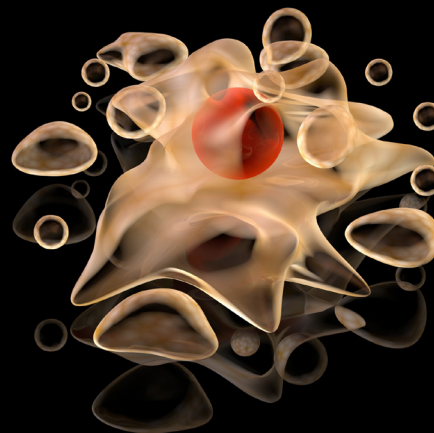


Death Becomes Us: Apoptosis and Carcinogenesis

by

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Part I – What Is Cancer?

“Hi, Derek. Studying for your Cell and Molec final?” asked Alyssa as she entered the suite.

“Just finished it. Glad that’s over. I’m actually reading about cancer.”

“Cancer? Why?”

“I got a summer internship working for a small biotech company in Philadelphia.”

“Awesome! When do you leave?”

“Not till next week. Mrs. Fletcher, the administrator of the summer internship program just sent me my airline tickets and housing information.”

“Are you done with all your finals?”

“Oh, yeah,” said Derek, stretching back in his chair.

“Me too. Why don’t we meet up with the gang and celebrate that this school year is *over!*”

Before Derek knew it his week was up and it was time to head to the airport. He was excited and couldn’t wait to get to Philadelphia. The day after he arrived, Derek took a bus to Elehcim, the biotech company to meet Dr. Hammell and begin his summer project.

“You must be Derek. I’m Daphne, Dr. Hammell’s graduate student.”

“Hi! Nice to meet you.”

“Dr. Hammell is stuck in a meeting and running late, so he asked me to meet you and help you get settled in.”

“Thanks a lot. I’m a bit nervous.”

“Everyone is their first day. You’re going to do great and who knows maybe you might get your name on a publication.”

“That would be awesome.”

“Have you read the articles Dr. Hammell sent you?”

“Yes, but there are some things that I’m not clear on.”

“Okay why don’t we have a test to see what you know and I can help fill in the rest.”

Derek silently groaned and thought, *Great, school just finished and now I have another test to take... .*

Questions

1. What is cancer and how does it develop?
2. What can lead to cancer?
3. What is the difference between malignant and benign?
4. Based on what you have learned do you think cancer is hereditary? Explain your answer.

Part II – The Paths of Certain Death

“Fantastic!” Daphne was impressed with Derek’s understanding.

“Indeed!” Daphne and Derek turned around in surprise to the new voice. It was Dr. Hammell.

Dr. Hammell and Derek walked over to his office.

“Well, it’s obvious you have a good grasp on what cancer is. The project you will be working on is to investigate novel cancer treatments by triggering the paths of programmed cell death, also known as apoptosis.”

“I’m not sure how apoptosis and cancer fit together,” said Derek.

“The maintenance, growth and development of eukaryotes depends on the production of cells and the mechanisms to destroy them. To ensure the health of the organisms, most cells die when they become damaged or infected. Cell death in this case and most other cases is not random, but occurs by a programmed sequence of cellular events in which the cell methodically destroys itself from within and is subsequently consumed by other cells, leaving no trace. This process of cell death is called apoptosis. Why don’t you read these and we’ll continue our discussion when you’re done,” explained Dr. Hammell. He handed Derek several review articles so that he could familiarize himself with the mechanisms involved in apoptosis.

“Great! Thank you.”

Derek sat at his desk with his stack of review articles. “Wow, there’s so much to know. I better start taking notes.”

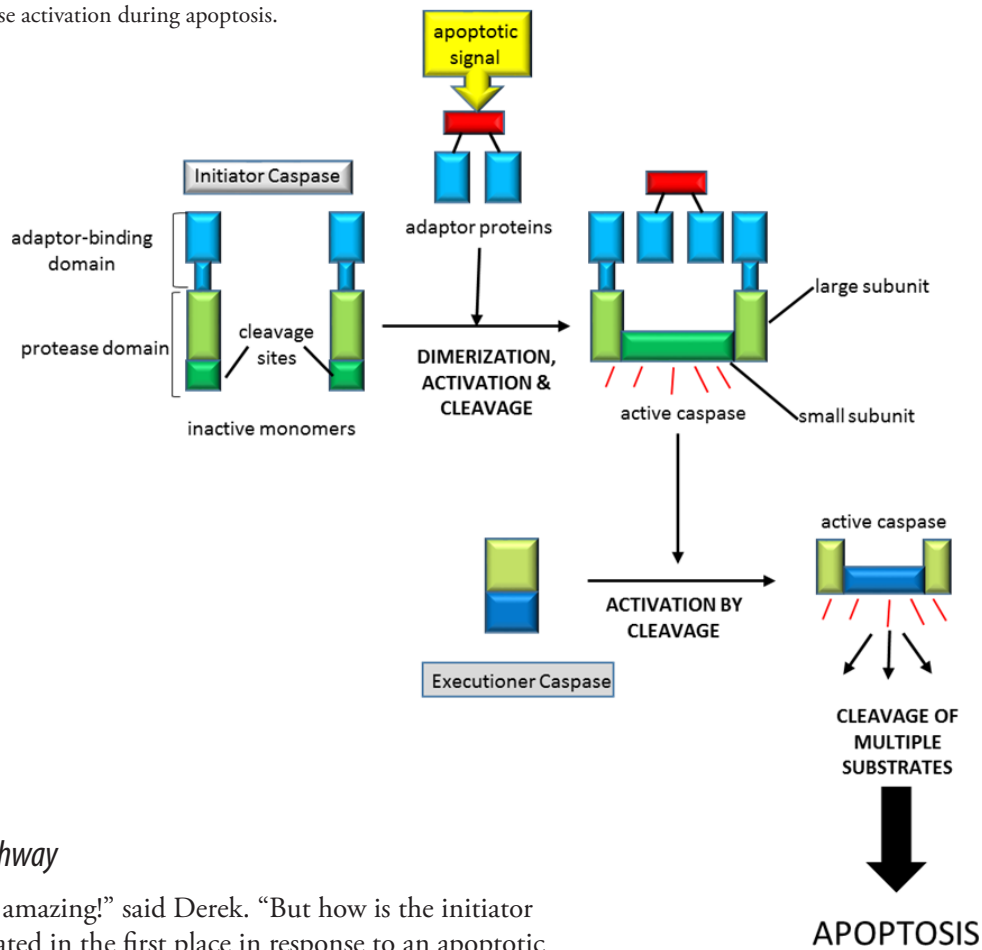
Typically when cells die as a consequence of acute injury they spill their contents all over their neighbors. This process is known as necrosis and has the potential to trigger an inflammatory response. However, in apoptosis the cytoskeleton collapses, the nuclear envelope disassembles, and the nuclear DNA breaks up into fragments. The cell shrinks and collapses precisely without damaging neighboring cells.

The dramatic cellular events that lead to cell death and engulfment is triggered by members of a family of specialized intracellular proteases called caspase. Caspase is synthesized as an inactive precursor and is activated within the cell only during apoptosis. There are two major classes of apoptotic caspase: initiator and executioner caspase.

The apoptotic process begins with the initiator caspase. Initiator caspase normally exist as inactive, soluble monomers in the cytosol. When an apoptotic signal reaches the cell, it triggers the assembly of large protein platforms that bring multiple initiator caspase together into large complexes. Pairs of caspase associate to form dimers within these complexes resulting in protease activation. Each dimer cleaves its partner at a specific site in the protease domain allowing for the active complex to stabilize. This stabilization is required for the enzyme to function properly in the cell (Fig 1).

The primary role of the initiator caspase is to activate the executioner caspase. Executioner caspase normally exists as an inactive dimer. Active initiator caspase cleaves the executioner caspase at a site in the protease domain causing a rearrangement of the active site from an inactive conformation to an active one. Once activated, executioner caspase catalyzes the wide scale protein cleavage events that eventually lead to cell suicide (Fig. 1)

Figure 1. Caspase activation during apoptosis.



Extrinsic Pathway

“Wow! That’s amazing!” said Derek. “But how is the initiator caspase activated in the first place in response to an apoptotic signal?” Derek wondered. He continued to read and take notes.

Initiator caspase can be activated when external signal proteins bind to cell surface death receptors triggering the apoptosis via the extrinsic pathway. A well understood example of how activation of death receptors triggers apoptosis is the activation of Fas by the Fas ligand. Fas ligand on the surface of a cytotoxic (killer) lymphocyte engages the Fas death receptor. A conformational change occurs, in this case it leads to trimerization, which further induces more clustering at the cytosolic surface. Activated receptors recruit adaptor molecules such as Fas-associating protein with death domain (FADD). FADD bind initiator caspase (procaspase-8) forming a death-inducing signaling complex (DISC).

Once dimerized and activated in the DISC, the initiator caspase (caspase-8) cleave their partners and then activate downstream executioner caspase (caspase-3) to induce apoptosis. Once activated caspase-8 can in turn activate caspase 3 through one of two mechanisms:

Mechanism 1: Caspase-8 cleaves procaspase3 directly and activates it (Fig 2).

Mechanism 2: Caspase-8 cleaves Bid (protein that stimulates apoptosis) and its COOH-terminal part translocates to mitochondria. The C-terminus of Bid triggers the release of cytochrome c which binds to apoptotic protease activating factor-1 (Apaf-1). Together, cytochrome c and Apaf-1 bind to procaspase-9, activating caspase-9. Caspase-9 cleaves procaspase-3 and activates caspase-3. For both mechanisms activation of caspase-3 leads to the induction of apoptosis (Fig 3).

Figure 2. Extrinsic pathway (Fas ligand), Mechanism 1.

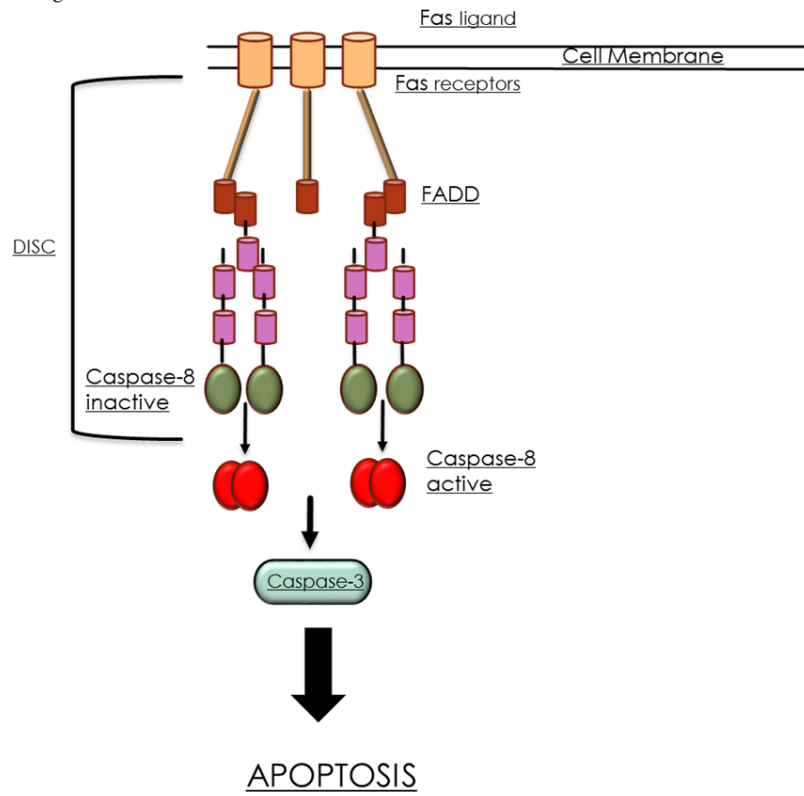
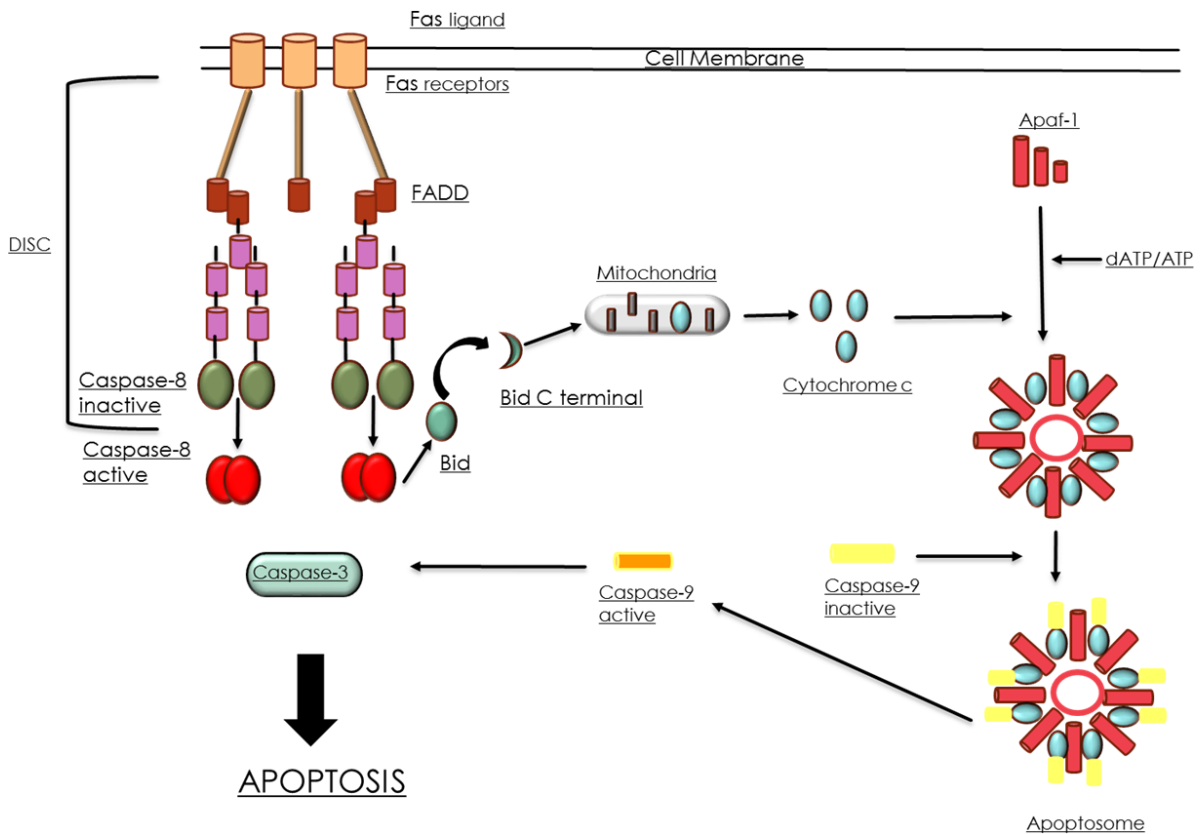


Figure 3. Extrinsic pathway (Fas ligand), Mechanism 2.



“Ok, so the initiator caspase is triggered by a signal outside the cell, but what signals would the cell receive that will cause it or want it to kill itself?” Derek thought. More reading, more notes....

Viral infection can induce apoptosis and occurs when one or several cells of a living organism are infected with a virus. Every time you get a viral infection, let's say influenza (flu), the virus invades certain cells of your body. The general life cycle of a virus is to attach to the host cell, gain entry, synthesize/replicate itself, assemble and finally leave the host cell to further infect other cells. Once inside, the virus hijacks the machinations to propagate more of the virus.

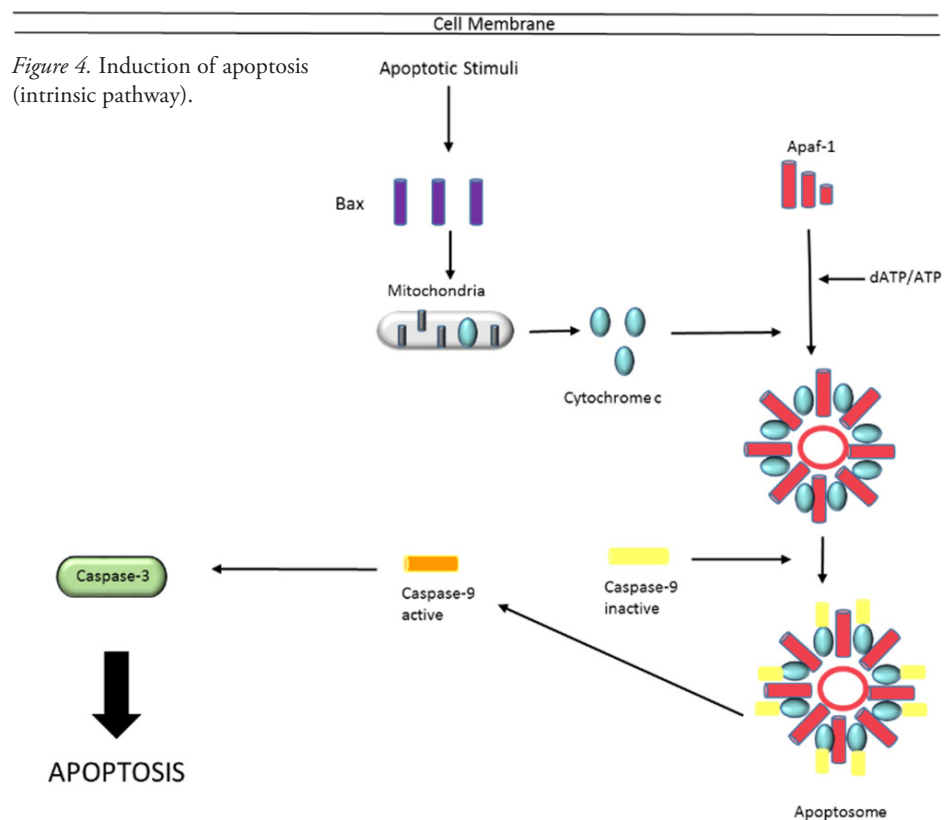
Viruses can trigger apoptosis of infected cells via a range of mechanisms. One such way is by recognition of viral proteins on the infected cell surface by cells of the immune system (for example cytotoxic T cells). When these cells are activated the expression of surface protein Fas ligand increases, which can then bind to Fas on the surface of the infected cell. The interaction of Fas and Fas ligand triggers the caspase cascade, leading to cell apoptosis and prevents further infection of other cells.

Derek looked up from his work as he heard someone let out a loud sneeze. Derek smiled at the thought of the possible hundreds of cells at this point undergoing apoptosis to prevent further spread of infection to other cells. Then he wondered, *Is it possible for the cell itself to know that something is wrong and initiate cell death on its own?*

Intrinsic Pathway

The apoptosis program can also be activated from within the cell, often in response to cellular stress such as DNA damage, chromosomal rearrangement and wound repair. This pathway depends on mitochondrial proteins that normally reside in the intermembrane space being released into the cytosol. These released proteins, particularly cytochrome c, activates a caspase proteolytic cascade in the cytoplasm, leading to

apoptosis. In the cytosol cytochrome c binds to an adaptor protein called Apaf1 (apoptotic protease activating factor-1), which causes it to oligomerize into an apoptosome. Apaf1 proteins in the apoptosome then recruit and activate initiator caspase-9 proteins. The activated caspase-9 molecules in turn activates downstream executioner caspase (caspase-3) to induce apoptosis (Fig. 4).



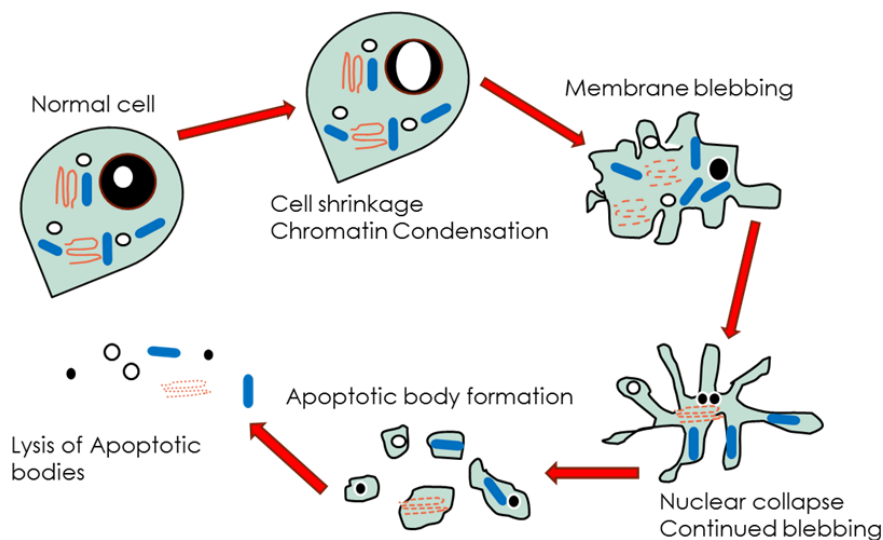
Apoptosis also occurs during embryonic development. Cell death helps sculpt hands and feet as they start out as spade-like structures, and the individual digits separate only as the cells between them die.

Derek examined his hands and laughed. *Who knew!*

Execution Phase

Cells undergoing apoptosis display distinct morphology. The cells typically begin to shrink following the cleavage of filaments (lamins and actin) in the cytoskeleton. This process causes a breakdown of chromatin in the nucleus, which often leads to nuclear condensation and a “horse-shoe” like appearance. As apoptotic cells continue to shrink they take on the appearance as membrane blebs or blisters and small vesicles known as apoptotic bodies. By packaging themselves into this form it facilitates their eventual removal by macrophages, large phagocytic cells. Macrophages are responsible for clearing the apoptotic cells from tissues in a clean and tidy fashion that avoids many of the problems associated with necrotic cell death (Fig. 5).

Figure 5. Execution phase of apoptosis.



Questions

1. Describe the difference between necrosis and apoptosis.
2. What are some events (external and internal) that could trigger apoptosis in a cell?
3. What is the function of the initiator caspase? What is the function of the executioner caspase?
4. What pathways are activated in response to an apoptotic signal?
5. Compare and contrast the apoptotic pathways identified in Question 4.
6. What morphological changes are typically seen with cells undergoing apoptosis?

Part III – Regulation of Apoptosis

Derek stretched and yawned, exhausted from reading and was still no closer to understanding what the connection between apoptosis and cancer was. Just then Dr. Hammell came into the lab.

“Excuse me, Dr. Hammell. I finished reading the articles you gave me. However, I’m still not sure how apoptosis and cancer fit together. Cancer is about uncontrolled cell growth and proliferation. That is the opposite of apoptosis.”

“There are many human disorders in which excessive numbers of cells undergo apoptosis and thereby contribute to tissue damage. Among the most dramatic examples are heart attacks and strokes. In these acute conditions, many cells die by necrosis as a result of ischemia (inadequate blood supply), but some of the less affected cells die by apoptosis. There are other conditions where too few cells die by apoptosis. Decreased apoptosis makes an important contribution to cancer cells; often their apoptotic program is regulated abnormally. Therefore, the cell employs multiple mechanisms to ensure that these proteases are activated only when appropriate,” explained Dr. Hammell

“So how does the cell regulate apoptosis?” asked Derek

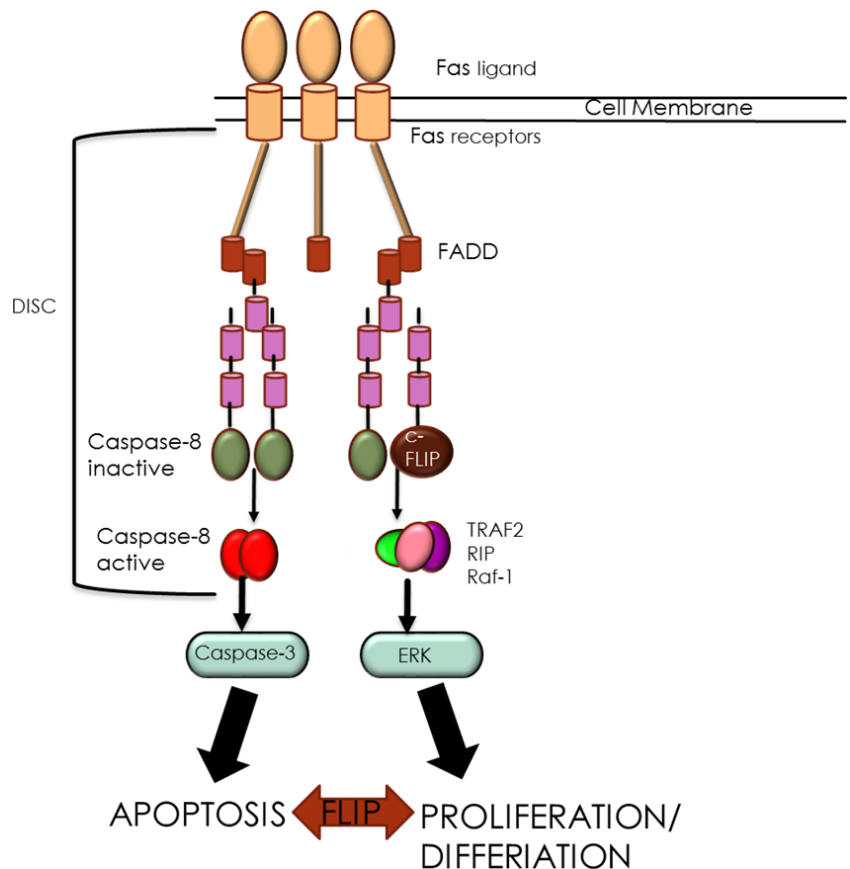
Dr. Hammell laughed. “Well, why don’t you do a literature search and tell me.”

Derek nodded and went back to his desk. He typed “apoptosis regulation” into his computer’s search engine and let out a sigh as he saw the multitude of results he got. “All right, here we go again.”

Extrinsic Pathway Regulation (c-FLIP)

Many cells produce inhibitory proteins that act to govern the extrinsic pathway. These inhibitory mechanisms help prevent the inappropriate activation of the extrinsic pathway of apoptosis. Some cells produce the protein c-FLIP, which regulates the activation of caspase-8 at the DISC. There are many variants of c-FLIP, but only three are expressed at the protein levels. These variants are c-FLIP long (cFLIPL), c-FLIP short (cFLIPS), and a short variant from the Raji B-cell line (cFLIPR). cFLIPL structurally resembles an initiator caspase, but has no protease activity because it lacks the key cysteine in its active site. cFLIPL dimerizes with caspase-8 in the DISC, which does not allow caspase-8 to be cleaved at the site required for its stable activation. Therefore, the apoptotic signal is blocked and the cell can proliferate and differentiate (Fig. 6).

Figure 6. Regulation of apoptosis by c-FLIP.



Intrinsic Pathway Regulation (Bcl2 family)

The main regulator of the intrinsic pathway is the Bcl2 family of proteins. Mammalian Bcl2 family proteins regulate the intrinsic pathway of apoptosis mainly by controlling the release of cytochrome *c* and other intermembrane mitochondrial proteins into the cytosol. The Bcl2 family can be categorized into two subfamilies, pro-apoptotic and anti-apoptotic. The pro-apoptotic proteins promote apoptosis by enhancing the release of cytochrome *c*. The pro-apoptotic Bcl2 family proteins consist of two subfamilies—the effector Bcl2 family proteins (Bax and Bak) and the BH3-only proteins. The anti-apoptotic proteins inhibit apoptosis by blocking the release of cytochrome *c*. The prominent members of the anti-apoptotic Bcl2 family proteins are Bcl2 and BclXL.

It was around 12:30 when Derek heard his stomach rumbling and decided he needed some lunch before he continued to tackle more. In the lunchroom he joined Daphne at a table.

“Hey, how’s it going so far?” asked Daphne.

“Okay I guess. Just finished reading about the Bcl2 family. It’s weird thinking that our cells have such well-coordinated and regulated systems for death.”

Derek began to test himself to see if he could understand the function of each Bcl2 family members. After a while he growled. “Wrong again. I’m never going to get these Bcl2 family members straight.”

“Take it easy, you’ll get it. I kinda developed a trick to remembering them. Well, at least for the pro-apoptotic proteins.”

“Yeah, what’s the trick?”

“For Bax, it’s just ‘Bring the Ax.’ Like an executioner. For Bak, it’s ‘Born A Killer.’ Again hinting to its role in promoting apoptosis.”

“I don’t know what’s weirder, your tricks or the fact that you sat around and came up with them.”

“Ha! Very funny, but it works for me.”

“Anything for the anti-apoptotic family members?”

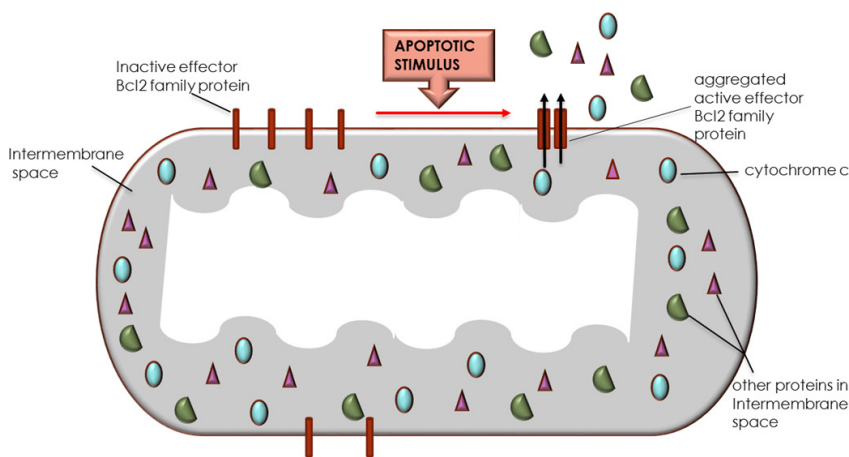
“You mean Bcl2 and BclXL? Yeah, I got nothing.”

Derek and Daphne laughed and before they knew it lunchtime was over. Daphne explained that she and several of the other graduate students and post docs were planning on taking Derek out to dinner to celebrate his first day after work. Derek thanked Daphne and went back to his desk.

“I’m going to get these families and their mechanisms straight if it’s the last thing I do! He went back to reading and taking notes on the regulation of apoptosis.

When an apoptotic stimulus triggers the intrinsic pathway, the pro-apoptotic effector Bcl2 family proteins (Bax and Bak) become activated and aggregate to form oligomers in the mitochondrial outer membrane, inducing the release of cytochrome *c*. (Fig. 7.) Bak is bound to the

Figure 7. Role of pro-apoptotic effector Bcl2 family proteins in the intrinsic pathway of apoptosis.



mitochondrial outer membrane even in the absence of an apoptotic signal. Bax is mainly located on the cytosolic surface of the outer mitochondrial membrane and will translocate to the mitochondria only after an apoptotic signal activates it.

Bcl2 family proteins members Bcl2 and BclXL inhibit apoptosis mainly by binding to pro-apoptotic Bcl2 family either on the mitochondrial membrane or in the cytosol and thus inhibiting apoptosis. Their binding to Bax or Bak prevents them from aggregating on the surface of the mitochondria. This in turn inhibits the release of cytochrome c and other intermembrane proteins in the mitochondria that might have otherwise induce apoptosis. Bcl2 and BclXL are either produced or activated by the cell in response to an apoptotic stimulus (Fig. 8).

In some case, when an apoptotic stimulus is present, BH3-only proteins become activated and bind to the anti-apoptotic Bcl2 family proteins so that they can no longer inhibit Bax and Bak proteins. This allows for Bax and Bak to become activated, aggregate in the outer mitochondrial membrane and allow the release of intermembrane mitochondrial proteins into the cytosol; thereby promoting apoptosis (Fig. 9).

Figure 8. Role of anti-apoptotic Bcl2 family proteins in the intrinsic pathway of apoptosis.

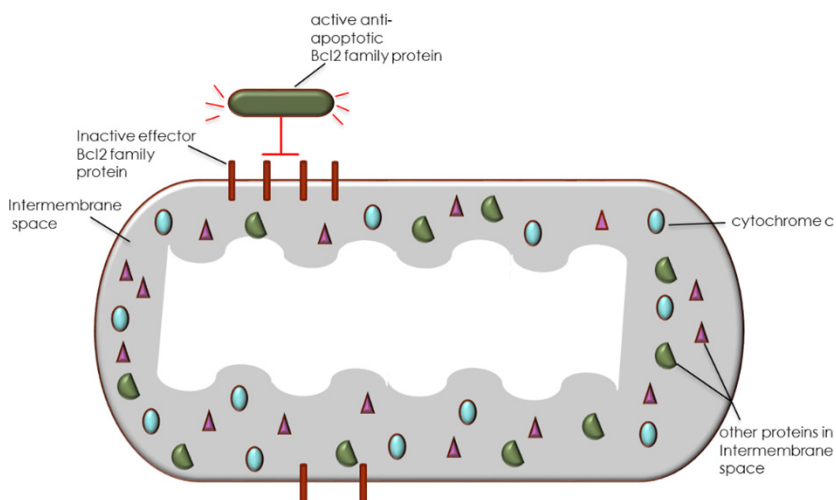
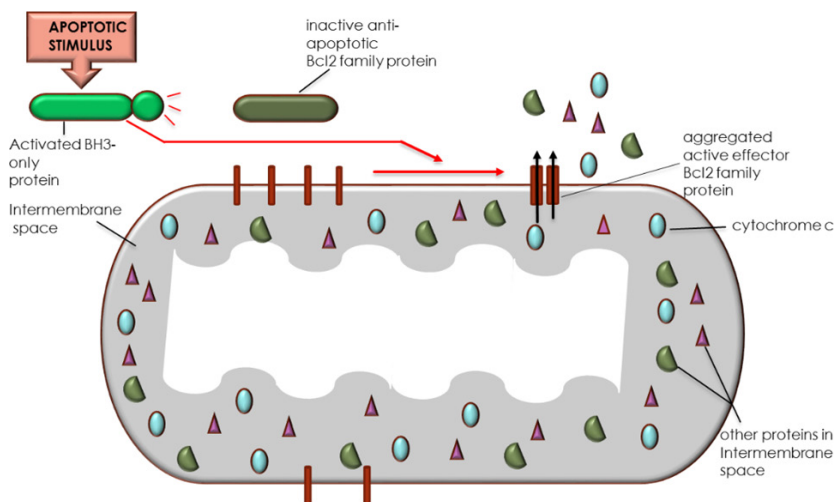


Figure 9. BH3-only protein activation during apoptosis.



“Okay, I understand how the extrinsic and intrinsic pathways are regulated. However, what regulates the caspase?” Derek asked himself aloud.

“IAPs!”

Derek turned around to see Daphne at his desk. “What?”

“IAPs, inhibitors to apoptosis.” Daphne said cheerfully. “IAPs are a family of proteins with various biological functions including regulation of innate immunity and inflammation, cell proliferation, cell migration and apoptosis. In mammals, IAPs can also regulate apoptosis by controlling caspase activity.”

Derek saw that Daphne was holding an empty coffee mug. “Let’s go get some coffee and I’ll explain.”

Derek got up from his desk and followed Daphne to the breakroom. “The human genome encodes eight members of IAP family and among them X-linked IAP (XIAP) and cellular IAP1 and 2 (cIAP1/2) have the capacity to bind to caspase. As you know, the core component of the apoptosome is the adaptor protein Apaf-1.”

Derek nodded and took a sip of his coffee.

“Binding of cytosol cytochrome c to Apaf1 causes an ATP-dependent conformational change and oligomerize Apaf1 into an apoptosome. Apaf1 proteins in the apoptosome then recruit and activate initiator caspase-9 proteins. The activated caspase-9 molecules in turn activates downstream executioner caspase (caspase-3) to induce apoptosis. XIAPs regulate caspase activity by a couple of methods.” Daphne sipped her coffee and continued “One method is by regulating the apoptosome and thus caspase-9 activity. XIAPs are present in the apoptosome and can directly bind to activated caspase-9 and inhibit it. A second method is by binding directly with the executioner caspase (caspase-3) and inhibiting their activity.”

“So let me see if I have this straight” said Derek. “cFLIP regulates the extrinsic pathway by inhibiting caspase-8 activation allowing for proliferation and differentiation of the cell, essentially inhibiting apoptosis. The Bcl2 family of proteins regulates the intrinsic pathway by 1) binding of Bax and Bak to the mitochondria causing the release of cytochrome c and other proteins allowing apoptosis to occur, 2) binding of Bcl2 or BclXL to the Bax in the mitochondrial membrane or Bak in the cytosol thereby inhibiting apoptosis, and 3) binding of BH3-protein only to Bcl2 or BclXL promoting apoptosis. Lastly, binding of XIAPs to either caspase-9 or caspase-3 inhibits apoptosis in both the intrinsic and extrinsic pathways.”

“You got it!” cheered Daphne.

Questions

1. What is the function of c-FLIP? Why would a cell develop this mechanism?
2. Compare and contrast the Bcl2 family proteins.
3. Describe what would be the outcome of the cell if a mutation knocked out Bax or Bak.
4. Describe what would be the outcome of a cell if a mutation knocked out BH3-only proteins.
5. Hypothesize what you think the function is of anti-IAPs.

Part IV – Apoptosis and Carcinogenesis

“This is all great, but how does any of this fit with cancer?” asked Derek.

Daphne laughed, “I’m not going to give you all the answers. Just think about it for a second and it will come to you.”

Derek thanked Daphne and went back to his desk to read over his notes. After a while Derek rubbed his eyes and looked out the window. *Hmmm, let's see ... cancer can be viewed as the consequence of a series of genetic changes in which the cell transforms into a malignant cell. Normally when a cell is damaged apoptosis is activated and the problem is solved. However, if the mechanisms of apoptosis are defective then these malignant cells can continue to proliferate and eventually evade other tissues...*

Derek quickly began reviewing his notes again. *Okay, if my theory is right, then defects in which mechanisms in these pathways would allow for a cell to transform into a malignant one?*

Derek’s eyes opened wide as he exclaimed, “Of course! If there are defects in the regulation of apoptosis or impairment in the transduction of the apoptotic signal then damaged cells would not die.”

Derek began making a list of all possible mechanisms where apoptosis could be defective and possibly cause cancer: 1) imbalance with Bcl2 family of proteins, 2) reduction in caspase activity, 3) imbalance of cFLIP and 4) impairment of the death receptor signaling.

Derek looked up and saw Dr. Hammell heading his way. “Dr. Hammell! I think I figured out the connection between apoptosis and cancer.” Derek handed Dr Hammell his notes.

Dr. Hammell read over what Derek had written and smiled. “Very impressive. Interestingly, every defect or abnormality in the apoptotic pathway can potentially be a therapeutic target for the treatment of cancer. Given the pivotal role the Bcl2 family plays in apoptosis a substantial amount of research is being done to develop drugs that mimic BH3. There are also efforts to silence coding genes of Bcl2 anti-apoptotic proteins. The IAPs are very attractive molecular targets as well. Many companies are designing drugs that would bind to IAPs, specifically XIAP. Others are working on IAP antagonists, which would inhibit IAPs. Still others are looking at agonists for death receptors, while many others are looking into drug and gene therapy treatments to activate caspase, particularly caspase-3.”

“It sounds very exciting!” exclaimed Derek

“Although it is very exciting most of these treatments are still in the pre-clinical phase of research. Here in this lab we are targeting Bax and Bak of the Bcl2 family. You will be looking at the effects of agonists to Bax and Bak to see their effects on cancer cells when administered alone and in combination with traditional chemotherapy drugs. It’s going to be a lot of work and we may not be successful. Are you game?” asked Dr. Hammell.

“You bet I am!” exclaimed Derek.

Questions

1. If the following apoptotic mechanisms are rendered non-functional or defective, explain what the outcomes would be and how this could possibly lead to cancer for each of the scenarios below.

Imbalance of Bcl2 protein family:

- Bcl2 and BcXL (anti-apoptotic proteins):

- Bax and Bak (pro-apoptotic proteins):

- BH3-only proteins (pro-apoptotic proteins):

Imbalance of cFLIP:

Reduction in executioner caspase-3:

Impairment of death receptor signaling:

2. Dr. Hammell described several treatments that are being investigated for cancer. For each of the scenarios below, describe how these treatments may help in the fight against cancer.

BH3 mimics:

Antagonists to IAPs:

Agonists to death receptors:

Agonists to Bax or Bak: