Cell Biology: Cellular Organization, Division, and Processes

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Lecture 33

The Cancer Cell

Hello everyone, I am Shikha Laloraya, Professor of Biochemistry, at IISc. Welcome to this lecture on cancer cells. In the course on cell biology, we have learned a lot about normal cells, how they are organized, how they function, and how they reproduce. In this lecture, we will understand how a normal cell becomes a cancer cell.

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So, what is cancer? Cancer is a disease characterized by uncontrolled cell division of cells that have lost a normal regulation of cell division that keeps it in check. The cells may also lose their normal organization and they can break away from the surrounding cells and invade other tissues. There are two main key defining characteristics of cancer cells that is, abnormal or unrestrained proliferation, which is defying the normal controls on their growth and division, and their ability to invade surrounding normal tissue and to spread to regions, which are normally occupied by other cells.

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A tumour is a mass or growth resulting from an abnormal proliferation of cells that divide more than they normally should. Tumours can be either benign tumours, that is they remain restricted to the original location and they are non-invasive, or they could be malignant that is they can invade the surrounding normal tissue and they can spread to other sites in the body by the circulatory or lymphatic systems. So, malignant tumours by definition are invasive. These types of tumours can form secondary tumours termed metastasis at sites which are distant from the original location of the tumour. And this process of spreading to distant sites is term metastasis; the metastasis can actually kill the patients.

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Cancers are classified or named based on the cell type and the tissue type that they arose from. For example, carcinomas arise from epithelial cells, these are the most common kind of cancers, sarcomas arise from connective tissue or muscle cells, leukemias and lymphomas arise from hematopoietic cells and lymphocytes, likewise the chondrosarcoma arises from cartilage, a melanoma raises from skin pigment cells. Various cancers arising from the cells of the nervous system are named as per the cell type that they arose from, for example a glioma from a glial cell, neuroblastoma from immature nerve cells etc. The cancers, which arise from these different specialized cell types can actually be considered as different diseases altogether.

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Most cancers arise from a single abnormal cell that is they are clonal. Cancer cells have got somatic mutations. Mutagenic agents can enable the development of cancers. And the development of cancer is a multi-stage process resulting from a gradual accumulation of mutations. Tumour progression refers to the evolution of a mild disorder of cell division into a rapid proliferation and acquisition of invasiveness. And this process involves successive rounds of heritable changes, which could be genetic and sometimes also epigenetic, which are then followed by natural selection selecting for the fittest cells that can survive in the inhospitable environment.

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This multi-step process leading to progression of stages of a gastric cancer to a metastatic cancer are shown here, where the gastric cancer, which is originally limited to the inner lining proliferates and then it invades into the interstitial space. And thus, it crosses this supportive tissue and the muscle and even the outer lining of the stomach. Various stages of course, are defined by the size of the tumour and also the ability to invade the surrounding tissue. So, in the most advanced stage you can see the escape of this cell into the interstitial space from where it can spread to other parts of the body and perhaps initiate another tumour elsewhere.

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Cancer cells also exhibit genomic instability and many chromosomal aberrations. So, these cells are basically genetically unstable. Chromosome aberrations such as deletions or amplifications or duplications of part of the chromosomes or even inversions or translocations are quite commonly seen in cancer cells.

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Cancer cells also exhibit a transformed phenotype, that is, the cancer cells are abnormal in shape, in motility, and in their response to growth factors. Cancer cells can divide even in suspension and show anchorage independent growth. Normal cells cease dividing after reaching confluence on a

substratum, for example on a tissue culture plate, and this is referred to as contact inhibition. But cancer cells continue to divide and pile up, that is they have lost this property of contact inhibition.

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Importantly, cancer cells exhibit altered sugar or glucose metabolism. And this phenomenon is termed Warburg effect after the scientist who discovered it. This basically refers to a shift from oxidative phosphorylation to aerobic glycolysis. So, cancer cells can import large quantities of glucose into their cells and of course, this property is also useful in imaging of tumours by PET-scan using fluoro-deoxyglucose. So, a lot of glucose is imported into the cancer cell. And also, the rate of glycolysis is increased tremendously and as a result of this the cancer cells they can produce a large amount of lactate from pyruvate even in the presence of oxygen. So, normally this pyruvate is sent over for oxidative phosphorylation but in this case the oxidative phosphorylation is reduced. And many of the glucose-derived carbon atoms instead are used as building blocks in the biosynthesis of proteins, nucleic acids, and also various other molecules, which are required for this rapidly dividing cell.

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Cancer cells also show resistance to stress. Now cancer cells have accumulated DNA damage and chromosome breakage. And normally these processes lead to apoptosis also. Yet, despite this genotoxic stress they do not undergo apoptosis. Cancer cells can also divide beyond the normal limit of cell division in culture, which we discussed in an earlier lecture is referred to as the Hayflick limit. Human cancer cells avoid replicative senescence, and this could be perhaps by maintaining telomerase activity as they proliferate, unlike normal cells. Or it could use an alternate pathway based on homologous recombination, which is referred to as the ALT pathway for maintenance of chromosome ends.

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Now the supporting connective tissue or stroma surrounding the cancer cells in the tumour is also referred to as a tumour microenvironment; it also contributes to the development of the tumour. The tumour microenvironment consists of fibroblasts, white blood cells, endothelial cells and pericytes and smooth muscle cells. So, cancer cells secrete signalling proteins that alter the properties of the surrounding cells in the tumour and also some proteases that can modify the matrix surrounding them. The stromal cells on the other hand also secrete proteins that stimulate the growth and division of the cancer cells.

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This slide shows how a tumour microenvironment can help in the development of the tumour. So, for example the tumour-associated fibroblasts, which are shown here, they are somewhat altered- they become different from normal fibroblasts. And in a tumour both the tumour cells as well as stromal cells, they increase the production of TGF beta -transforming growth factor beta.

TGF beta acts on the surrounding stromal cells, immune cells, endothelial cells, and also smooth muscle cells, you can see in many of these it is playing a role. And it causes immunosuppression and angiogenesis, which makes the cancer even more invasive. TGF beta also converts the effector T-cells which normally attack cancer with an inflammatory reaction into regulatory or suppressor T-cells, which turn off the inflammatory reaction.

So, a tumour may become dependent on its stromal cells because it has brought about so many changes in its surrounding stromal cells and an example of this is that the growth of transplanted carcinomas in mice requires the tumour-associated fibroblasts for proliferation. The normal fibroblasts are not sufficient for this indicating that the tumour-associated fibroblasts are somewhat different, and they provide something, which helps further proliferation of the tumour cells.

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Some cancers also have a small population of cancer stem cells. Now these cells, although they are rare, they can divide indefinitely. The cancer stem cells like normal stem cells, self-renew. And they also produce large numbers of dividing transit amplifying cells that have limited capacity for self-renewal. The transit amplifying cells are of course, much more numerous and they constitute the majority of the cell population in tumours. So, the cancer stem cells are rare, they are hard to find. However, the cancer stem cells can be identified based on the presence of stem cell specific cell surface markers and also their enhanced ability to form tumours.

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This figure shows the importance of cancer stem cells in tumour establishment, even at a secondary site. So, these cells when they become mobile and invasive, they can cross the endothelial cell layer and enter into the bloodstream. From there they can also cross the boundary and establish a metastasis at a secondary site. And of course, they have the potential of self-renewal and also producing the cancer cells. So, if these cells escape from the normal tumour and reach at a different site then they can produce a secondary tumour or a metastasis.

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The steps in formation and progression to invasive ductal carcinoma of the breast is shown here. So, this initiates with intraductal hyperplasia followed by its progression to atypical intraductal hyperplasia and then into an intraductal carcinoma. These cells, as the cancer progresses can become invasive and cross the boundary of the duct to metastasize, as is shown here in the fourth stage of invasive ductal carcinoma.

The steps in breast cancer to brain metastasis are explained here. The invasive cancer cell crosses the two layered epithelium and the basement membrane of the duct, and it migrates and it enters into the bloodstream after squeezing between the cells of the endothelial layer. So, now it has entered the bloodstream and it can actually go to other parts of the body. It can extravasate and it can also cross the blood-brain barrier to form a new tumour in the brain.

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So, to summarize, the hallmarks of cancer are mentioned in this figure. And this has been adapted from an interesting review article by Hanahan and Weinberg. The original hallmarks that were described earlier are increased or unlimited proliferation and replicative immortality of cancer cells, the ability to resist cell death, activation of invasiveness and metastasis, induction of angiogenesis that is blood vessel formation. Then evading growth suppression and sustained proliferative signalling which again helps in the uncontrolled division of these cells. There also certain enabling factors for cancer and these include the deregulation of cellular energetics, the immune evasion that is avoiding destruction by the immune system, and tumour promoting inflammation and genomic instability and enhanced mutations.

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So let's try to understand what causes cancer. So, there are agents that can bring about mutations, which can cause cancer and these are referred to as carcinogens. So, carcinogens can be of different types but basically they are agents that cause cancer by inducing mutations. And examples of these are many; many harmful chemicals in the environment that we are surrounded by. And not only that, solar UV radiation can cause mutations, aflatoxin, carcinogenic chemicals present in cigarette smoke for example, and there can be many others as well.

Tumour promoters are agents that contribute to cancer not by causing mutations but by stimulating cell proliferation. So, when the cells are dividing a lot, then there is a chance that spontaneous mutations can occur more readily obviously in rapidly dividing cells as opposed to non-dividing cells. And examples of such tumour promoters are hormones such as estrogens, asbestos, and a few other chemicals as well.

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Some viruses cause cancer, and this is well known, in fact, one of the oncogenes was discovered in cancer-causing viruses. So, how viruses do this is, actually there are many different ways: one way is by inactivating the tumour suppressor genes. And also, there are different classes of viruses so, for example retroviruses can bring in oncogenes and we have discussed the example of Rous sarcoma virus which had the SRC oncogene. The viruses which cause cancer are referred to as tumour viruses. And mentioned here are some of the human tumour viruses, human papilloma virus, Hepatitis B virus, and Epstein-Barr virus and also HTLV.

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Now mutations in certain genes, which have recently been termed cancer critical genes contribute to cancer development. So, examples of these genes are proto-oncogenes, these genes are normally present in cells. And a gain of function mutation in these genes can produce an activated version of the gene and it will code for an activated protein or an over expressed protein that then functions as a dominant transforming oncogene.

So, examples of proto-oncogenes are *c-src, c-myc* or *c-abl.* So, basically the prefix C refers to cellular and this implies that this is the proto-oncogene version of this transforming gene. An oncogene is the activated form of the proto-oncogene and examples of this are any of the activating mutations that convert a proto-oncogene into an oncogene, but very often these genes were present in the viruses, which caused cancer. And examples of this are as *rasH, rasK* not mentioned here *src, myc, abl* and *B-raf* and of course there are many more examples these are not the only examples, you can look up many more interesting examples of oncogenes in the literature.

Another class of cancer critical genes are the tumour suppressor genes. So, in this case the loss of function mutation in the tumour suppressor gene contributes to cancer. And by definition, these cancer-causing mutations in these tumour suppressor genes are recessive, because they are loss of function. And examples of this are also many, a few are mentioned here for example, retinoblastoma or *Rb, p53, APC, BRCA1, BRCA2, INK4* etc. And there are also micro RNAs that can function as tumour suppressors; some examples are the *let-7* micro-RNA and the *mir-34* micro-RNA.

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Since cell cycle control is disrupted in cancer cells resulting in their untimely and unrestrained proliferation let us revisit cell cycle control. So, shown here are some key regulators of the cell cycle transitions. In animal cells there are many CDKs or cyclin dependent kinases. The progression through the restriction point, requires the CDK4/6 complex with Cyclin D, the G_1 to S transition requires the CDK2 complexed with Cyclin E, progression through S is controlled by CDK2-Cyclin E complex, the G_2 to M transition is controlled by the CDK1-Cyclin A complex again. And progression through M phase is controlled by the CDK1-cyclin B complex.

Extracellular growth factors control the progression through G_1 and the restriction point of these cells. So, normally many cells in the body are not dividing, they are resting, but some of them can actually under proper conditions and signals, they can come out of G_0 and enter G_1 and you know if they cross this restriction point they are committed to initiate DNA replication.

So, Cyclin D synthesis is induced in response to growth factor stimulation. And this happens in part by signalling through this RAS, RAF, MEK, ERK pathway. So, Cyclin D continues to be made as long as these growth factors are present, and this drives the cell through the restriction point. Now cyclin B is also rapidly degraded by APC-C Ubiquitin Ligase in G_1. So, if the growth factors are removed, then its levels fall rapidly.

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Another layer of control is shown here. There's additional regulation of CDKs by the cyclin-dependent kinase inhibitors or CKIs in mammalian cells. And examples of these are shown here the INK4 family, they act to inhibit progression through the restriction point in G_1. They are mentioned here: p16 and 18, 19, various variants are there. The KIp family, which includes p21, p27 and p57; they bind to CDK2-Cyclin E or CDK2 cyclinA and they block entry into S and progression through S and G_2.

So, the growth factor signalling reduces the transcription and translation of p27. This is a CDK2 inhibitor, and they lower its levels, hence the S-CDK is relatively active. Activated CDK2 also phosphorylates p27 and targets it for degradation by ubiquitin-mediated proteasomal degradation driving the S phase progression.

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So growth factors or mitogens, they bind to cell surface receptors and this initiates intracellular signalling. An important pathway involves the activation of the small GTPase RAS, which activates the MAP kinase cascade we already mentioned that RAS is a oncogene. So, activated RAS can function as an oncogene. Now this activation of RAS results in increased expression of many immediate early genes including *MYC* which is also another oncogene in it is activated or over expressed form.

Myc regulates transcription and it increases the expression of many delayed response genes including Cyclin D. So, G_1CDK, which is a CDK4/6 complexed with Cyclin D is activated. And the cell can cross the restriction point in presence of these growth factors.

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After activation of the G_1CDK, the G_1CDK it phosphorylates Rb. Rb is retinoblastoma, a tumour suppressor. As cells pass through the restriction point in G_1. So, unphosphorylated RB binds E2F transcription factors. E2F binds to its target sequence, whether or not Rb is present. But when Rb is present, and it binds to this E2F it acts as a repressor. So, E2F-Rb complex represses transcription of these E2F regulated genes.

Phosphorylation of Rb releases it from this complex and hence the repression mediated by Rb is no longer there. And hence these E2F dependent genes can be transcribed. So, this E2F now activates the transcription of its target genes. And examples of such genes are Cyclin E of G_1 CDK and this triggers entry into S phase, also cyclin A. The growth factor signalling reduces the transcription and translation of p27 CDK2 inhibitor lowering its levels.

So, activated CDK2 also phosphorylates p27 and targets it for degradation by ubiquitin-mediated proteasomal degradation. Thus, G_1S CDK is active. And the entry into S phase can occur. Cyclin A was also transcribed and hence the SCDK is also active. So, S phase can proceed further.

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So, from the previous description we can see that Cyclin D is a critical target of growth factor signalling and defects in Cyclin D regulation can result in loss of growth regulation as seen in cancer. And in fact, in this pathway we mentioned many oncogenes as well as a tumour suppressor gene. So no doubt mis-regulation of this could contribute to cancer. For example, the mutations causing continued unregulated expression of Cyclin D contribute to lymphomas and to breast cancers, it has been reported.

And mutations that inactivate Ink4, which is a CKI that binds and inhibits CDK4 and 6, are also quite common in the human cancers. So basically, if you mutate or inactivate the CDK inhibitor, then obviously the CDK would be active, and it could drive cell cycle progression forward. The CDK4, 6 Cyclin D target retinoblastoma, which we discussed as a repressor of E2F transcribed genes is frequently mutated in retinoblastoma and a variety of human cancers.

Retinoblastoma is a tumour suppressor and I already mentioned it binds E2F. And it represses E2F mediated transcription of S phase genes. So, its inactivation may allow S phase entry and progression similar to when it is phosphorylated by G1 CDK and dissociates from E2F.

We also mentioned MYC whose expression was upregulated upon growth factor signalling and it actually also helps in activating Cyclin D expression. So, interestingly long time ago it was found that *c-myc* expression is upregulated in Burkitt's lymphoma as a consequence of a chromosomal rearrangement that inserts the *c-myc* gene into the highly expressed immunoglobulin locus in B lymphocytes such that the expression of *c-myc* was increased. So, it was able to contribute to the uncontrolled proliferation of these cells in Burkett's lymphoma because of its enhanced expression.

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Another tumour suppressor is p53, it also affects cell cycle progression in addition to its other roles. So, p53 regulates cell cycle progression as well as apoptosis. DNA damage leads to induction of p53, it is phosphorylated and also there is more of it. And this activates transcription of cell cycle inhibitory proteins, and it also activates the transcription of pro-apoptotic genes and so, the transcription of pro-apoptotic genes causes apoptosis. And in this case, there will be a cell cycle arrest. And in response to DNA damage p53 induces p21, which is a CKI, cyclin dependent kinase inhibitor that blocks the cell cycle progression in G_1 by inhibiting CDK2 cyclin complexes. So, this cell cycle arrest that results from the activation of p53 allows more time to the cell for DNA repair.

On the other hand, high levels of DNA damage or extensive DNA damage can result in high levels of p53, which drives the cells to apoptosis. So, p53 induces apoptosis by activating the transcription of pro apoptotic members of the BCL2 family. In absence of p53 cells having damage accumulate the damage that is unrepaired and they continue to proliferate and do not undergo apoptosis.

Incidentally, do you know that elephants have 20 copies of p53 in their genome in contrast with just two in humans? Recent findings indicate that this may explain why elephants, despite their large

size, rarely get cancer. In elephants instead of repairing damage they prefer to eliminate damaged cells by apoptosis at a higher rate than humans in order to minimize tumour formation. I hope you enjoyed this lecture and learned something about how cancer cells differ from normal cells. Thank you.