

Wiggles Isn't Wiggling: Apoptosis Edition

by

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Part I –Wiggles Goes to the Vet

Claire had brought her 10-year old Golden Retriever mix, Wiggles, to her local veterinary hospital because she had recently noticed a lump on her dog's neck. Claire was very worried. Claire had adopted Wiggles as a puppy, when her daughter was nine years old. Now her daughter was in college.

The physician, Dr. Mary Green, entered the exam room.

Dr. Green: Hello, Claire, it's nice to see you again. What seems to be the issue with Wiggles?

Claire: Hello Dr. Green. I'm so glad you could see Wiggles today. He has been lethargic and just doesn't seem to be himself. On Tuesday I noticed this small lump by his throat.

Dr. Green: I see. Let me do a quick physical exam and examine his peripheral lymph nodes. Has he been eating regularly? Have you noticed a loss of appetite?

Claire: He's not been eating as much as usual and has also been drinking lots of water.

Dr. Green: I'm glad you brought Wiggles in this afternoon, Claire. That lump you felt on his neck is an enlarged lymph node, and the other lymph nodes appear to be enlarged as well. I would like to perform a fine-needle aspiration and get some blood work to get a better idea of what's going on. I'll call you in a few days when the samples come back from the lab.

A few days later Dr. Green called Claire.

Dr. Green: Hello Claire, this is Dr. Green. The fine-needle aspiration I conducted the other day showed some abnormalities. I'm sending the sample to Dr. Henry Crab, a veterinary oncologist, for further analysis. I'll let you know as soon as possible.

Claire: Oh my gosh! Let me know as soon as you find out.

Dr. Green sent the aspiration and blood sample to her colleague Dr. Crab. Claire returned to the office a week later to receive the results.

Dr. Green: Hello again, Claire. I have the results for Wiggles. I sent the blood sample and cells to my colleague who is a veterinarian specialist. Wiggles' blood contained high calcium levels, and the aspiration slides contained abnormal lymphocytes. Dr. Crab indicated that these lymphocytes are malignant and according to his analysis it appears that Wiggles has lymphoma.

Claire: Oh my poor Wiggles! What are my options? I want to make sure he gets the best treatment possible!

Dr. Green: Well, Claire, my colleague, Dr. Crab specializes in treating cancer in dogs. My suggestion would be to make an appointment to see him.

Claire thanked Dr. Green and went to see Dr. Crab. At their appointment, Dr. Crab suggested HDAC (histone deacetylase) inhibitors (or HDACi) as a course of treatment for Wiggles. Dr. Crab explained that histones are proteins that help organize and compact DNA. Compact DNA can't be transcribed. In cancer cells, expression of genes that inhibit the cell cycle and induce apoptosis are turned off; treating cancer cells with HDACi turns these genes back on.

As shown in the pathway (Figure 1), two genes that are turned on by HDACi treatment are p21 and Bim. The tumor suppressor protein, p21, inhibits cell cycle progression by binding and inhibiting Cdk 2. Bim is a protein that activates Bax and Bak so that these proteins can each form pores in the outer mitochondrial membrane. Bim also binds to Bcl-2 preventing Bcl-2 from binding to Bax or Bak and preventing pore formation. HDACi treatment also turns off expression of Bcl-2, further reducing the concentration of Bcl-2.

Questions

1. Define the cell cycle.
2. Define apoptosis.
3. Use Figure 1 to explain why formation of Bax and Bak pores lead to the activation of Caspase-9.
4. Caspase-9 activates caspase-3 and other caspases that cleave cellular proteins. Give three examples of proteins cleaved by caspases and the resulting cellular changes.
5. Use Figure 1 to explain why HDACi treatment tips a cell's balance in favor of apoptosis. Incorporate Bcl-2, Bim, Bax, Bak, and Caspase-9 into your explanation.

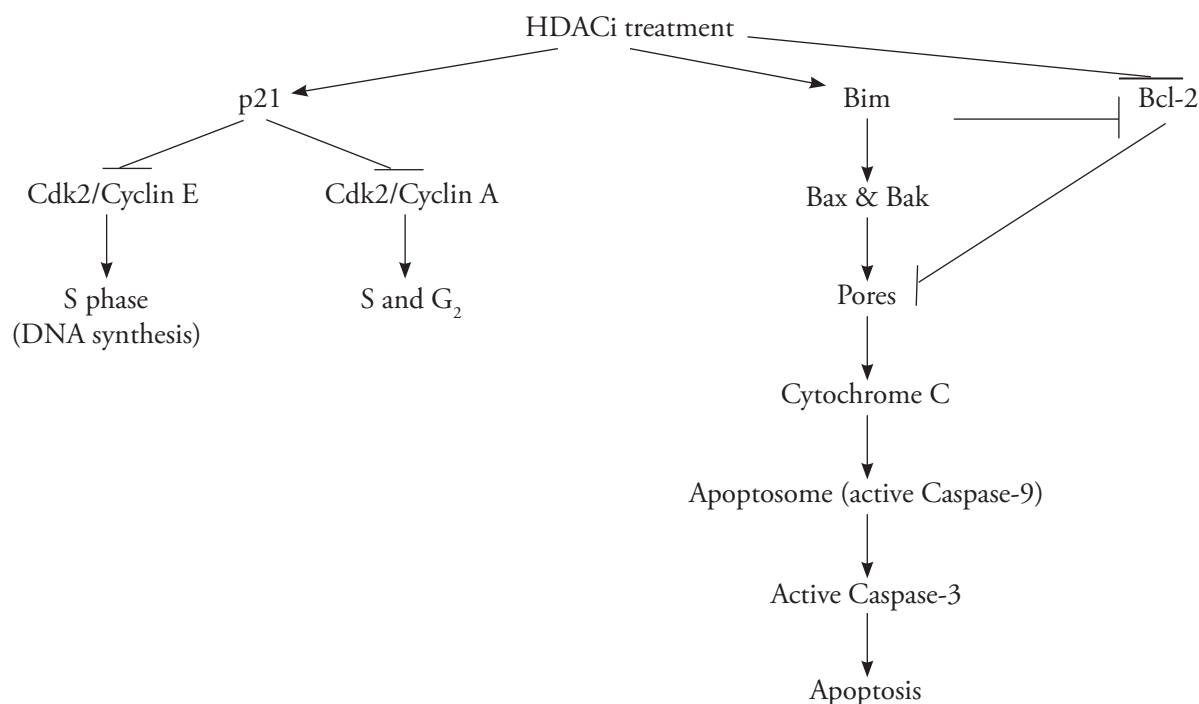


Figure 1. HDACi treatment causes cell cycle arrest and induces apoptosis in cancer cells. The apoptosome forms when Cytochrome C binds to Apaf-1, which allows Apaf-1 to bind to and activate Caspase-9.

Part II – Alice’s Lab Experiments

Later that evening Claire called her daughter Alice to share the news.

Claire: Hi honey, I have some bad news about Wiggles.

Alice: Oh no! What’s wrong?

Claire: You remember how Wiggles didn’t have any energy when you were home over break? Well, it turns out that he has lymphoma.

Alice: That’s awful. Can the vet do anything?

Claire: The vet said they could try treating him with something called an HDAC inhibitor (HDACi).

Alice: Mom, my cell biology class tested HDAC inhibitors in lab!

Alice’s mind was reeling when she got off the phone. She couldn’t believe her dog might be treated with the same kind of drug that her cell biology class had tested. What if her class discovered an HDACi that could be used to treat cancer? Maybe even Wiggles! Alice reviewed the lab results from her class.

Alice’s cell biology class had been testing potential HDAC inhibitors that were synthesized by the Organic Chemistry II class last year. The organic students had completed the synthesis of two potential HDAC inhibitors and named them Xyzi and QB.

The students determined whether Xyzi and QB could kill cancer cells by first incubating U937 cells (a human lymphoma cell line) with different concentrations of Xyzi or QB and then determining the number of live cells after 48 hours of treatment. The data was expressed as the IC^{50} value, which is the concentration of chemical that kills half the U937 cells. Table 1 gives the IC^{50} values for Xyzi and QB.

Table 1: IC^{50} values for potential HDACi synthesized by Organic II students.

<i>Potential HDACi</i>	<i>IC^{50} for U937 cells</i>
Xyzi	0.28 μ M
QB	2.8 μ M

Questions

- Based on the results in Table 1, is Xyzi or QB more effective at killing cancer cells? Explain your answer.
- Although Alice’s class showed that Xyzi and QB both kill U937 cells, they did not know if the cells die because these compounds induce apoptosis or necrosis. Why is it preferable for HDACi to cause apoptosis instead of necrosis?
- List three characteristics of cells undergoing apoptosis.
- Design an experiment to test the hypothesis that Xyzi induces apoptosis in U937 cells. Read about apoptosis in your textbook. Then choose a characteristic you listed in Question 3 that could be tested by methods covered in class. Be sure to check the section of your textbook that discusses methods used by cell biologists. Your design must specify treatments, controls, and methods for determining apoptosis.

Special instructions for Question 4:

- Submit your answer to Question 4 as a separate document; this portion of the assignment will be reviewed by others in your class.
- Keep the experimental design to one page and include the following information:
 - Your hypothesis and prediction. (One sentence for each.)
 - Explanation of the methods. A flow chart is best.
 - Assuming Xyzi induces apoptosis:
 - Draw the expected results in a form consistent with the methods.
 - Explain the results.

Part III – What’s Best for Wiggles?

Claire: Hi honey, I have more news about Wiggles and I just don’t know what to do!

Alice: Ok Mom. Tell me what Dr. Crab said.

Claire: Dr. Crab suggested treating Wiggles with an HDACi, but I’m just not sure. Should I treat Wiggles or not?

Alice: Oh Mom, this is so hard! Let me do some research and I’ll call you back.

Your Task

Assume you are Alice. Write a one-page letter to Claire (Mom) explaining whether or not Wiggles should be treated for lymphoma. Include the following in your letter:

1. What is the average age at which dogs are diagnosed with lymphoma? Compare this to the life expectancy of a golden retriever.
2. What is the treatment for lymphoma?
3. What is the expected survival rate after treatment?

Paper requirements:

- Approximately one page (1.5 line spacing).
- Typed and printed with black ink. (Pink, yellow, and blue make instructors blind!)
- Standard font and size required. (Times, Times New Roman, Cambria, or similar. Use 11 or 12 font size.)
- 1-inch margins.
- Sources:
 - At least two different sources.
 - Websites are acceptable as long as they are scientific. Please ask if you are not sure. Examples include:
 - A university veterinary college website.
 - (NSF) National Science Foundation.
 - Specific website for the cancer.



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