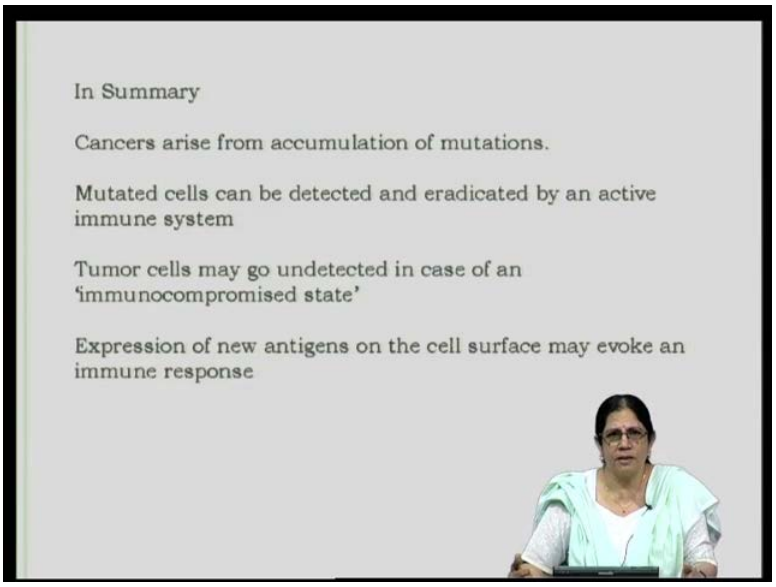

So, let me just end here by, I just like to go to the summary now, the last here before we come to the… In summary, cancers, all cancers arise from accumulation of mutations; that we have seen. It has this enough, ample experimental proof, that is, the mutations. In fact, what people have shown, that they have been able to establish cancers in-vitro, in experiments by transecting genes, which are known as oncogenes and also by creating in, you know, inducing mutations. Now, cancers therefore, arise from accumulation of mutations.

Now, these mutations, now all of us are so exposed to various, you know, all types of mutations, especially in the environment we have so many kinds of (( )), which are present. You all, you all must be, you all must have heard of, you know, carcinogens, which come from the emission of vehicles. So, therefore, there is no way, that we can avoid being exposed to carcinogens, but thankfully, our immune system is so robust, that they, the immune system can take care of these mutations, because the mutations, that take place in, in normal cells by these carcinogens, bring about expression of proteins on the cell surface of these cells, which can be recognized as non-self.

You may have by now studied natural killer cells, which can recognize altered cells. Natural killer cells, if you remember, can mount an extremely unaffected immune response to the altered, virally altered cells, as well as cancers. If you think in terms of the innate immune response with respect to cancer, in fact, natural killer cells come to the forefront. So, natural killer cells should be able to eliminate any mutated cell, which can be recognized as non-self.

Now, if, you know, I told you, that cancer arises, it is, it is cloned, it is monoclonal. So, one type of cancer is from one type of cell, one cell. So, before the cell becomes a mass of cells, immune system, if it is robust, is able to mount an immune response, then this cells are deleted. In fact, this is what is happening to us all the time; cancers go, of course, are detected effectively and thrown out of the system. However, some mutated cells can be detected and eradicated, sorry, some mutated cells can persist because they go undetected.

So, let us now understand this mechanism. A person, who is immunocompromised, may allow the tumor cells to go undetected. In an immunocompromised situation, a tumor cells has a better chance of slipping by and once it slips it is able to establish itself and the tumor cells become, the tumor becomes an entity. We do know, therefore, that an effective immune system or robust immune system is able to recognize cancers as non-self and is able to abrogate or delete this. However, we also know from whatever we, we have seen so far, that most cancers are able to express new molecules on the cell surface, which can effectively mount an immune response curtailing the cancers.

Now, in the next class I will continue with this particular topic and tell you how cancers establish themselves, what is this mechanism by which the cancer is able to evade the

immune system? In fact, there is not just one mechanism, there are several and establish itself such that even if the immune system is robust, the cancer is free to roam around and establish metastatic (( )) in the rest of the body. So, I will see you next time with the topic on immune system and cancer, continue with it.

Thank you.