

# Wiggles Isn't Wiggling: Gene Expression 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 the 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. Histone deacetylases (or HDACs) are a group of enzymes that remove acetyl groups from the amino acid lysine on histone tails. The removal of acetyl groups allows histone tails to bind tightly to DNA and prevent transcription of particular genes. In cancer cells, expression of genes that inhibit the cell cycle and induce apoptosis are turned off. Treatment with an HDACi turns these genes back on.

### *Questions*

1. Define epigenetic inheritance.
2. What three mechanisms initiate epigenetic change?
3. Based on Dr. Crab's treatment plan, which mechanism that you identified in Question 2 is most likely to play a role in the development of Wiggles' lymphoma?
4. Define a nucleosome core particle.
5. Explain the histone code hypothesis.
6. How does the histone code hypothesis relate to oncogenesis?

## Part II – How Do HDAC Inhibitors Work?

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.

Alice recalled that inhibiting HDACs causes an overall increase in histone acetylation particularly in the core promoter elements of specific genes. HDACi treatment turns on the expression of genes that favor cell cycle arrest and apoptosis. For example, HDACi treatment increases the expression p21 and Apaf-1, which halts the cell cycle and facilitates apoptosis, respectively.

### Questions

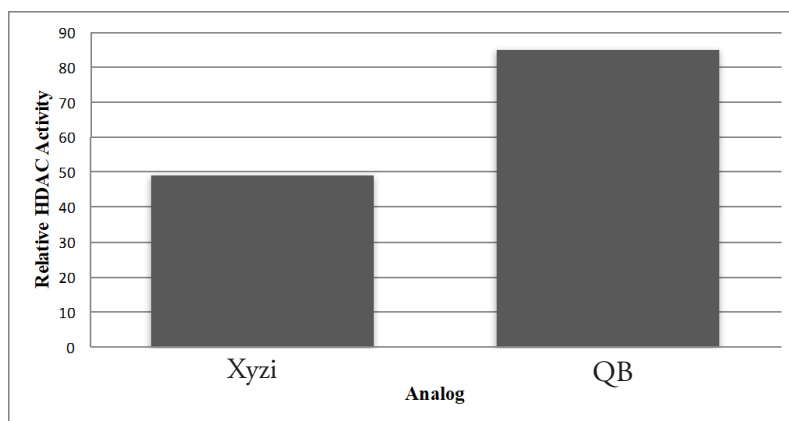
1. Which RNA polymerase most likely transcribes p21 and Apaf-1 genes? Explain your answer.
2. State three characteristics of core promoter elements for RNA polymerase II transcribed genes.
3. State three characteristics of enhancer elements for RNA polymerase II transcribed genes.
4. Enzymes called HATs (histone acetyltransferases) and HDACs regulate histone acetylation. Explain how histone acetylation within a promoter helps facilitate the expression of a gene.

## Part III – Alice’s Lab Experiments

Alice was excited about the HDACi lab. 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 class last year. The organic students had completed the synthesis of two potential HDAC inhibitors and named them Xyzi and QB.

The first experiment they conducted was an inhibition assay. HDAC 1 protein was mixed with buffer and HDAC substrate. Two additional samples also contained either 13  $\mu\text{M}$  of Xyzi or QB. All three samples were incubated for 30 minutes. The reaction was then stopped, the amount of product was determined, and the relative HDAC 1 activity was calculated by dividing the amount of product produced in the presence of Xyzi or QB by the amount produced without either chemical (analog). Figure 1 gives the relative activity of HDAC 1 with Xyzi or QB.



*Figure 1.* The effect of potential HDACi on HDAC 1 activity. Relative HDAC activity is calculated by dividing the amount of product produced in the presence of the potential HDACi by the amount produced without the potential inhibitor. One hundred percent activity means the potential inhibitor did not inhibit HDAC 1.

### Questions

- Based on the results in Figure 1, is Xyzi or QB more effective at inhibiting HDAC 1? Explain your answer.
- In a follow-up experiment, Alice’s class determined that Xyzi killed cancer cells at a lower concentration than QB. Based on the results in Figure 1, why is it not surprising that Xyzi kills cancer cells at lower concentrations than QB?
- Alice’s class was to design an experiment to determine if Xyzi and QB cause changes in gene expression like known HDACi. They decided to examine the expression of p21.
  - Briefly explain the role of p21.
  - Design an experiment to determine if Xyzi and QB each activate the expression of p21 in U937 cells. Your design must specify treatments, controls (positive and negative), and methods for determining expression. Your cell textbook is a wonderful resource for methods. (*Hint:* Read the methods pertaining to Northern blotting and immunoblotting.)

#### *Special instructions for Question 3b:*

- Submit your answer to Question 3b 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 p21 expression is activated:
    - Draw the expected results in a form consistent with the methods.
    - Explain the results.

## Part IV – 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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