Cell Biology: Cellular Organization, Division and Processes

Prof. Mohanish Deshmukh

Cell Biology and Physiology, Neuroscience Center

University of North Carolina at Chapel Hill

Lecture 17

Apoptosis Part I

(refer time: 00:24)

Well, hello everyone! My name is Mohanish Deshmukh I am a Professor in Cell Biology and Physiology department, I am also in the Neuroscience Center at the University of North Carolina in Chapel Hill and I am excited to talk about Apoptosis today. This is an incredibly fun topic, and we will discuss three things in today's lectures. First is we will talk about the definition of apoptosis. I suspect most of you already know this. Second, we will talk about why it is an important pathway both in the context of development as well as in several pathological situations and third, which is the bulk of a lecture, we will discuss the historical perspective on how the genes were first identified and the actual mechanism by which apoptosis occurs in mammalian cells. So let us get started.

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So what is apoptosis? Apoptosis is basically activation of a pre-existing genetic program that results in cell death. So in that sense it is no different from any other genetic program that the cell can engage for example to divide, or to differentiate or to move, this program just happens to have the outcome that the cells die. So it is incredibly important for the cells to make that decision very carefully.

It is evolutionary conserved in fact people think that all multicellular organisms have an apoptotic pathway. And a very important aspect of apoptosis is that the morphological features are very characteristic. For example, the cells shrink, nucleus condenses into small fragments, membranes bleb, and you may have seen pictures of this and the DNA actually ends up getting fragmented into tiny fragments.

And most importantly, you know, I want you guys to remember that it is an active process which requires ATP and many cases also protein synthesis and the reason to emphasize this is because you know years before people used to think of death as a passive process: death by neglect / death

because cells just starve. Apoptosis is different; this is a decision a cell takes in consideration of all factors, activates the program, and then executes it where the cells die.

(refer time: 02:52)

So classically, folks considered cell death in two broad contexts. The context of of necrosis and the context of apoptosis. Now I know that there are many different forms of cell that that have been discovered; you may have heard about them. For today's lecture we will discuss on these two broad definitions, of the broad context of cell death, necrosis versus apoptosis, because I want you all to appreciate the difference.

So what is the difference? So during necrosis the cells end up swelling and then the membrane ruptures. So this is very important, the cell lyse essentially, organelles are in disarray, and one very important aspect of necrosis is that there is massive inflammation. Why? Because the cells actually lyse and so that is how you end up with massive recruitment of immune cells; typically in an injury situation when you see redness that is basically a lot of inflammation because of necrosis.

In contrast to this apoptosis is different, right, so instead of swelling, the cells actually shrink. Organelles are preserved, cells do not end up rupturing the membrane. The membrane instead blebs and what I mean by blebs are these structures that are beautiful to look at and they are engaged because of cytoskeletal changes, apoptotic bodies where small bits of the cells actually get separated are also a feature of apoptosis.

Chromatin actually condenses into these small structures, and dying cells can eventually get phagocytosed. So either it can be professional phagocytes that come and engulf dying cells or it can be even neighbouring cells, but the important characteristic is that this does not result in massive inflammation, unlike necrosis. I do not want to give the impression that there is no immune cell involvement; there is, in order to get rid of the dying cells but by and large it is immunologically very silent. And that is important because this occurs routinely during normal development.

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So when does apoptosis occur? I want to talk about two contexts: first, during development. During development this is actually an incredibly important process from the earliest parts of development where the developing blastocysts needs to sculpt, there is cell death that occurs during this stage. Another good example is in different developmental stages there are some cells that are needed at one stage but not needed at another stage. One really good example is the tadpole tail, which is needed during the tadpole stage but in the adult frog stage the tail is gone and essentially the cells that form the tail, they undergo apoptosis. Apoptosis is also very important in controlling cell numbers, you know, we need to make sure that we have a certain number of cells - not excess and so this is controlled by apoptosis. This, actually is particularly important in the context of the developing brain; you do not think of cell death occurring in large numbers in the developing brain but it turns out in that the developing brain, almost half the neurons that you are born with end up dying. And one of the main reasons why almost half the neurons end up dying is because you need

to make proper connections and wiring up the brain is very complicated task, so you produce more cells, make the perfect connections and those that are in excess are eliminated.

And so the last one in the context of development is when you are trying to put together a complex organism some cells are just going to end up in the wrong place. So you need a mechanism that gets rid of those kinds of cells and so that occurs by apoptosis. I do not want to give you the impression that apoptosis occurs just during development, this is actually an incredibly active process during normal homeostasis.

Since the time we started talking about apoptosis, a couple minutes ago, cells in us have undergone apoptosis and the reason for that is that we are constantly surveying the status of our cells and we eliminate cells that are damaged, eliminate cells that have become harmful or eliminate cells that have become infected with viruses or have become cancerous.

So this is a a very important homeostasis mechanism that is quietly just trying to fix the problems and getting rid of cells that have the potential to cause harm.

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So apoptosis is also implicated in many pathological situations. I want to give you some examples, which occur because of too much and some which occur because of too little apoptosis. So what are the diseases associated with too much apoptosis? So some examples of that are stroke, spinal cord injury, neurodegenerative diseases such as Alzheimer's and Parkinson's disease. You know, these are complex situations where cells, neurons, end up dying ; neurons may end up dying because of multiple different factors, not just apoptosis. But clearly, apoptosis is also involved / implicated your markers of apoptosis are seen in these situations and there are efforts to see whether blocking apoptosis can rescue some of the phenotypes of these diseases. It is likely to be more complicated than that.

Another good example is is AIDS; this is a situation where the HIV virus induces apoptosis of the T-helper cells and that is why you have depletion of the immune system resulting in the acquired immunodeficiency syndrome. Examples of too little apoptosis, a big one, is cancer. This is a great example because cancers emerge because of a whole different other factors; cells essentially lose their ability to control their cell division.

There is excess cell division but a common feature of all cancers is that the cells should have undergone apoptosis and they do not. So the ability to eliminate any abnormal cells is a key feature of apoptosis. When that does not occur, then you can have situations just such as cancers emerge and in many of the exciting medicines that are being developed for cancer therapy, particularly what you people refer to as personalized therapy they are trying to figure out exactly why the apoptotic pathway is not able to be activated and then force its activation.

Other context is autoimmune disease, this is a situation where the normal development, the self-reactive cells, are eliminated and that elimination occurs by apoptosis; if the apoptotic pathway is blocked then that can result in autoimmunity.

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So here is a historical timeline of apoptosis research. I have taken the liberty to make this timeline. I have broken it into four different sections because I want you guys to get an appreciation for how this field had developed. Now the first thing is literally hundreds of years ago, scientists had been studying and defining a form of cell death that had common morphological features during development. I am sure some reports might even go before 1800s.

And when electron microscopy was developed, they had you know used that as a tool to define these particular, morphological characteristics of cell death that occurs during development. What happened in 1972 was a landmark paper was published by Kerr, Wyllie and Currie and that is the paper in which they defined the term apoptosis to this type of cell death. So this is why I put a historical context of 1972. From here on the word apoptosis became popular in the context of defining this type of cell death. So then for about 20, 30 years, people , one of the most interesting things that happened is that the genetic pathway of cell death was discovered as we will discuss in the next slide first in the context of *C-elegans* ; Bcl2, caspases were identified as regulators of apoptosis in mammalian cells.

And also, the death receptor pathway and the Fas ligands and the TNF receptor were also all defined to be important features of apoptosis. I put an emphasis on 1996 because another landmark paper was published in 1996, and what this paper this was done by Xiaodong Wang's Lab was that he identified his group identified cytochrome C as a key activator of apoptosis.

And this really made mitochondria as a central player in apoptosis and from here on mitochondria became a key, recognized as a key regular apoptosis. We appreciated the increasing complexity of apoptosis, expanded the function of Bcl2 family proteins particularly for BH3 family proteins IAP's and of course the exciting research on this continues to this day and in the future.

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So the mutagenesis screen in *C. elegans* led to the identification of three genes that regulate cell death in *C. elegans*. See these are called the *ced* genes, *ced* standing for cell death abnormal, and two of these *ced* genes, *ced-4* and *ced-3*, were required for cell death. In other words, if you had mutant worms that were mutated in either *ced-4* and *ced-3* then these worms the cells in these worms did not die.

In contrast, Ced-9 is an inhibitor of cell death and so mutations in Ced-9 caused more cells than normal to die. And so this you know of course things, a few other genes have also been identified since then in *C. elegans,* but this is the core pathway of apoptosis in *C. elegans* that was identified through the mutagenesis screen. Now what is striking about it is that there are homologues of all three of these genes that regulate the cell death pathway in mammalian cells.

And at the main fundamental level the structure kind of is the same. So what I mean by that is the homolog of Ced-4 is Apaf-1 and a homolog of Ced3 are caspases, and so both Apaf and caspases are required for apoptosis in mammalian cells. And the homologue of Ced-9 is the Bcl2 family genes. And the Bcl2 family genes, at least the initial member Bcl2, is an inhibitor of cell death. Things in mammalian cells are have evolved into having multiple members and we will talk a little bit more detail about that, the Bcl2 family now has both pro and antiapoptotic members.

Caspases are all it is a large family, which is all inducers of apoptosis and Apaf to the best of our knowledge is still a single gene family. But I hope you guys appreciate how the *C. elegans* the initial identification of genes in *C. elegans* really paved the way for the identification of the genes regulating apoptosis in mammalian cells.

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So for the rest of the lecture, we are going to focus more on the pathway of apoptosis in mammalian cells. And typically one would start with the pathway from outside to all the way inside and what happens in the end. But in this context , I actually want to start with inside out. So let us look at you know what are the key features of apoptosis and then let make our way backwards to see how are these key features of apoptosis brought about.

So what are the key features of apoptosis? So we learned earlier, the dying cells exhibit fragmentation of DNA, chromatin condensation, membrane blebbing, so this involves cytoskeletal changes and in the end phagocytosis. And the family of genes that regulate, that effect, these changes are actually the caspases. You know we talked about Ced3 and mammalian caspases, well, this is where the caspases come in.

The caspases are among the last is among the last event to be to happen during apoptosis and once caspases are activated at least the ones that are involved in executing cell death once those caspases are activated, then they can actually effect these apoptotic changes. So what are caspases?

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Well, the caspase family is a pretty large family in mammalian cells. There are at least 12 known caspases in mammalian cells; some of them are involved in in apoptosis, some are involved in inflammation, and others could have some other functions. So just to keep it simple we will focus on broadly caspase family but then talk mostly about the ones that are known to be involved in apoptosis.

So caspase family structurally has two subunits, so the p20 so the large subunit, and a small subunit. And most of caspases have a pro domain; now this pro domain can be relatively short or can be much longer and it turns out that the caspases that have the longer pro domain, actually the pro domain is very important in activation of caspases. So caspases as I said are the mammalian homologues of the *C elegans* Ced-3 gene. They are basically cysteine proteases. What that means is that their active site has a catalytic cysteine but their specificity is that they cleave after aspartic acid residues. So not every aspartic acid residue in their substrates, but they utilize a substrate sequence specificity in which proteins are going to be cleaved. For example, an executioner caspase called caspase 3 uses the, cleaves after the sequence DEVD, whereas another caspase called caspase 1, which is involved mainly in inflammation cleaves after the sequence YVAD, but the cleavage is after aspartic acid residues.

And what these caspases do is that once they are activated it they can cleave specific cellular proteins to induce rapid apoptosis at least the ones that are involved in apoptosis. And so if you ask me how many proteins are actually cleaved by caspases during apoptosis that number may well be in the hundreds if not a thousand. So a lot of proteins, there are some key ones that are, that we will talk about, but a lot of cellular proteins seem to be cleaved during apoptosis. And the consequence is that the cell really ends up getting dismantled and dies quickly.

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So the caspase family that mediate apoptosis can be roughly divided into two parts. So there are the initiator caspases;,as the name suggests these are the caspases that initiate apoptosis, and there are the executioner caspases, and these are the caspases that actually do the job of killing the cell. In the context of initiator caspases the two that have received the most attention actually these two: caspase-9 and caspase-8.

And I should mention that that one of the distinguishing characteristics of the initiator caspases is this presence of a larger PRO domain. So this is the sequence that is in front of the large domain, you can see these initiator caspases contain one of two kinds of domains, either the CARD domain, which is called the caspase activation and recruitment domain, or the DED domain and that is referred to as the death effector domains.

And what these initiator caspases do, is utilize these domains, either the CARD domain or the DED domain to is basically interact with other proteins that form a complex in which these caspases are activated. So caspase-9 for example is activated on the apoptosome complex via as you will see interaction with the CARD domain. And caspase 8 is activated on the death receptor domain while the DED domains.

These interactions lead to the activation of these initiator caspases and once these initiator caspases are activated, they can cleave and activate the executioner caspase. The one that has received the most attention is caspase-3. But there are other contexts in which caspase-6 and caspase-7 are also activated. But once you have the executioner caspases activated and as I said these initiator caspases can even activate the executioner caspase once you have caspase-3 activated for example then it cleaves hundreds of cellular proteins to induce rapid cell death.

I want to talk about two examples just to show you how the activated caspase 3 can exert these apoptotic changes.

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So, the first example focuses on how you get DNA fragmentation. And the enzyme that causes DNA fragmentation is the enzyme called caspase-activated DNase, it is called CAD. And this enzyme is normally in a complex with its inhibitor called ICAD so inhibitor of Caspase activated Dnase and this is the normal situation in a healthy cell because it is very important for these enzymes to be in a complex with its inhibitor because otherwise you would get DNA fragmented all the time.

And so what happens is that once you have caspase-3 activated, this can actually cleave the ICAD and you essentially have the release and activity of CAD which then causes DNA fragmentation. So this is how caspase-3 can actually cause DNA fragmentation. In another example caspase three can actually cleave another protein for example called ROCK1.

So Rho Activated Serine Threonine Kinase, this protein is normally in an inactive conformation but cleavage of caspase-3 causes it to become active and then once ROCK 1 is active, it can phosphorylate the myosin regulatory light chain that then causes membrane blebbing. So here is a situation where the cytoskeletal changes are mediated by caspase-3 via activation of the ROCK 1 kinase.

And so these are two just examples of what happens when you have caspase-3 activated.

So let us talk about how these caspases, caspase-3 in particular, is activated in the context of apoptosis. We know what it does once it is activated but let us talk about how caspase 3 is activated. And there are two main pathways first we will talk about the extrinsic pathway; it is also called the death receptor pathway. The reason is called the death receptor pathway is because there are molecules like Fas for example or TNF, tumour necrosis factor, both these can actually bind to their receptors, Fas will bind to Fas receptor / TNF will bind to its TNF receptor. And once these ligands bind to its receptor what happens is that these receptors get trimerized and then FADD gets recruited to these death receptors. FADD is an adapter protein and what this adapter protein does is it helps recruit caspase 8 to this death receptor complex. And this interaction occurs while these DED domains that we talked about before, remember, caspase 8 is an initiator caspase- it has these DED domains with the DED-DED interaction helps recruit caspase-8 to the death receptor. And once caspase-8 is recruited to the death receptor it gets activated. It gets activated by a process called induced proximity.

And once caspase-8 is activated, what it does is that it can directly cleave and activate caspase-3. And so this is actually the fastest way to initiate apoptosis in a cell, and because it is it is a direct short circuit from the external receptors directly to the caspase-3 and so this is called the death receptor pathway or the extrinsic apoptotic pathway. It is the fastest way to kill a cell it is usually employed by immune cells when they need to do something fast. If they are infected with a virus for example, they need to execute the death process very quickly.

Another way of activating caspase-3 is via the intrinsic pathway. This is also called the mitochondrial pathway of inducing apoptosis. And in that context, the initiator caspase is caspase-9. So not the death receptor but caspase-9, and so there is an incredibly interesting mechanism by which caspase-9 is activated in the intrinsic pathway. And this really came as a big surprise in the field because it turns out that the key trigger for activating caspase-9 was this protein in the mitochondria that is called cytochrome C.

Cytochrome C had been well studied in the context of mitochondrial oxidative phosphorylation. It is actually a key protein for generating ATP and for survival and it turns out the very same protein that helps you survive and generate ATP, actually in the context of apoptosis comes out. It comes out, it is triggered out and then that is cytochrome C what happens is that it binds to a protein called Apaf1 and then there is this complex called the apoptosome complex that is formed that results in the activation of caspase 3.

Remember from the *C. elegans* slide I mentioned that the mammalian proteins Apaf and caspases are important. Well, this is how it fits in, in the context of the mitochondrial pathway. And this really was a major surprise because nobody considered cytochrome C to be a key trigger of cell death and it is one of the most fascinating aspects of the cell death mammalian cell death field.

Again, the mechanism by which caspase 9 is activated on the apoptosome complex is via interaction with the CARD domain. So it turns out that Apaf also contains a CARD domain, caspase-9 contains CARD domain and these CARD-CARD interaction again on this complex causes the activation of caspase-9 by process that people refer to as induced proximity but once caspase-9 is activated then 3 is activated and the cells die.

So what regulates whether the cytochrome C is going to stay in the mitochondria or come out? And that turns out to be the function of the Bcl2 family of proteins; the original member of the Bcl2 family was called Bcl2, B cell lymphoma. But now this family has evolved into multiple members. We will talk more about it but the important thing to keep in mind right now is that this is the family that is the gatekeeper of determining whether cytochrome C stays inside or comes out.

And there are two great key proteins in this family that basically help cytochrome C come out but in a normal healthy situation Bcl2 makes sure that cytochrome C stays inside the mitochondria. And if you are wondering what are the external or what are the stimuli that help Bcl2 determine whether it should promote the release of cyrochrome C or not? These are the stimuli that are the triggers various triggers for apoptosis. For example growth factor withdrawal in neurons for example, or DNA damage in virtually any cell, stress- your stress for example or other stimuli whenever cells are faced with these kind of stress stimuli they basically figure out whether they can cope with it whether they can fix it or whether they should induce apoptosis and die. And if they decide to induce apoptosis signalling pathways they use different components to signal to the Bcl-2 family.

We will talk more about it very soon but once the Bcl-2 family proteins are sort of urged to induce this release of cytochrome C then it is the common pathway of apoptosis in all these different situations. And as I said that is called the intrinsic pathway of apoptosis. So basically, just to summarize you have two ways of activating caspases in cells. The fastest way is through the death

receptor pathway via caspase-8 as the initiator complex, this is also called the extrinsic pathway. Or there is the mitochondrial pathway, the intrinsic pathway the key trigger here is cytochrome c and the apoptosome formation, but the outcome then is still activation of caspase-3. So this is basically the entire picture in a nutshell, it looks complicated but I hope you guys appreciated how all these pathways lead to activation of caspase-3 that kills the cell and then there are two ways of reaching this point.

So, in the next slides we are going to talk about a little more detail about how the Bcl2 family is regulated and other ways of regulating caspases.