Cell Biology: Cellular Organization, Division and Processes

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Lecture 18

Apoptosis Part II

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So what I want to do for the remainder of this lecture is to focus on the Bcl-2 family proteins right here, talk a little bit about how interesting this is, and also very interesting family proteins that regulate apoptosis at this stage. So, that is what we will cover; some regulation at this stage, some regulation at this stage. And then I want to give you a specific example of how this pathway is a little different in neurons and I am sure you will find that interesting. Let us continue.

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So, the Bcl2 family, remember in *C. elegans* there is just one Ced-9, which is antiapoptotic. However, in mammalian cells the Bcl2 family has three subcategories of family members. One is the anti-apoptotic family members, which is Bcl2. Another example that you may have heard is Bcl-XL. These two are the best studied and that. So, they are all anti-apoptotic. So, they inhibit apoptosis.

However, a subset of the Bcl2 family are proapoptotic and these the two that get the most attention actually a protein called Bax and a protein called Bak. As we learn later they both can act redundantly to induce apoptosis. Now you will notice one of the difference between the antiapoptotic and the proapoptotic members is that the antiapoptotic proteins are a little bit longer, they contain an additional BH4 domain.

The BH4 domain is basically called the Bcl2 homology domain. So, they have transmembrane domains and then they have the BH domains but basically the antiapoptotic members are Bcl2, Bcl XL cell and others, and the proapoptotic members are Bax, Bak and Bok. Now uh there is a third subset of the Bcl2 family proteins that are called the BH3 only proteins. Now the BH3 protein only proteins contain only the BH3 domain it is only the BH3 domain.

And some of them contain, most of them contain a transmembrane domain but one of them actually does not, but the most important difference is that they do not they are not multi-BH domain family proteins they are just single ones and that is why actually this subset of family was the hardest to actually discover once these were discovered. So, the BH3 family proteins are actually the most early sentinels of apoptosis. These are the proteins that actually interact with the anti and proapoptotic members to either inhibit or promote apoptosis. These are the ones, that are actually the targets of the signalling pathways that then activate the BH3 proteins. And once the BH3 protein is activated then the cell can either determine whether it is going to die or live. So, let us figure out how that works.

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So, let us talk about how the Bcl-2 family proteins interact with each other to induce apoptosis . So, as I said the Bcl2 and Bcl XL are inhibitors of apoptosis; Bax and Bak are inducers of apoptosis. In normal cells healthy cells, this system is held in check, what I did not mention is that these proteins can interact with each other. Bax and Bak are two proteins that can actually physically go to the mitochondria and form channels on the mitochondrial outer membrane to promote the release of cytochrome C.

Bax is actually permanently in the mitochondria, Bak is already in the mitochondria, Bax can actually shuttle between the mitochondria and the cytosol regardless. The most important thing you want to know is that Bax and Bak can form channels in the mitochondria that can cause the release of cytochrome C and Bcl 2 and Bcl XL can prevent this formation of channels.

So, how is it determined who wins and what happens when cells want to undergo apoptosis. What happens is that these BH3 family proteins get activated. So, these are these proteins that are first activated in response to these various stimuli and I will give you specific examples later. But I want to tell you that these proteins can be activated either transcriptionally, So, in other words some of these BH3 family proteins might not be present in a cell and so they need to be transcriptionally induced. Remember I told you in the beginning that death is an active process. In many cells these are the genes that need to be synthesized in order for this death pathway to proceed. But there are other situations where the BH3 family proteins can be activated by phosphorylation or dephosphorylation. And then other examples are that they change sub-cellular localization.

Some of these BH3 family proteins interact with some other with the cytoskeletal for example and and a change in localization can then release them to then interact with these proteins to induce apoptosis. And the last one is by cleavage, Bid for example is a protein that gets activated by cleavage. Now what happens when these proteins are activated? What happens when these proteins activated is that a subset of them can actually inhibit these antiapoptotic proteins.

So, these are called the BH3 sensitizers and a subset of them can directly interact with Bax and Bak and activate it these are called direct activators. And so, consequence is that you have these BH3 primary proteins that do two things, one is they activate Bax and Bak to promote the release of cytochrome C and they inactivate Bcl 2 and Bcl XL and others. So, you got to remove the breaks and step on the gas.

You are stepping on the gas by activating these and removing the brakes in different context different BH3 family proteins are induced. I do not want to give the impression that that these all these BH3 family proteins are needed or even are activated in all situations; it is very context dependent. And so, these are considered to be a large redundant family of proteins that subsets are activated during different stimuli and then they can induce apoptosis by directly activating these and directly inhibiting those. And you know to different extents different things happen but that is essentially how it works.

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Here is one specific example I want to give you of what happens during DNA damage induced apoptosis. I am sure all of you are familiar with the p53 protein, this is a protein that is activated in response to DNA damage we will not talk about how it is activated. But I do want to give you an example of what happens when p53 becomes activated in the context of DNA damage.

And how does p53 then induce apoptosis? And the way it does it is by a transcription dependent mechanism and a transcription independent mechanism also. The transcription dependent mechanism is where p53 directly acts as a transcription factor to induce a subset of these BH3 family proteins. In particular, Noxa and Puma and these are proteins that can then activate Bax and then Bax can cause cytochrome C release.

But actually p53 can also directly sort of translocate to the mitochondria and directly somehow induce Bax activation and the release of cytochrome C. So, this is one example of how the BH3 family proteins are activated during DNA damage induced apoptosis.

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Let us focus our attention on a regulation that occurs even at the late point during apoptosis, this is after cytochrome C is released and this is where the apoptosome is now activating caspase 3. There is a very interesting family of proteins called the IAPs, inhibitor of apoptosis proteins, that can actually inhibit caspases at this stage and actually even at this stage. So, these inhibitor of apoptosis proteins were initially discovered in viruses, bacterial viruses in fact, these are proteins you can imagine viruses have all kinds of interesting proteins that they express to restrict apoptosis to allow them to replicate and then then get out of the cell.

But mammalian cells have also evolved these proteins and this act as very good brakes in this pathway. One particular cell type, which use utilizes these brakes very effectively are again neurons. And so, if you have the inhibitor of apoptosis proteins, and the cells need to die, there has to be some way of inactivating these brakes because otherwise the cells are just not going to be able to induce apoptosis.

And it turns out that the mitochondria in addition to releasing cytochrome C also releases other proteins, some of which can actually inactivate IAPs. So, one such protein is called Smac. And so Smac is an inhibitor of IAPs and so, you can see how it is very clever which is that the mitochondria

end up releasing not only cytochrome C which activates caspases but they also release proteins such as Smac that can then inactivate IAPs and permit cytochrome C mediated caspase activation to fully proceed to kill the cell.

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So, I want to give you an example of a cell type in which these controls over apoptosis are particularly important and those are neurons. Neurons are post mitotic cells that really have to last for a long time. Neurons do not divide so, they actually do not get cancer and so, restricting apoptosis as much as they can is just good for their long-term survival. Neurons do die during development but once that developmental period is over now you really want your neurons to live for a long time. And I want to give you some perspective of how neurons do that. So, how do they ensure that they are stricter controls, one is to maximize its anti-apoptotic checkpoints . So, the IAP family of proteins that as I mentioned before are brakes on caspases are particularly important in neurons; it is not the case that IAPs are not present in other cells, they are, but I do want to give you, I want to emphasize the fact that they are particularly important in neurons.

And you can imagine that neurons may have a situation where the mitochondria get damaged, cytochrome C might accidentally spill out, you do not want your caspases activated every time that happens and so, neurons exert a very strict control over that. In fact, the Smac that is released from neuronal mitochondria may not even be sufficient to eliminate IAPs and neurons do something else to degrade IAPs to permit apoptosis. So, it is a very good safety brake in neurons.

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The other thing that neurons do is minimize their pro-apoptotic checkpoints. So, what is the example of that? There are, remember I told you that Bax and Bak are two Bcl-2 family proteins that can both act redundantly. Well, it turns out in neurons Bak is not expressed. So, when cells become post-mitotic neurons they shut off the expression of Bak and they become completely dependent on Bax only.

So, if you delete Bax, post-mitotic neurons are unable to undergo apoptosis. And so, it is a very clever way in which they have reduced the redundancy and focused everything on Bax and that way they can control Bax much better. If you are a mitotic cell you want to maximize your proapoptotic potential. Why? Because there could be many situations where the cells become harmful for the organism and they are dividing. And so, you want to make sure that you have as many proapoptotic proteins regulated but as many as possible. So, the neurons do that by minimizing the proapoptotic components.

So, if you look at what the apoptotic pathway looks like in mature neurons. You can see that they have done a few things to restrict this pathway. One of them is to get rid of Bak to minimize the redundancy. The other is to engage the strict control of caspases with the IAPs.

And then we talked about that. There is actually something else neurons do, mature neurons in particular, once development period is over what mature neurons do is end up completely shutting off Apaf1. So, effectively they do not have any capability of activating apoptosis via this apoptosome pathway once the development period is over. So, that is very interesting now really interesting question is why these mature neurons do not completely shut down apoptosis?

Why do they still continue to express caspases, continue to express Bax, some of the BH3 family proteins are still engaged. And so, this actually was perplexing; you know if you do not want to die and want to live for a long time take all these proteins and just put a lock on all of them. But it turned out the mature neurons discretely do small things, but do not shut down apoptosis completely.

And in the last five or so years we have gotten a better understanding of what neurons need these caspases for. And it turns out that neurons utilize these same apoptotic proteins for non-apoptotic purposes. I will give you one example.

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So, here is an example of what neurons do with these caspases and apoptotic proteins. Neurons have a very interesting architecture. So, they have a cell body over here with dendrites, they have long axons and then they have these synaptic terminals over here. And it turns out that there are situations particularly in development when the entire neuron needs to die. But then once that development is over the neurons really do not want to die.

But they still want to maintain their ability to get rid of synapses and to get rid of axon branches it turns out this is something we do fairly frequently it is called synaptic remodelling or axonal branch remodelling. It is also called pruning for example. And one of the big surprises was the finding that neurons engage key components of the apoptotic machinery when they want to get rid of their synapse and when they want to get rid of their axon branches.

So, the reason why this was surprising is because activating the apoptotic machinery, Caspase in particular, brings a lot of risk for a neuron potentially of the entire neuron dying. And so, exactly how neurons do it where they activate caspases in a very discreet and very finite localized manner is still not entirely clear. But at least we know that that is what the neurons are engaging the caspases for even in the adult situation.

So, this just summarizes this point that neurons utilize many of the key components of the apoptotic machinery, Bax and caspases in particular, for synapse and axon pruning. This is this last slide of the apoptosis lecture. I had a wonderful time talking about it. I hope you guys enjoyed it and in the future when you hear about caspases, apoptosome or the Bcl-2 family proteins, that you will remember what these proteins do.

And maybe these are some of the pathways that you might even study and research in the future. I want to thank Dr. Shikha Laloraya for inviting me to give this lecture on apoptosis and thank you all.