Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis

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Lecture 41 : Molecular genetics in tumorogenesis

Namaskar. Welcome back. Today we are going to start another new week in the NPTEL course of Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis. In this week we are going to discuss the importance of molecular diagnostics in management of one very important domain that is cancer. So, before we directly go into the diagnostics I want to discuss a bit of the role of molecular genetics in cancer pathology or pathogenesis. So, today's class is on molecular genetics in tumorogenesis.

The concepts which we are going to broadly cover are the different characteristics of a cancer cell, cell signaling process which helps in carcinogenesis, different genes which are frequently played role in which frequently play role in carcinogenesis like oncogenes, tumor suppressor genes also DNA repair genes. Then the associated mutations which you can see in cancer and different mutagen starting from chemical mutagen, then radiation, tumor viruses which causes mutation in cancer and then we are going to discuss the invasion and metastasis their principles how they help or they cause in carcinogenesis. So, coming to a very pertinent part of tumorogenesis is basically cell cycle and its regulation. So, in a normal cell the proliferation and progression of the cells through the cell cycle are basically strictly regulated, regulated by a group of proteins which are basically the regulator and how they regulate they basically control different events or incidents which are occurring in the cell cycle.

In different phases it G 1 phase, G 2 phase, synthetic S phase or mitosis everywhere there are events which are occurring and they are regulated by these pool of proteins. Among them a very important one is cell cyclin, cell cyclin dependent kinases or CDKs they known as the master protein kinases. They basically check whether the cells are properly passing through the check points. So, here you can see these are the check points actually. These are the check points are basically the transition of one phase to other.

So, this cyclin dependent kinases they basically strictly regulate that cells must complete

the the events of one phase properly and no incompletely covered incident containing cell passed to the next phase. Suppose a cell has not properly evolved in a specific phase suppose it in it is in G 1 it has not properly evolved in G 1 phases events. So, CDKs basically regulate in such a way that till it completes its all the events in G 1 phase it will not pass into the S phase. So, here you can see there is one CDK 2 along with some other proteins which help in regulate the cell cycle through it is check points. So, another protein is basically cyclin.

So, the cyclins actually activates the subunits and they are activated throughout different phases of this cell cycles and regulate rather help cyclin dependent kinases in their activities. Then there are different inhibitors as well inhibitors which basically inhibit kinases. So, when a cyclin dependent kinase is inhibited basically a cell is allowed to pass to the next phase by after crossing the check points. Apart from that there is another point that is R point which is restriction point. In the restriction point suppose a cell is actually halted for completion of it is anomalies or completion of it is events or if a cell is put out from the cell cycle to complete is complete the events it can reenter into the cell cycle via the restriction point.

Then there are extracellular mitogenic signals which basically regulate the regulatory proteins what are those proteins like transcription factors. So, one very important thing is transcription factor which actually helps in the synthetic phase where basically replication is occurring. So, the replication the S phase CD case are basically regulated by this transcription factor and it gives the signal that the DNA synthesis or the replication now can start. So, there are different extracellular mitogenic signals. Now, why I am saying all these things because in cancer you know there is unregulated proliferation of the cell.

So, if the cell cycle is somehow the this regulatory cyclin dependent kinases or cyclins or CDK inhibitors their functions are jeopardized in some phases what will happen the cell will go on in this cell cycle for proliferation without any inhibition without any regulation even if there is any anomaly present in a cell without completing it or without correcting it will pass on to the next phase. So, these are the possible ways how cells enter in can enter in carcinogenesis phase. Now, one very important transcription factor p53 which is also known as the gurgian of the genome it basically helps in arresting the cell cycle as well as inducing apoptosis. Apoptosis is basically a programmed cell death you all must be knowing this term. So, what this p53 does it basically identifies if there is any damage any anomaly in a cell.

So, what it does it arrest those cell to pass on to the next phase. So, it arrests the cell cycle and tries or gives the cell an opportunity to heal its anomalies, but if that damage is beyond repair in that case that cell undergoes apoptosis. So, basically p53 it acts as a

gurgian and does not allow one anomalous cell to pass on for proliferation, but what is seen this p53 is basically deranged in majority of the cancers. So, what happens if there is an inactivation of this p53 protein a cell can pass on to the next phase once again containing all its anomalies. Now, in cancer you know that there is immortalization of cell.

So, what is that immortalization the cell can have an infinite life span it can go on proliferating generating newer cells containing those anomalies. Now normal mammalian somatic cells they proliferate for a limited number of times before undergoing senescence. Senescence are those senescence cells are those cells which they do not they do not undergo proliferation, but of course, they remain metabolically active. Now one very important enzyme is telomerase if you remember we discussed telomerase. So, this telomerase is basically responsible for maintaining the telomeres the ends of the chromosomes.

Now, if this telomerase remains active what will happen the telomere shortening will be restricted. So, the cell can undergo multiple proliferation beyond the limited number which actually happens in mammalian somatic cells. Now, this you can see majority of the tumors basically they contain one active telomerase enzyme which can keep which keeps the cells actively proliferating even the somatic cell they can actively proliferate beyond the normal limited life span. So, this is how cells can cancer cells can undergo immortalization. Now cell signaling plays one very important role in carcinogenesis.

Normally what happens the growth factors and the they are respective receptors basically pass on the signal for cell proliferation. So, what is the normal phenomena one growth factor? So, consider this as a growth factor the growth factor binds to it is respective receptor which is present over cell membrane and that growth factor receptor is activated transiently and that activates that activation of this growth factor basically activates several downstream signal transducing proteins like Ras protein is one such example. Now, Ras protein undergoes downstream cascades of activating kinases those kinase activate some proteins which can regulate the genes and regulate the gene expression at different level it can be cytosolic regulation or it can be nucleus regulation. So, what happens finally, in the nucleus there is activation of the transcription of specific genes which gives the signal for cellular proliferation this is the normal phenomena. Now in case of cancer cells what happens this growth signals or proliferatory signals are persistent.

How they are persistent? Either the cancer cells are generating their internal stimulatory signals which is not dependent over the normal signals. It can be such like there is one mutation in this growth factor receptor which causes a constant activation of the growth factor receptor devoid of the growth factors. Then even the downstream kinase

expression these genes, genes for the downstream kinases, genes for the downstream enhancer proteins they can also be mutated in such a way that there is a constant active downstream element present. So, these are the causes which finally, activate this proliferative cell signaling in carcinogenesis. Now, there are few genes the terms are very common which we will commonly learn in tumorogenesis are oncogenes, proto oncogenes, tumor suppressor genes along with some DNA repair genes.

So, proto oncogenes are basically the mother of oncogenes. So, when this proto oncogenes are mutated definitely they give rise to oncogenes. Now what happens this proto oncogenes are basically these gene products take part in normal physiological process. What are those process? They stimulate cell division, cell division they stimulate they inhibit the cell differentiation they halt cell death. So, basically they give a signal for cellular proliferation for normal development and maintenance of tissue and organ function.

But if this proto oncogenes are mutated they give rise to oncogenes in such a way that their expression is either increased or there is some there is suppose there is point mutation and deletion or insertion these are the type of mutation based on that there is over expression of this proto oncogenes. For that what you will get one hyperactive gene product that is constantly giving signal for cell division constantly inhibiting the cellular differentiation constantly inhibiting the cellular death. How it can be done? Suppose a proto oncogenes basically activating a promoter region. So, if there is the mutation a promoter is constantly activated or an enhancer is constantly activated there can be gene amplification which can give rise to extra chromosomal copies of proto oncogenes. So, there are multiple proto oncogenes which is giving a greater signal for cellular proliferation.

Similarly there can be chromosomal translocation where a proto oncogenes is basically translocated. So, it has the proto oncogenes if it is translocated to some other chromosome to some other location and is added to another gene in such a way that that gene product is constantly activated. So, these are the different mechanism by which proto oncogenes when converted to oncogene can gives a constant cellular proliferative signals. Now, these are the list of oncogenes and their associated cancer. So, you can see the proto oncogenes can be growth factors can be growth factor receptors then can be the downstream proteins which transduces or transfer the signals.

Nuclear regulatory proteins which are basically the transcription factors which stimulate the increases the transcription also cell cycle regulators like cyclin cyclin dependent kinases and you can see from the previous list as well when they are mutated they can give rise to this list of cancers list of tumors here. So, these are the very common examples of proto oncogenes when they are activated or rather mutated they can give

rise to different types of malignancies. So, here are a long list you can go through it you can learn what are the associated oncogenes with different tumors. Then coming to tumor suppressor gene of course, by the name it is evident that this tumor suppressor gene somehow help in suppressing the tumorigenesis how. So, basically this tumor suppressor genes encodes proteins, proteins which are which acts as receptor for secreted hormones that basically inhibit cell proliferation.

It can be a negative regulator of cell cycle. So, basically cell cycle inhibitor negative regulator of growth signaling pathway. So, whenever there is a signals for cellular proliferation it acts as inhibitor for that. In cell cycles it act act it basically gives negative signal to the check points it checks on whether any damage DNA is passing on. So, downstream inhibition for the cell cycle then also it induces apoptosis and synthesizes some DNA repair gene.

So, these are the normal functions of tumor suppressor gene. So, basically when there is a transformation of a normal cell to a cancer cell you can understand that this tumor suppressor genes function is somehow inhibited. So, there is loss of function of one or more tumor suppressor genes or there are defective copies of their downstream encoded proteins. So, one very common example is retinoblastoma protein coming from RB gene which basically controls the cell cycle transition from G 1 to S phase. So, what it does this RB protein it binds with the transcription factor and that transcription factor is required for a transition or rather activation of the transcription.

So, when this RB binds to this transcription factor it is basically the transcription factor is basically inhibited. So, there is inhibition of the transcription. Now if there is inhibition of this RB protein what happen the halt over this transcription is now over. So, the cell can easily enters the S phase. So, it can start replication and downstream transcription.

So, what happens in carcinogenesis is basically disruption or deletion of the RB gene function and finally, causing one uncontrolled cell proliferation. Again these are the list of tumor suppressor gene. So, you can see here is negative regulator for cellular signaling these are DNA repair genes when inhibited give rise to damage DNA, then protein kinases here you can see these are the downstream signaling Ras GTPase enzymes, transcription factor repressor, RB or retinoblastoma protein another transcription factor. These are the different kinds of activity of the tumor suppressor gene when inhibited it can either give rise to different inherited cancer or also some spontaneous cancer can occur from the from the mutation in tumor suppressor gene. Then coming to the pathogenesis of cancer basically there are multiple types of mutation which are associated with cancer.

So, what happens there is a life time accumulation of somatic mutation which build which is building inside the cells which finally, gives rise to tumorogenesis. Now it is very common in somatic mutation not much common in germ line, but there are few rare cases of inherited cancer which are associated with germ line mutation as well. Now normally there is mutation in normal people normal human being non human being who are not having tumors, but the basal mutation rate for them is very very low. What happens these mutations get an enhancement when the cells comes in the cells come in contact with some carcinogens. Carcinogens like chemical carcinogens it can be radiation it can be different viruses which can gives rise to tumor.

Now chemical mutagen are basically some groups of chemical that can modify DNA it can rather damages the DNA like alkylation or deamination of DNA bases. Then there is intercalation of different base care and formation of DNA adducts also oxidative damages. Then x ray and radioactive radiations they also damages DNA via double strand DNA breakage. In UV radiation it gives rise to formation of pyrimidine dimers by cross linking the adjacent pyrimidine bases. So, these are the chemical mutagens and radiation associated mutagenesis.

There are few virus which can cause cancer like one very common example is human papilloma virus. So, these are oncogenic virus oncogenic virus contains oncogenes. So, their genomes the part of their genomes basically resembles or different oncogenes. So, there is a postulation that probably these oncogenic viruses they their genome is basically by evolution evolutionally evolutionary platform they are basically derived from proto oncogenes. Whereas, other viruses they do not contain such gene.

So, this oncogenic viruses are typical having a pattern of their genome which can be similar with the proto oncogenes. And how they stimulate the proliferation in the host cell they basically inserted in a promoted region. So, it induces it acts as a proto oncogene and induces the cellular proliferation. Then some expression of the protein can inhibit the tumor suppressor protein also some protein can inhibit apoptosis. So, this is how the integration of the viral oncogene can finally, behave like oncogene or can inhibit the tumor suppressor gene.

But remember viruses they rarely complete the carcinogenesis what they need is some additional factors which fully activate the carcinogenesis inside the cell. There are some processes which basically activate this viral oncogene inside the cell. Apart from these mutagens there are certain mechanisms in cell which can give rise to tumor. One such event is genomic instability in tumor cells. Now genomic instability can be chromosomal instability can be microsatellitic instability.

Now chromosomal instability is basically when the chromosomes are unstable. Normal

in such a way that the whole chromosome or a part of chromosome are duplicated or deleted. So, they are basically not behaving like the normal ones. And finally, what they give what is the output they can give rise to aneuploidy where there is loss or gain of a whole chromosome. So, there is incorrect number of chromosome or the haploid loss or haploid gain can be there which is known as polyploidy.

Now these aneuploidy or polyploidy are definitely associated with poor prognosis in cancer patients. They are associated with different types of solid tumors and hematological tumor. Now what is the probable mechanism there is lack of active p53 protein which. So, if there is inactive p53 protein. So, you know these loss or gain of chromosome they can pass on to the further the progenies of the cells on further proliferation.

Apart from that other mechanisms like mitotic defects can be there which result in chromosomal missegregation. So, this can cause chromosomal instability. Similarly, microsatellitic instability are basically unstable regions of DNA sequences. Now what are those regions these microsatellites are basically repetitive DNA sequence in the genome and they are much more prone for shortening or extension if the mismatch repair enzymes are defective. So, if there is mismatch DNA repair system inactivation what happens this microsatellite regions they give rise to some sort of instability which causes the tumorogenesis.

Then there is loss of heterozygosity. So, what is loss of heterozygosity? In a diploid cell mutation of one only one allele of a tumor suppressor gene is not sufficient to cause cancer because the other normal one is present. But what they give rise to is a functional phenotype where if there is where one wild type allele is basically the normal one whereas, the other one the other tumor suppressor gene is mutated. But when there is second heat which causes the normal allele deformation they give rise to this loss of heterozygosity. So, initially the pattern was heterozygous where one normal allele and one mutated allele of tumor suppressor gene was present. So, there was heterozygosity, but when there is second heat these heterozygosity is lost.

So, what we get is a full fledged cancerous phenotype. So, that is loss of heterozygosity. Then of course, there is DNA hyper or hypomethylation we have discussed that DNA methylation are one very well established method of epigenetic changes in our system. Now in cancer DNA hyper methylation is often involved with suppression or silencing of tumor suppressor gene also conversely DNA hypomethylation may contribute in activation of oncogene. So, both way separation of tumor suppressor gene or activation of oncogenes due to hypo or hyper methylation can give rise to cancer.

Next we are proceeding for invasion and metastasis in tumorigenesis. So, the very the very common pattern of tumor is to invade in other tissue or metastasis or spread to different body parts via either blood stream or lymphatics. So, this is metastasis. So, these are the causes of spreading of tumor cells from the primary site to different other sites. Now, this metastatic cells which are basically they which are basically away from their primary sites they are less adhesive to the normal cell.

So, more they have greater tendency for spreading also they can degrade and penetrate the tissue barriers tissue barriers of extracellular matrix which surrounds the tissues extracellular matrix or basement membrane surrounding blood vessel this is how they can penetrate and get into other tissues or can penetrate inside the circulation in the systemic circulation for secondary spread and raising secondary colonies. How it happens? So, what happens initially from the primary site there is local invasion. So, tumor cells invade the local tissues then there is intravasation. Intravasation means they gain entry into the blood or lymphatic vessels of the same tissue. Through this blood vessels or lymphatic vessels they are transported to distant site.

In a distant site suppose there is arrest of those tumor cells. Now what will they do? They will try to escape from the circulation and enter in those distant organs. So, what happens there is extravasation followed by micro metastasis in a tissue which is different from the primary tumor. Here they colonize and finally, causes metastasis or spread to different other regions different from the primary tumor site.

So, this is the basic principle of metastasis. In metastasis there are multiple factors which help in spreading this metastasis spreading the tumor cells. One such important phenomena is EMT or epithelial mesenchymal transition. What happens the tumor cells it initially behaves like the epithelial cells, but soon it loss it loses its epithelial characteristics and gain the newer mesenchymal phenotype which gives it the access into the blood stream. So, when the blood stream it behaves like mesenchymal leukocytes, mesenchymal leukocytes which are which are of mesenchymal origin. So, they behave like that and can be circulated through the blood stream.

So, basically the expression of their different markers this metastatic cells they expresses markers which are common for mesenchymal cells. So, initially they have they have markers of epithelial cells like Epcams, Ecadherin, Cytokeratins, but now there is up regulation of mesenchymal pattern when there is EMT. So, they expresses Vimentin, N-cadherin which are marker for epithelial marker for mesenchymal cells. So, what it does this EMT basically gives one metastatic potential gives one more aggressive invading property to the cell to the tumor cell also this metastatic this mesenchymal CTCs or mesenchymal tumor cells they are basically very common for resistance to cancer therapy. Then, the cellular motility is also helped by small g proteins

those are activated by cytoplasmic signaling pathways, their over expression is a phenomena in tumor cell also different matrix metalloproteinases are over expressed which causes which helps in rather digestion of the extracellular matrix.

Then, newer blood vessels are required for tumor spread that is angiogenesis. Now, this angiogenesis are also helped by over expression of VEGF that is vascular endothelial growth factor basic fibroblastic growth factor or BFGF. So, these are over expressed to assist the metastasis and angiogenesis. Now, stromal micro environment is also helping carcinogenesis. So, what happens this cleavage of matrix components releases some angiogenic factors which promotes newer vessel formation and favors the cell cancer cells motility.

So, there are different growth factor signals which gives the signal for angiogenesis. So, coming to the summary which is basically the hallmarks or characteristics of cancer cell. So, what happens cancer cells are self sufficient in growth. They are getting growth signals without actual growth factors, they are insensitive to the inhibitory signals for proliferation. They can pass the they can rather dodge the apoptosis even if they are carrying some damage DNA, they can dodge the DNA repair mechanism.

They have one limitless replicative potential also there are certain factors like angiogenic factors which are getting constant stimuli for angiogenesis which causes metastasis in helps in metastasis of the cancer along with some expression of digesting enzymes which causes digestion of the surrounding extracellular matrix which gives the cancer cells a potential to spread from the primary site to the distance site. So, these are the key points for this lecture. These are my references and see you in the next class. Thank you.