Inorganic Chemistry of Life Principles & Properties

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Lecture - 50
Inorganics in medicine - Apoptosis

Welcome you to the next class on Inorganic Chemistry of Life Principles and Perspectives. In the previous class we have looked at inorganic ions, inorganic metals, inorganic salts, inorganic complexes in medicinal aspects of it. And we found that almost every element in the periodic table, almost I am not saying all of them have for have been found in some context or the other in the medicinal aspects of their compounds or salts or ions we have seen all that.

Now, we just move a little forward in this particular class in order to take that the therapeutic levels; so therapy. So, we talked about the drugs earlier inorganic chemistry, inorganic complexes as medicinal items, now we look at how they function some of them functions etcetera. So, when it comes to the therapeutic approaches what I mean is, you know very well popularly known treatments for example, for cancer.

So, what is one popularly know for the cancer is something called radiate therapy or radiation therapy, this is something called chemo therapy, is called drugs given to the patient and variety of the things of the; or a combination of these two. So, all of these are there. So, in this particular you know lecture, let us look at some of the therapeutic approaches particularly targeting the cancer. So, when it comes to the cancer, I think we need to look at something called the chemical based or the drug based kind of a approach. So, its called Chemotherapy in this.

So, before we go into this, we need to understand certain aspects; aspects such as the apoptosis, apoptosis is nothing, but the programmed cell death and this is an important aspect to be understood. So, once we understand that, then we will look at how this apoptotic process is affected affecting the cancer cells by these compounds or cancer agents.

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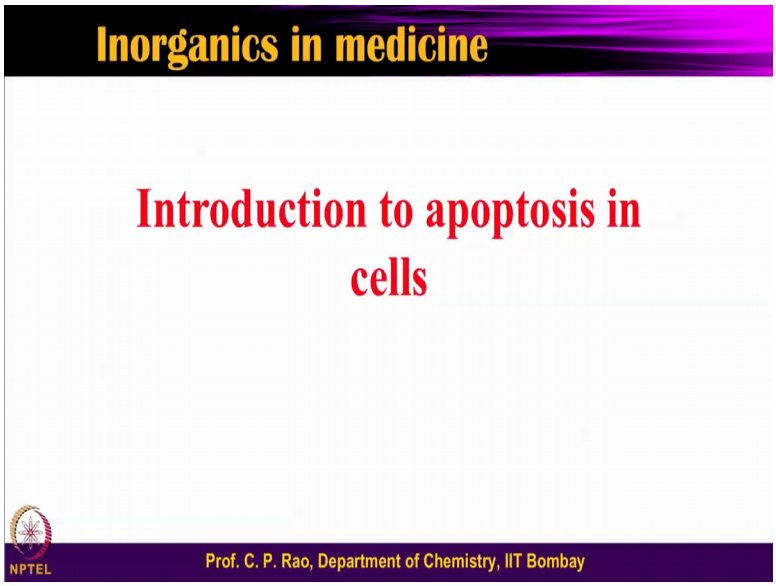

So, let us begin with some kind of a introductory understanding aspect of apoptosis in cells.

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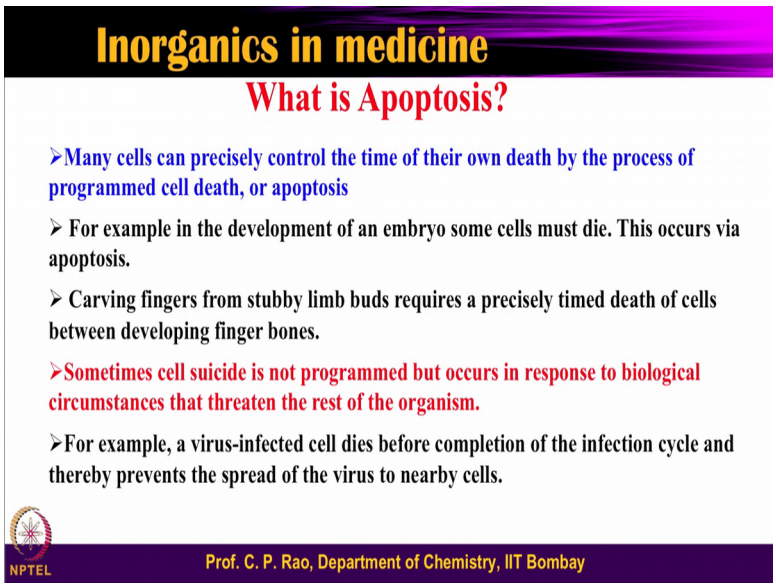

So generally it is very well known in the biological systems, many of the cells can definitely control their time of their own death. So, it is a planning their own death ok. So, by a kind of a programmed process called this programmed process of death cell death, which is known as the apoptosis. There could be other kinds of deaths that will

come to that in a while. So, that is why we are talking about the apoptosis as a programmed one.

Now, so, look at the point number 2. So, this is because if you look at some of the development of the cells for example, embryo. So, when the embryo is developed, there are to be some of the cells has to die. So, this again occurs in a planned way in a programmed way therefore, it is an apoptosis. This is explained better from this point the carving fingers from stubby limb, limb buds requires a precisely timed death of cells between the developing finger bones and that is what we were talking about. This is something where you know there is a need for some of the cells to go for death and that is what is referred as the programmed cell death.

On the other hand the other one is sometimes the cell suicide is not or cell death is not programmed, but occurs because of various kinds of external factors. So, the external factors could be threatening the cell to die and such kind of things can happen when you have an external toxic agents or been or toxins have been surrounded, or there is a pressure on the cells a physical, chemical chemicobiological kind of a you know circumstances that may threaten these cells to cells to die. Such kind of things that do not come under the program death they are the other kinds of things which we will see in a while.

Such kind of a non program nonapoptotic thing for example, a virus infected cell dies before completion of the infection cycle, and thereby prevents the spread of the virus to the to the nearby cells. So, in some cases that is a necessity based or biological circumstances based. So, there is a kind of a threat for the rest of the organism. So, therefore, the system decides the some of the cells have to die and that is what you are looking at.

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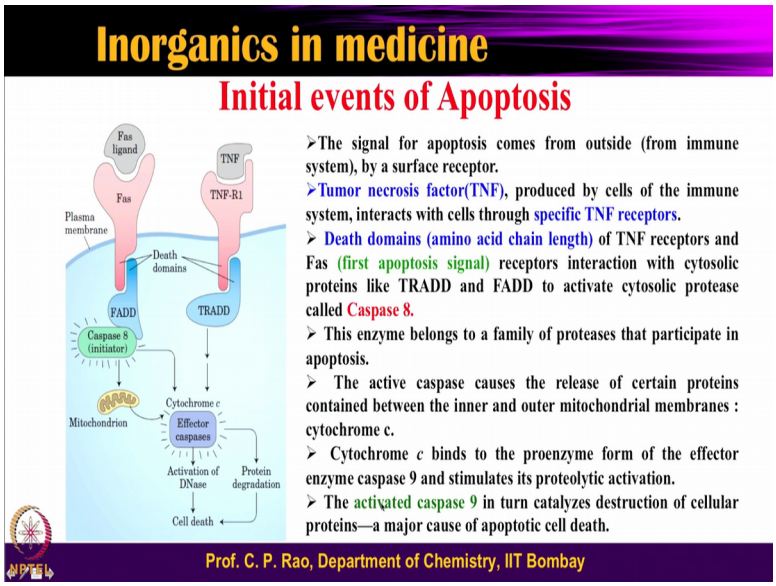

So, before we go to that part of it which is generally known as necrosis, let us look at the apoptosis little bit more in detail ok; so apoptosis to happen. So, there has to be some kind of a signal. So, the signal has to reach this cell, where the signals come? The signal for the apoptosis comes from the immune system.

So, from the immune system the signal comes in the form of for example, here this is nothing, but tumor necrosis factor. So, this is a tumor necrosis factor and such factors are noticed understood by r 1 means receptor tumor necrosis factor receptor. So, tumor necrosis factor is emanated from this immune system and as a signal to initiate the process of the apoptosis.

So, such signals that such species will come and interact with the receptor at this stage and then the further events will follow it up ok. And this indeed in fact, interacts when this interacts with the TNF r 1, which is tumor necrosis factor receptor this is in contact with the proteins like TRADD. And there are some other proteins like fadd these are in contact with the FAS. So, that is first apoptosis signal. So, this is the FAS signal interacts with the FAS receptor thereby interacts here with the proteins what all these do? These do create something called a caspase initiation.

So, it is basically initiation process the signal has come signal has knocked the door of the cell and therefore, you have basically a caspase system has generated. This caspase system will indeed activate the intro and extro you know cellular this cell wall or cell

membrane mitochondria and to release the certain kinds of a effector molecules or the proteins and this could be in the cytochrome C or effector complexes.

So, all these effector complexes will generate a host of proteases host of nucleases. So, you must be knowing what is a protease. Protease is an enzyme which chews away the protein or which hydrolyzes the peptide bonds. So, when the cell has to die what all you have to do? Cell has got a lot of proteins cell has got a nucleic acids, in other words a nucleus therefore, this has to be disintegrated.

So, if we disintegrate this and engulf those species, then it is really a good apoptosis if you cannot then it will create other kinds of a problems. So, you have a FADD protein chain on one side TRADD protein chain on the other side and this will generate to the effector systems like cytochrome C effector complexes. And all these generate this is this generates a caspase mechanism. So, which is referred as caspase 9 and this will give the enzymes which can chew the DNA which is called DNAs and which can chew the protein it is called the proteases

So, a lot of proteases and the DNAs just are generated and therefore, the protein material as well as the DNA material is being broken into pieces and that is what basically happens. So, they activated caspase 9 in turn catalyzes the destruction of the cellular protein a major cause of the apoptotic cell death ok.

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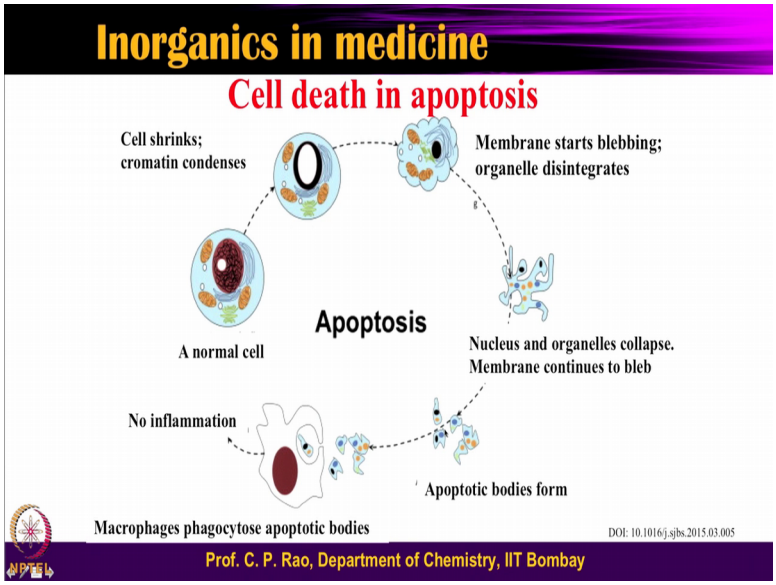

So, the same thing whatever I mentioned in the previous slide in the form of write up, let me give you in the form of a kind of a scheme because its always easy to understand the schemes as compared to the reading material ok. Let us take this as a normal cell which has everything perfect that is called normal cell.

Now, when this kind of a signals come at the cell surface, that is the apoptotic signals as I talked to you on the previous slide, the apoptotic signals or basically coming from TNF or the FAS. So, these signals will interact with the cell at the receptor sites and that induces a variety of changes in the cell one of the thing is cell can shrink and chromatin will condense a lot of material of the chromatin, all these material which is the DNA kind of material will condense. And we will start blob babbling kind of thing. Like you see that these kinds of things are called babblings. So, the cell is nice here and cell is not so, nice here. So, it is kind of a thing that is coming over there. So, the membrane starts babbling. So, the organism the organelle starts disintegrating.

So, this will disintegrate you can see that, the nucleus and organelle collapse the membrane continues to the bleb the thing. You see that small small pieces, small small pieces you got and these are referred as the apoptotic bodies. Now once these apoptotic bodies it is like taking some material and grinding in a motor kind of a thing. So, it is a cell is made into pieces cell is made into pieces. So, that cell does not exist that is what you call cell death basically I hope you understand that, it is not a very anything other than that.

So, these are small apoptotic bodies these apoptotic bodies if they are not engulfed if they are released, then they are very dangerous. But another hand if they are engulfed is a macro phase phagocytose cytose. So, these kind of a aproptotic bodies will form, and they will engulf thereby no bad effect to the system at all.

So, this is a very nice happy way of killing the cell or cell taking the death. So, cell death is a happy cell death is known as the apoptosis; a programmed cell death is also known as apoptosis. I hope you understand normal cell at the cells when it receives the apoptotic signals, it will show start shrinking a cromatin will condense then blebbling starts, then pieces will start forming and then pieces will separate, and the pieces are engulfed. So, by macro phase therefore, no inflammation no side effect will be there.

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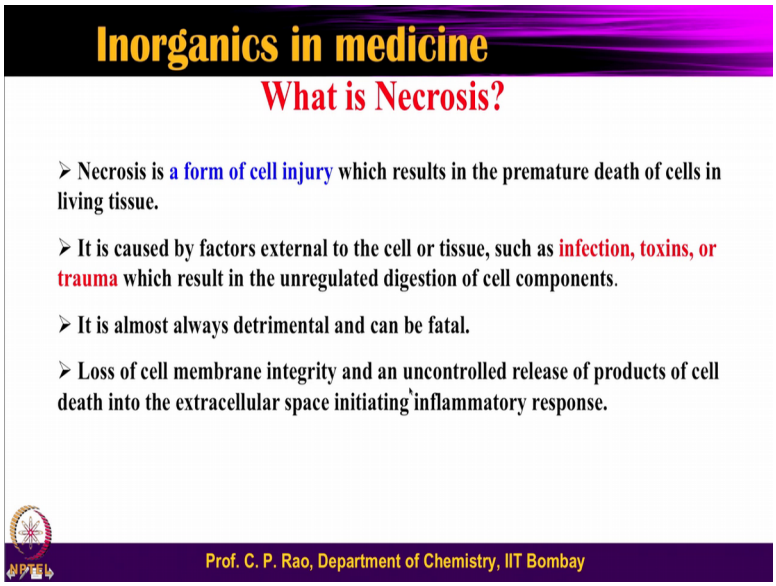

On the other hand the other process as I told you, when due to certain biological conditions, due to certain kind of a external forces, due to certain kind of a infection due to certain kind of a toxins attacking the cells may also die such a kind of death is a forcible death and its not a apoptotic death.

- So, there is for example, necrosis is a form of cell injuring, you just cut the cell or break the cell hit the cell which results in a premature death of the cells in living systems. So, this premature death is basically is through the necrosis. So, its caused by factors external to the cell, these factors could be physical, chemical, chemicobiological too. If you why we are saying chemicobiological it could be due to certain thing is secreted and those things will act on it.

So, cell or tissue such as infection, toxins or trauma, which results in the unregulated digestion of the cell components; so therefore, it is not fully utilized. So, its most of the times is not good the process is a detrimental and can be sometimes can be fatal as well.

So, loss of the cell membrane integrity and the uncontrolled release of the products like the small pieces that we have seen earlier all those things will be released into extracellular space. So, that will cause inflammation. So, therefore, it is dangerous.

So, therefore, necrosis unlike the apoptosis is a forcible death, unforeseen death, due to physical, chemical, chemicobiological kind of factors and therefore, the cell does not

have a plan to or program to do that and this is a dangerous because the species that comes into the medium will infect the system itself infect the tissue.

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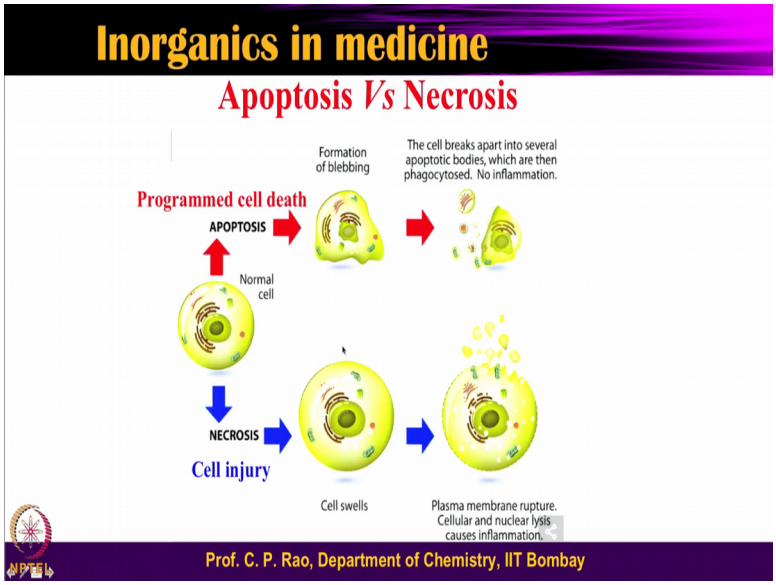

So, therefore, we look at the comparisons and contrasts this. So, take a normal cell, it is apoptotic process, and you see nicely blebbling and then pieces and then engulfed. On the other hand you have a cell gets some kind of an injury necrosis cell swells. And then it breaks into pieces, the plasma membrane ruptures and cellular and nuclear lysis causes all these things will come out, but not being engulfed and they cause inflammation.

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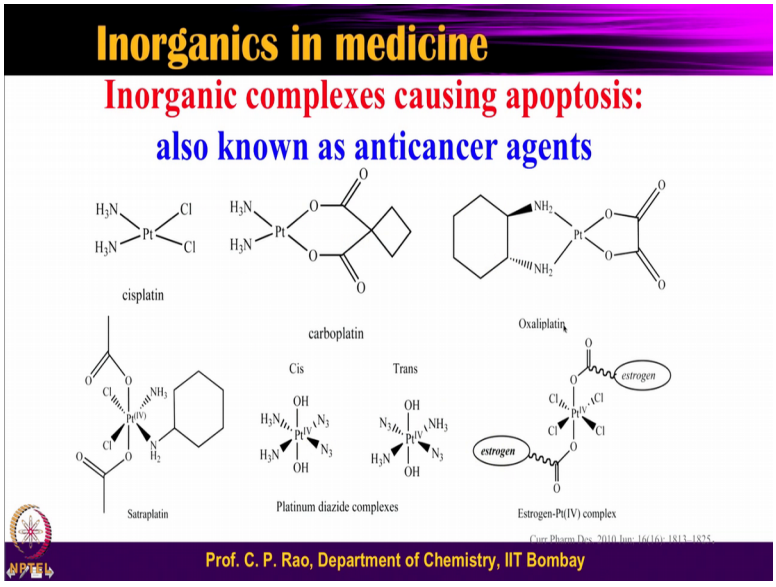

So, now you understand there are 2 types of cell death or there one is programmed cell death, which is the best for the system and the other one is necrosis which is not at all good for the system, but that will create a lot of problems to the system itself inflammation and other things.

Now, we have already seen earlier in the previous class, there is certain platinum kind of a drugs or known as can anticancer agents and these are been very well studied in the literature, and these are found to cause apoptosis. And in the first row here you have three compounds all are based on the based on the cisdiamino complexes, cis complexes and platinum 2 based ones. And there are four more compounds are though they are below. And these are all platinum four complexes. These are the new generation of the platinum complexes, which have not really got into the market yet, but the platinum 2 complexes have already come into the market. But these have been shown laboratory as well as many other trial experiments too.

The reason is instead of taking the platinum 2, one would like to take platinum 4 and the cell, the cell reducing components of the cell themselves will reduce to platinum 2 and then the rest of the story is like the other 1. So, these are known as anticancer agents also these basically kill the cells by apoptosis process.

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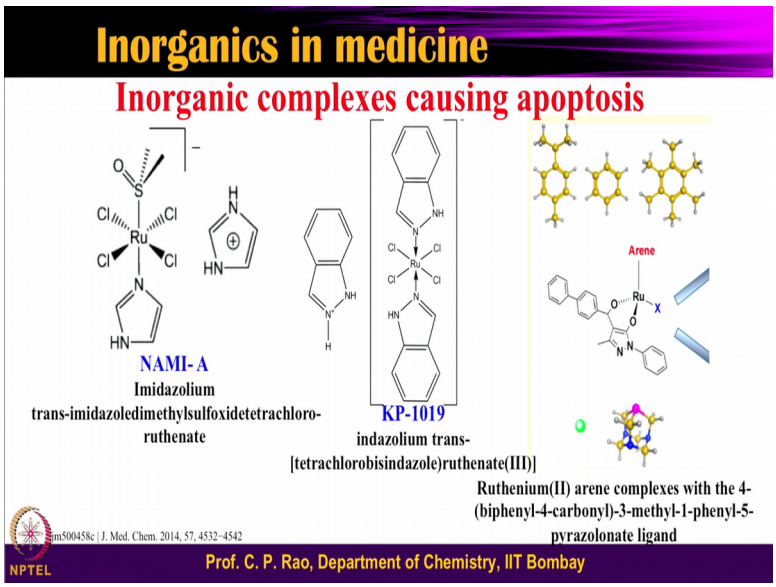

So, what is apoptosis is non dangerous way of killing the cells program way of killing the cells.

Now, there are some more compounds shown based on the ruthenium you can see imidazolium kind of a compounds is a counter cation and imidazolyl thing, and then sulfoxide methylsulfoxide thing and chloro and this is again anion and this is a counter cation, this is with the benzimidazole this is with the imidazole. You can also have complexes of the pi arene; you know the pi system of the molecule can also interact with the metal as a metal pi bond as like this kind of a pi system or simple benzene or hexa methyl kind of a benzene etcetera all these things.

So, and this has x x could be halide or x could be some other kind of a species too and then you have sorry this is this is basically a counter ion and this is the x is the halide. So, these are several other a known or which I have shown, apoptotic conditions for the cell death and they are all anticancer agents. Does not mean all of these are in the market. So, please do not take that way.

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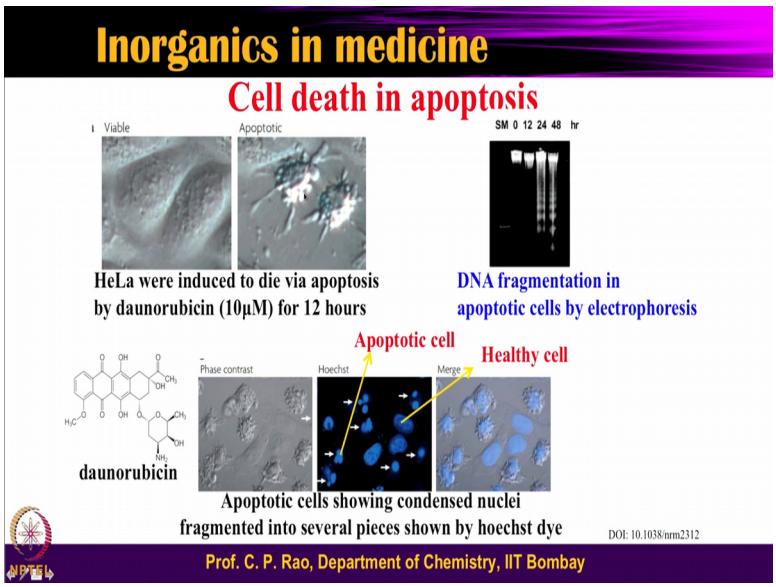

Now therefore, let us look at the cell death enough of process. So, here we have taken the HeLa cells. So, induced to die by apoptosis and what is the drug use? The drug is used this is a daunorubicin daunorubicin daunorubicin is shown over here structure of this one and this is infected for twelve hours. And on the left side this figure very nicely seen cells, intact cells you can see this is one cell this is another cell intact cells, but on this side these are treated with the daunoru rubicin and there is the drug that is the nothing, but anticancer agent, when you treat with this you see that babbling blebbling is coming

and that cell is dead. So, this is basically is called the apoptotic. So, this is bright field kind of a picture.

Now in fact, to check whether this is really done or not you can take the DNA components of this and you can look at the electro forces. And if it is really kill broken down if DNAs has chu in the nucleus part or nuclear component what you should get? The broken pieces of the DNA; and you see that huge smearing of the band that shows that DNA is not in one piece, then thousands millions of pieces that you have so that means, the drug has really caused an apoptotic death in these and thereby breaking the breaking the DNA part of it into pieces, as well as the protein parts into the pieces.

Let us look at the things this apoptotic system by using certain kind of a dies, which get accumulated into different aspects of it. Some of them go into the cell mitochondria, some of them go into the DNA power etcetera. Here we have taken this is the pure cells, this is a confocal microscopy, and now when you add these apoptotic cell showing the condense nuclei fragmented into the into the several pieces and I shown by the hex. No this is you treat with the daunorubicin and start babbling and to this if you add hexed as the dye agent, you add that you can see this kind of individual blobs there blobs blob blob blob kind of thing and some of them are (Refer Time: 21:24) intact. So, these are these intact ones are called healthy and these pieces are called apoptotic.

So, the apoptotic and this one, but now if you overlay this over this you will find this as the picture. So, this is a confocal microscopy bright field, this is a the when you put the hex into that the color imaging which comes from the its fluorescence emission and then you overlay with these things. So, this is the kind of a thing that you can see. So, they are probably this is a real system real system using the daunorubicin with the cells here, the cells used or hela cells the hela cells are nothing, but the cancer cells.

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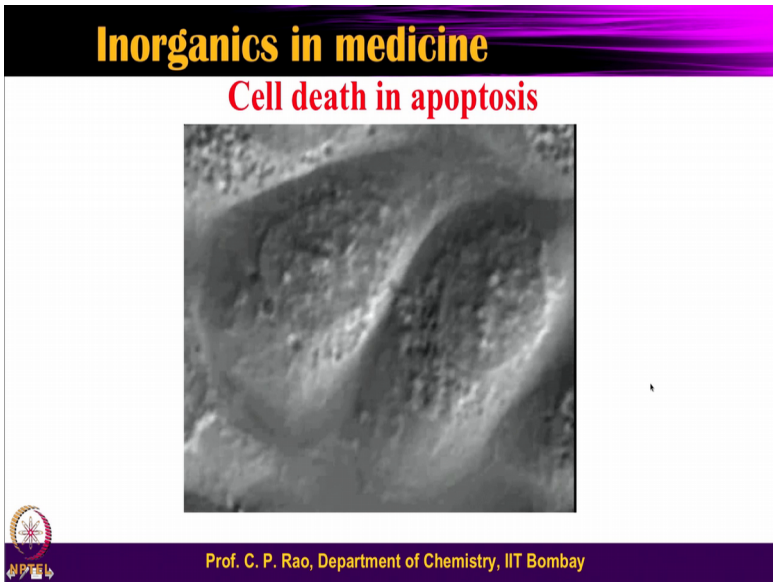

Now so, we can see that the cell death in apoptosis. So, let us have this video thing, then video you can see that we started with the 2 intact cells and now you can see is a little bit of babbling babbling and see more babbling, much more babbling.

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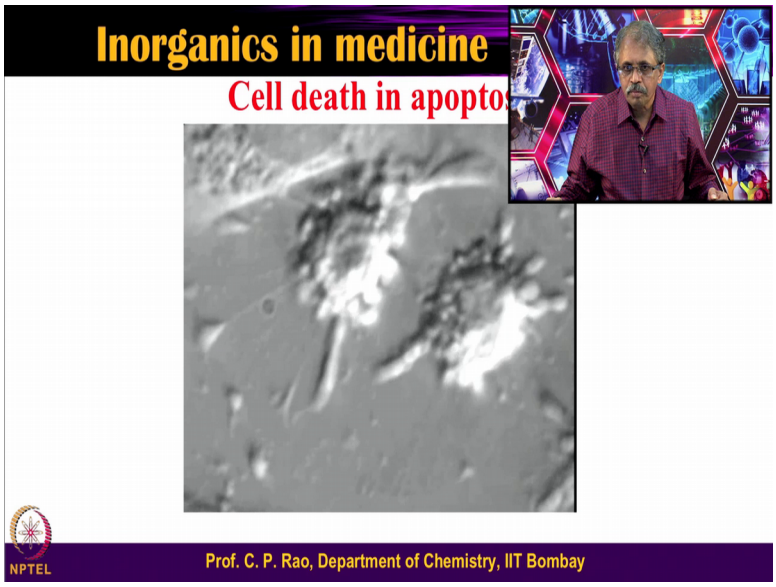

And they become pieces see the pieces; see the pieces, they go into there and they are getting that white regions are engulfing, that white regions are engulfing. So, they are engulfed the pieces are getting engulfed this bright white region is engulfing. So, that is engulfed and you can see that getting engulfed full and we can see the total probably the

video, which is just about a minute and quarter minute and ten seconds or something. So, you can see there, very nicely this has been utilized.

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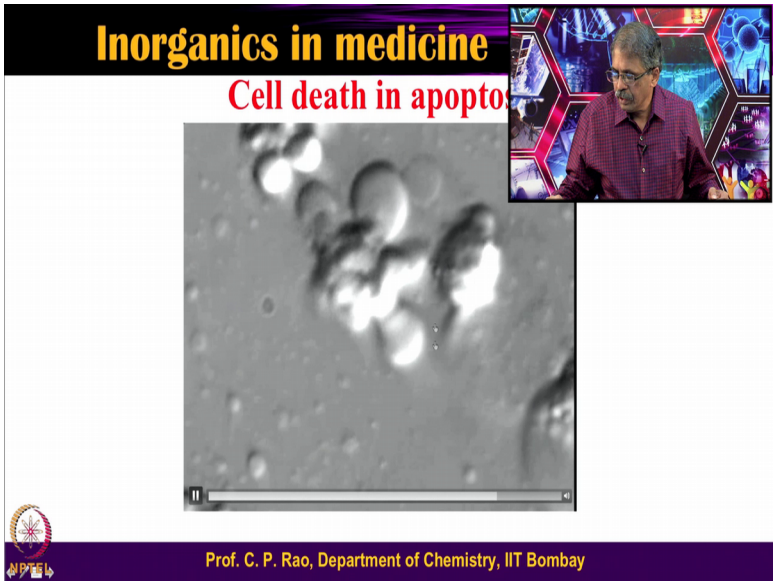

So, bake broken it into pieces and the pieces have been engulfed as you can see the things happening in that ok.

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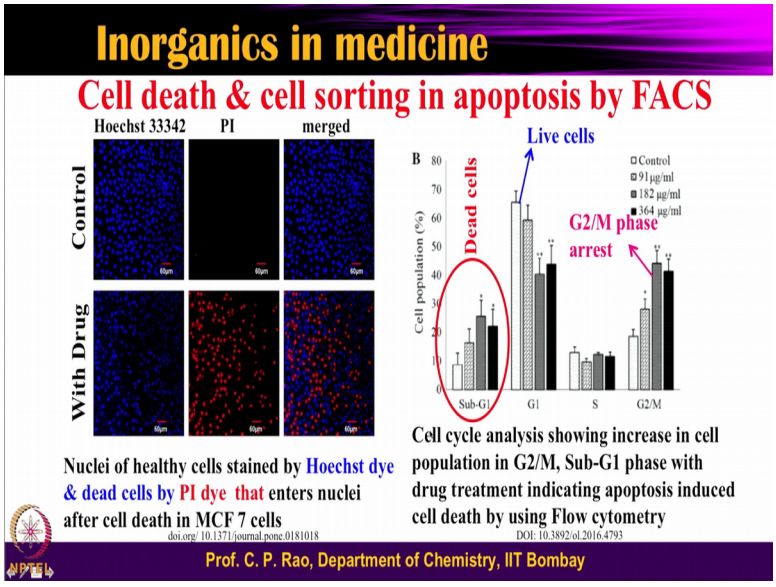

So, that is what the cell that about, how we do? Now let us look at different staining agents a control with the drug. So, also we can sort out the cells and in what fees

etcetera. See here this is the MCF 7 cells, and this is a control means the top 3 are only with the cells no drug is treated the bottom ones are treated with the drug ok.

And the first one is treated with the hexed die, the next one is a PI is propidium iodide, which will go into the into the nucleus and this is the merged picture. You see that this is the nice individual cells and the cells take the hexed and the cells nothing has happened no death in the sense because there is no drug is given. So, then when you put the PI nothing is seen. PI goes only into the DNA of the dead cells, now you overlay perfectly this picture and these pictures are almost the same is called merged.

Now, you take the cells and add the drug, and keep that, but particular time 10 12 hour, you see there are several cells are missing. Here, fully cells are there here only some cells are there; that means, those cells are dead and they will not pick up hexed and those dead cells will pick up the propidium iodide and you get the red fluorescence. So, the hex gives the blue fluorescence and propidium iodide gives the red fluorescence and if you overlay this left one with the middle one you get the right one, you see that this is almost equal to as a full one.

So; that means, the blue cells are intact red cells are dead. So, you this is a kind of a good nice way of demonstrating these things ok. The same thing can be studied by a method called FACS. this is the method FACS is referred as FACS fluorescence this is based on the fluorescence, assorted cell sorting.

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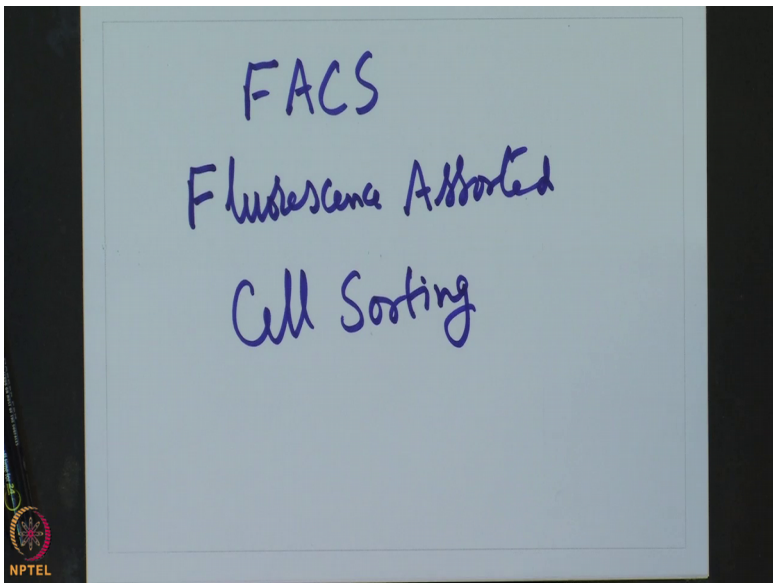

So, cells are sorted based on their fluorescence and the fluorescence agents are used and there is a instrument, which where you can call it as a FACS machine and then the FACS machine you can get these ones.

Now, the results are the cell population different cells are their, cell population absolutely very nice you can see, then you would see the dead cells, you can see live cells, you can see some phases. So, example is cg 2 m.

Now, what happens this is the first one is a control no drug, second one with the little amount or drug, third one is a little more of drug fourth one is much more of drug. There is 91 microgram 182 microgram, 364 microgram and then control cells. So, therefore, the cells are sorted out for this kind of a work. So, they are very nicely you can see.

As you increase initially you have this much with some amount of drug death is increased more death is there 30 percent around that. So, these are dead cells what happens to the live cells it must get reduced. You have a maximum of live cells then the live cells are reduced a bit reduced further, reduced further. So, you can see live cells are there

Now, the question is how are they what is the kind of a phase that is going through. And there is a s phase is here and not much change, but there is a G 2 M phase you can see small bigger much bigger in this so; that means, it is the G 2 M phase in which the cell cycle is getting apoptotic attack. So, cell cycle analysis showing increase in cell population in G 2 over M sub G one phase with drug treatment indicating apoptosis induced cell death. So, apoptotic induced cell death and this is studied by flow cytometry is also known as the FACS fluorescence assorted cell sorting out.

So, what we have learned in this lecture. So, we have learned in this lecture there are certain cells can plan their own death and that is much better it is known as the programmed cell death is known as the apoptosis. And it is also the best way because when the cell dies all the fragments that comes out or being engulfed, and not thrown into the medium therefore, no infection.

The other procedure is when cell should only getting certain kind of a unusual or unexpected kind of a signals it will be a physical chemical chemicobiological in such cases the cell is forced to die, but such kind of deaths not will lead to the programmed

case because all the pieces that formed in that are not engulfed. So, therefore, program death for this is better and it is required in. several cases.

The next thing is we have looked at the platinum based complexes, ruthinium based complexes and these are all called anticancer agents and these are many of these cases many of these compounds were shown for their apoptosis. Though all of them are not in the market for the cancer treatment, several of them are available in the treatment.

So, therefore, we looked at the cell death apoptosis by they taking the adding the drug to the system without having the drug to the system. So, we were looking at the control, we have looked at the drug treated once. In fact, we have seen all of these even through a kind of a short movie, which tells you how this apoptotic cell death takes place. So, initially it is condensing then fragmentation, the fragments being the engulfed etcetera without getting into the flowing into the medium of this.

And then we also looked at additional aspect of it, how to understand this by microscopy. We have looked at and fluorescence microscopy, we also looked at using certain dyes, which will stain the live cells which will stain the dead cells. A hex will stain the live cells propidium iodide will sain stain the dead cells.

So, using that one, we could identify in the control everything should be live cell and when the drug is treated partly live cells some of them are dead cells we could find out that. And we also I have also explained towards the end that this can be identified in what phase actually the apoptosis is occurring. So, these are the some kind of a aspects based on the apoptosis and relevant to the inorganic drugs, platinum ruthenium or this is mainly focus for the cancer drugs.

Of course, in the next class I will try to take up some more therapeutic based inorganic therapeutic based ones.

Thank you.