Cell Biology: Cellular Organization, Division and Processes

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Lecture 16

Cell Death, Aging and Senescence

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Hello, I am Shikha Laloraya, Professor of Biochemistry at IISc. Today's lecture is on cell death, aging and senescence. And we will be discussing aging and senescence, which are complex topics. So this is just an introduction to these topics.

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So what is ageing? Everyone has witnessed it, but if we were to define it we could say that it is a time-dependent progressive decline of an organism. It is characterized by loss of physiological competence and results in degenerative pathologies and enhanced susceptibility to age-related diseases. It results from time-dependent accumulation of cellular damage. Senescence is a phenomenon in which the cells stop dividing. They undergo a cell cycle arrest, which is irreversible. This differs from quiescence because in this case in case of senescence the arrest is irreversible whereas quiescent cells can come out of the arrest under certain circumstances.

Cell death, it is a loss of cells. Cells cease to exist and this is associated with aging or various other reasons. Cells can die, when they are damaged or infected or even during the normal course of development and this occurs via programmed cell death that will also be discussed. Programmed cell death occurs by process known as Apoptosis. This is an active cellular death process characterized by distinct changes such as chromatin fragmentation, condensation shrinkage and cell fragmentation to form Apoptotic bodies. These are cleared by phagocytic cells and therefore there is no spillage of cell contents or inflammation. And this process is different from the necrosis during which cells die by swelling and bursting and releasing their contents thereby triggering and inflammatory response.

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Ageing occurs due to a time-dependent accumulation of cellular damage. What kinds of cellular damage contribute to aging? A group of researchers, Lopez-Otin et al have defined 9 different hallmarks of aging shown here: genomic instability, Telomere attrition, Epigenetic changes, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and altered intercellular communication.

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So, changes in the DNA and chromatin are an important hallmark of ageing. DNA can get damaged due to either exogenous or endogenous reasons when cells exposed to genotoxic chemicals or harmful radiations such as UV or ionizing radiations or if ROS. reactive oxygen species, are produced in excess inside the cell or even spontaneously during the process of replication certain errors could occur. Now these errors can be of various types and they are repaired by different repair pathways present inside the cells which are active.

However, when either, there is too much of DNA damage or there is a decreased efficiency of these repair pathways then damaged DNA can accumulate and this can contribute to aging. Another hallmark of age is telomere attrition or shortening of telomeres and this can happen when the telomerase enzyme, which maintains ends is not present or not active and when this happens and the telomeres shorten and ultimately the ends of the chromosomes are exposed there can be loss of genetic information near the ends the gene near the ends-We could do lose them or the ends can be recognised by the DNA repair machinery as double-strand break and there could be abnormal fusions of these chromosomes to other chromosomes or parts of other chromosomes which can result in misregulation of gene expression at the junction or also it can result in the production of dicentric chromosome which are unstable and therefore they would be lost. So again are telomere shortening is harmful and it contributes to ageing.

Aging cells also accumulate epigenetic changes, for example that could be changes in the methylation status of the DNA. So, there could be increase in local methylation while there could be decrease in global methylation in these aging cells. They could also be alterations in the modification status of histone proteins. There could be changes in methylation or in the acetylation modifications present in the histones and this can bring about changes in expression of genes and affect many other chromosomal processes. There can also be alterations in chromatin remodelling or even organisation of chromatin due to changes in silencing proteins or nuclear remodelling factors, ultimately resulting abnormal chromatin structure. So all of these can bring about changes in transcription, in RNA processing operations and also defects in DNA repair and therefore cause genomic instability. And such epigenetic changes which are actually not changes in the DNA sequence per se but in the chromatin can also contribute to aging.

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Now when cells are subjected to stress either due to exogenous or there can be endogenous stress which can actually affect protein folding of existing proteins or during protein synthesis there could be protein unfolding. And normally in the cell there are ways to take care of it. For example, there is

Chaperone mediated refolding in cells, there is also ubiquitin-proteasome mediated degradation of unfolded proteins. And proteins and unfolded proteins also get sequestered and get removed by autophagy. However, if this impairment in any of these normal pathways then unfolded proteins may accumulate and ultimately they form aggregates and this can result in proteotoxic effects.

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A very important hallmark of aging is deregulated nutrient sensing which has been studied a lot in recent years. There are 4 different nutrients sensing pathways that influence aging. One is based on IGF-1. Now IGF-1 is insulin-like growth factor-1, it is produced in response to growth hormone, which in turn is produced by the pituitary and this is termed as the somatotrophic axis. So IIS can actually help in sensing glucose levels and this pathway is conserved aging controlling pathway. And some of its targets include FOXO family of transcription factors and also downstream is mTOR, the mechanistic target of rapamycin, and the signalling happens by the PI3 kinase and AKT kinases. So as already mentioned another pathway is mTOR. And mTOR senses elevated amino acid concentrations in cells. And this consists of two different complexes, there are two different complexes mTORC1 and 2 and they regulate anabolic metabolism. It is known that its downregulation extends longevity. Another pathway is dependent on AMPK and this senss a low energy state by detecting high levels of AMP, adenosine monophosphate. Sirtuins also sense low energy states by detecting high levels of NAD+.

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So the take home message from this complicated slide is really that there are nutrient sensing pathways which are impaired in aging cells and in fact two of these pathways, IIS and mTOR dependent pathways, they signal nutrient abundance and anabolism and these pathways accelerate aging and their downregulation favours longevity. Whereas the AMPK and Sirtuin dependent pathways, they signal nutrient scarcity and catabolism and their upregulation favours healthy aging.

Interestingly dietary restriction has been shown to increase the life span as well as health span in all investigated eukaryotic species, including nonhuman primates. And this supports the effect of altered nutrient signalling in aging.

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Another important hallmark of aging that is accompanied by metabolic changes is mitochondrial dysfunction. As you all know mitochondria are crucial for energy production in the cell, they produce ATP. So in aageing cells mitochondria can become dysfunctioln due to accumulation of mitochondrial DNA mutations and also due to nuclear DNA mutations because nuclear DNA also codes for mitochondrial proteins and hence it is important for mitochondriogenesis.

There could be impairment of the electron transport chain components; there could be altered fusion and fission of mitochondria, mitochondrial dynamics shown here. And also there could be impairment in mitochondrial quality control or defective mitophagy. So defects in all these processes could result in mitochondrial dysfunction. And dysfunctional mitochondria, of course would result in decreased bioenergetics of the cell.

Because the main function of energy production cannot be formed efficiently, also dysfunction mitochondria can produce high levels of ROS or reactive oxygen species. Now, below certain threshold, ROS can induce survival signals, which restore cellular homeostasis but at very high levels ROS can actually contribute to ageing. So, mild mitochondrial damage can induce hormetic response the mitohormeses that triggers adaptive compensatory processes , whereas severe dysfunction of mitochondria is harmful and it can contribute to ageing.

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Ageing organisms accumulate senescent cells. Cellular senescence is induced by damage and it is a state of irreversible cell cycle arrest. In young, there is a limited amount of cellular senescence and this can have beneficial effects such as anticancer or anti ageing and it helps in tissue homeostasis. However, in older organisms is pervasive damage, there is more cellular damage and there are large number of cells which are senescent which are accumulating perhaps due to deficiency in clearance and replenishment of the cells.

So, this results in deleterious consequences on tissue homeostasis and it can result in increased inflammation and have effects on neighbouring cells and this all contributes to ageing.

Stem cell exhaustion also has many negative effects for example defects in hematopoietic stem cells can result in anaemia. Dysfunction in mesenchymal stem cells can result in osteoporosis and defects in satellite cells can result in reduced repair of muscle fibres and so on.

Communication between cells, intercellular communication that is, can also become defective in aging cells and this can result in neuroendocrine dysfunction or deficiencies in the immune system and so on.

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Cellular senescence is a crucial hallmark of ageing. It was originally defined based on observations of the limited replication potential of human diploid fibroblasts in culture by Hayflick and Moorhead in 1961. It was observed that these cells that are derived from the human fetal tissue were not immortal, but they could yet divide several generations in culture while maintaining their karyotypes. However after about 50 sub cultivations or passages they would decline and this was later referred to as Hayflick limit.

It was thought that the cause of this phenomenon is due to intrinsic factors which are expressed as senescence at the cellular level. In a later work, it was inferred that the shape of the survival curves

was such that it is similar or suggestive of multiple target or multiple hit event and it was suggested that multiple inactivation events are required before the cells into this phase of decline.

While the cellular components that may be in activated were not identified at that time, the complex nature of initiation of senescence could be predicted consistent with our current understanding. Furthermore, it was proposed that the basic step in this phenomenon is the accumulation of hits or errors during DNA replication, which inactivates part of the genome. And therefore, it has later been referred to as replicative senescence.

Therefore, senescence is defined as a state of permanent cell cycle arrest resulting from the limited replicative capacity of normal human diploid fibroblasts in culture, called replicative senescence by Hayflick. Recently senescence has also been described as a state of stable cell cycle arrest in response to diverse stresses or intracellular damage.

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Now, various effector mechanisms of senescence have been identified and this indicates that senescence is a collective phenotype of these multiple effectors and its not a one hit process. And this highlights its complex nature, as was predicted by Hayflick. So, some of these characteristics some of them we have already mentioned, but the main characteristics of senescent cells are cell cycle arrest, activation of a DNA damage response which is accompanied by telomere dysfunction induced foci.

And also DNA segments with chromatin alterations reinforcing senescence, that also maintains and senescence via p53 activation. Increase in various cyclin-dependent kinase inhibitors, which perhaps is responsible for the cell cycle arrest. Alteration in the secretome of senescence cells, which is also termed as SASP or Senescence Associated Secretory Phenotype, that is various cytokines, chemokines, proteases, plasminogen activator, inhibitor Type 1 and these factors are secreted and this secretome is usually associated also with persistent DNA damage response, indicating that there might be connection between the two.

There are also alterations in the nucleus, in chromatin and therefore in gene expression in senescent cells. For example, epigenetic changes can cause transcriptional activation of p16, a cyclin dependent kinase inhibitor. There is also accumulation of senescence associated heterochromatic foci and their formation depends on the retinoblastoma pathway. Retinoblastoma is a tumour suppressor. And further RB suppresses subset of cell cycle genes which are involved in DNA replication.

There is depletion of a protein known as Lamin B in HDFs. This is a protein which is part of the nuclear lamina, and we will discuss it in further slides. There are alterations in histone modifications and in the organisation of chromatin domains. And there is also spatial repositioning of perinuclear heterochromatin. Now senescent cells are damaged. They are arrested. So, they are not dividing and yet they persist so they are resistant to Apoptosis despite having damage.

And this may arise from upregulation of anti-apoptotic gene in these cells. Senescent cells are not idle; they are actually metabolically active and undergo ER stress.

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One can see certain morphological changes in senescent cells are shown here. So these cells, they are greatly enlarged and they havean irregular shape compared to the original cells from which they arose and they have increase granularity. They also have altered composition of the plasma membrane. For example, there could be upregulation of caveolin. They have also very large and somewhat dysfunctional mitochondria that produce high levels of ROS.

They also show accumulation of a large number of lysosomes and increased activity of lysosomal senescence associated beta galactosidase, which is often used as a marker for senescence cells. We already mentioned SASP the Senescence Associated Secretary Phenotype that is the release of a specific particular type of secretome associated with senescent cells. There are several nuclear changes; there is compromised nuclear integrity due to loss of Lamin B1 which also results in the appearance of chromatin fragments in the cytoplasm referred to as cytoplasmic chromatin fragments or CCF's.

There is accumulation of DNA damage foci in the nucleus that is foci arising from phosphorylated histone H2AX which is a marker of DNA damage. There are also senescence associated heterochromatic foci which can be seen in the nucleus and there is spatial repositioning of the perinuclear heterochromatin.

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There are also certain diseases in humans which, are characterized by premature ageing and these are referred to as Progeria syndromes. Now, they arise from mutations in genes encoding protein components of the nuclear lamina which is a part of the nucleus just below the nuclear membrane. So, in these syndromes the patients unfortunately show premature symptoms of ageing, and highly accelerated ageing.

And one of the examples of such syndrome is HGPS or Hutchinson-Gilford Progeria Syndrome. In this syndrome the cells they express a mutant Lamin A, which is also another kind of lamin, and this very common mutation results in deletion of the last 50 amino acids near the carboxy-terminus of this protein. When the cells from the patients are cultured it is observed that they show several nuclear defects, for example one can see certain indentation or rather change in the shape of the nucleus can be seen.

There can also be lobulations in the nucleus, so far from regular oval shape. In addition, there is thickening of the nuclear lamina compared to a normal wild type cell. There is also loss of peripheral heterochromatin and what cannot be seen here but has also been observed, is a clustering of nuclear pores which are otherwise more or less uniformly distributed in the nuclear envelope. Now interestingly, in animal as well as cellular models such changes in the nuclear lamina, which are similar to HGPS often elicit stress pathways including activation of the tumour suppressor p53, deregulation of the somatotrophic axis, the nutrient sensing axis that we mentioned already, and attrition of adult stem cells, all of which can contribute to ageing.

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So finally we have discussed various known hallmarks of ageing, ageing effectors, and characteristics of senescence cells. This is a very active area of research and these findings set the stage to start designing interventions, not only to alleviate premature ageing in patients, but also to extend longevity and extend health span of individuals. As discussed in this interesting article by a group of researchers who work in this area of ageing. Stay tuned for the next lecture on Apoptosis. Thank you.