Sickle Cell Anemia — Hope from Gene Therapy

Secrets of the Sequence Video Series on the Life Sciences • Grades 9 — 12

Teaching materials developed by **VCU** Life Sciences

Virginia Commonwealth University

Classroom Tested Lesson

Video Description

"Secrets of the Sequence," Show 108, Episode 2

"Sickle Cell Anemia – Hope from Gene Therapy" – approximately 10 minutes viewing time

This inherited red blood cell disorder is the most common genetic disease in America, and one of the most painful and debilitating. Until now, bone marrow and cord blood transplants have been the only treatment available to patients, and matched donors are hard to find. But an experiment at Genetix in Cambridge, Massachusetts is raising hopes. By treating the bone marrow of mice with anti-sickling gene therapy, scientists saw great improvement in normal red blood cell production.

Ward Television
Producer: Kris Larsen
Associate Producer: Eric Wills
Featuring: Dr. Lewis Hsu, Emory University & Georgia Sickle Cell Center
Dr. Phillipe Leboulch, Harvard Medical School & Genetix Pharmaceuticals, Robert Pawliuk, Genetix Pharmaceuticals
Lesson Author; Reviewers: Susan Walton; Catherine Dahl, Dick Rezba, and Selvi Sriranganathan
Trial Testing Teachers: Leigh Dougherty, Martin Shields, Tommy Sommerville

National and State Science Standards of Learning

National Science Education Standards Connection

Content Standard C: Life Science
As a result of their activities in grades 9-12, all students should develop understanding of

- Molecular basis of heredity
- Biological evolution
- The cell

Content Standard F: Science in Personal and Social Perspectives.
As a result of their activities in grades 9-12, all students should develop understanding of

- Science and technology in local, national and global challenges

Content Standard G: History and the Nature of Science.
As a result of their activities in grades 9-12, all students should develop understanding of

- Science as a human endeavor
- Nature of scientific knowledge
- Historical perspectives

Selected State Science Standards Connections

Use http://www.eduhound.com (click on "Standards by State") or a search engine to access additional state science standards.

Virginia

BIO.4 The student will investigate and understand relationships between cell structure and function. Key concepts include

- a) characteristics of prokaryotic and eukaryotic cells.

BIO.6 The students will investigate and understand common mechanisms of inheritance and protein synthesis. Key concepts include:

- h) use, limitations and misuse of genetic information; i) exploration of the impact of DNA technologies

Florida

Biology 4 Demonstrate understanding of the principles of genetics with emphasis on molecular basis of heredity, genetic diversity and related biotechnologies.
SC.F.2.4.2. know that every cell contains a "blueprint" coded in DNA molecules that specify how proteins are assembled to regulate cells.

Overview

Sickle cell anemia is a genetic disorder of the blood in which abnormal hemoglobin molecules are produced. Hemoglobin is a protein molecule in each red blood cell that carries oxygen from the lungs throughout the body. The molecule releases the oxygen at the cells and changes conformation so that it can pick up carbon dioxide to return to the lungs.

The hemoglobin molecules produced in individuals with sickle cell anemia are abnormal. When the molecules release oxygen, they cannot return to their original conformation. Instead, they remain in rod-like structures. This causes the normal, doughnut-shaped red blood cell to curve into a sickle shape. These misshaped blood cells don't flow well in the blood vessels and may clump together. They may become trapped in the narrow capillaries, blocking the flow of blood. This results in severe pain as well as other problems. Due to the lack of oxygenated blood and the rapid destruction of the ill-formed cells by the body, anemia is also a major symptom.

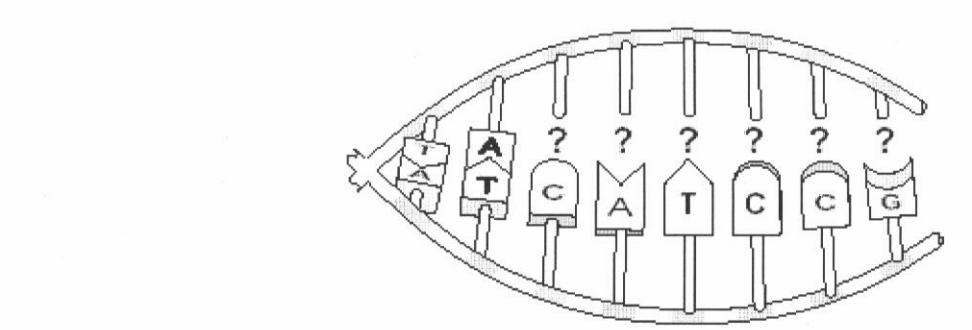
The gene for normal hemoglobin is known as 'Hemoglobin A' while the abnormal gene is known as 'Hemoglobin S'. An individual with the genotype 'AA' carries two copies of the Hemoglobin 'A' gene. An individual with the genotype 'AS' is a carrier of the gene for sickle cell anemia, but does not show symptoms of the disease. An individual with the genotype SS has sickle cell anemia because he or she does not have a gene that codes for normal hemoglobin.

The mutation that causes sickle cell occurs in the hemoglobin-Beta gene on chromosome 11. The abnormal protein results from the substitution of a single base in the gene sequence of the beta-globin chains. This base change results in the substitution of the amino acid valine for glutamic acid in the 6th position of the mRNA transcript. Details of the chromosome and protein structure can be found at
http://www.ornl.gov/sci/techresources/Human_Genome/posters/chromosome/hbb.shtml

The sickle cell gene originated in areas where malaria was a serious threat to health. Individuals of the AS genotype - sickle cell carriers - were better able to survive malaria. As populations migrated to areas where malaria was not a threat, the gene lost its usefulness.

Bone marrow transplants have been shown to be a cure for sickle cell, but the marrow must come from a healthy sibling. Gene therapy appears to be the solution of the future. Possibilities include transplanting a corrected gene into the bone marrow and turning off the defective gene while turning on the gene for fetal hemoglobin.

Testing: A sample related multiple choice item from State Standardized Exams



Which series of bases will complete this strand of DNA?

- **A** CCTGAT
- **B** ACTGGC
- **C** GTAGGC
- **D** TCAGGG

Source: 2001 EOC Biology Online Exam, Texas
Answer: C

Video Preparation

Preview the video and make note of the locations at which you will later pause the video for discussion.

Before Viewing

1. Ask: "Have you ever heard of sickle cell anemia?"
   If so, ask: "What do you know about the disease?" and "How is a sickle cell different from a normal cell?"
   Review the "normal" shape of red blood cells with hemoglobin.

2. Provide students with basic information about the disease. In addition to the video and the lesson overview, see the National Heart, Lung and Blood Institute Web site for information: www.nhlbi.nih.gov
   On the Web site enter "what is sickle cell anemia?" into the search engine to find information pages.

   Additionally, you can go to
   http://www.ornl.gov/sci/techresources/Human_Genome/posters/chromosome/hbb.shtml and show students the location of the HBB gene on chromosome 11.

3. Review with the students that offspring receive one allele from their mother and one from their father so different genotypes arise. Have students complete the Introductory Activity on Punnett squares showing the cross between AA and AS parents and then between AS and AS parents (see Appendix A: Punnett Square Worksheet and Appendix B: Answer Key to the Punnett Square Worksheet).

Ask, “What is the probability of having offspring with sickle cell disease from these crosses?”
(0% for AAxAS cross; 25% for the ASxAS cross)

During Viewing

1) START the video.

2) PAUSE the video (2:40 minutes into the video) after Chris' mother says, "That's what caused Christopher to be born with sickle cells."

Ask students:

- “What is Chris’ mothers’ genotype for hemoglobin? *(AS)*
- “What is his father’s genotype?” *(the same, AS)*
- “Which Punnett square predicts the possibility that Chris would have sickle cell disease?” *(the second one, ASxAS)*

Refer students to the two Punnett squares they completed in the ‘Before Viewing’ phase. Point out that Chris' parents hoped they were the AS x AA cross, but they turned out to be the AS x AS cross.

Ask students: “If Chris’ parents had another child, what would be the chance of that child having the sickle cell disease.” *(25%)*

3) RESUME the video.

4) PAUSE the video (4.55 minutes into the video) after the host says “ …and today he is sickle cell free”.

Review the location of bone marrow and its function.

Ask students:

- “How does a bone marrow transplant work to treat someone with sickle cell disease?
  (It provides the person with bone marrow that produces normal red blood cells)
- Why do bone marrow transplants not always work?
  (Because of graft vs. host disease. The new marrow is rejected by the person’s immune system)

5) RESUME and play to the end.

After Viewing

1. Ask students, “What is gene therapy?”
   Gene therapy is a genetic engineering technique in which genes that cause disorders or diseases are replaced with genes that produce the needed proteins.

2. Assist the students in summarizing on the board the steps of the technique that were suggested as gene therapy for sickle cell disease.
   - *Researchers isolated an anti-sickling gene that re-programmed stem cells in diseased bone marrow to produce healthy non-sickle blood cells.*
   - *They then found the right means (or vector) to deliver the anti-sickling gene to the stem cells. They used a virus derived from the HIV virus, with the dangerous genes removed, to get the new message through.*
   - *Bone marrow was removed from a mouse with the sickle cell disease.*

- *The marrow was treated with the anti-sickling gene.*
- *The treated marrow was exposed to the vector virus in vitro.*
- *The treated and exposed marrow was injected into a genetically identical mouse with the disease.*
- *The new bone marrow took over and the mouse was cured.*
- *Next step: Will it work in people?*

Teacher Notes for the Student Activity: The Mutated Sickle Cell Gene

Discussion

Engage students in a discussion about the ethical issues surrounding the use of gene therapy. Use one or more of the following questions to start the discussion:

Note: Inform students that the following questions focus on personal value and that there are no right or wrong answers. Serving as a moderator, facilitate student to student discussion of the issues. When interest in a discussion topic wanes, move on to another question.

- Who decides what is normal and what is a disability?

- How does this view affect an individual with a disability or disorder? Will they be viewed or treated differently by society than "normal" individuals?

- Are disabilities diseases? Do they need to be cured or prevented?

- How does this view affect an individual with a disability or disorder?

- Gene therapy in somatic (body) cells (*such as what was done to the mice in the video*) does not alter the genetic code of eggs and sperm and only affects the individual being treated. How do you feel about ‘germline’ gene therapy in which the eggs and sperm are altered, thus determining how the trait will be passed to future generations?

- Currently gene therapy is very costly. Who will determine which individuals will be offered treatment with gene therapy? Who will pay for the therapy? Should only people with life-threatening conditions have access to gene therapy?

Note: Some of these questions were adapted from the Gene Therapy page of the Humane Genome Information Web site. This site offers a great deal of information and additional resources on gene therapy.
http://www.ornl.gov/sci/techresources/Human_Genome/medicine/genetherapy.shtml

Procedure:

1. Distribute copies of the Student Handout: The Mutated Sickle Cell Gene showing a nucleotide sequence for amino acids 3 through 9 for normal hemoglobin.
   Have students :
   - Find the complement DNA,
   - Transcribe the DNA into messenger RNA
   - Use the RNA code to determine the amino acid sequence.

   The sequence can be listed on the board or on an overhead transparency.

2. A table of codons for the amino acids can be found at http://www.bmrb.wisc.edu/referenc/codons.html

3. Change the 2nd base in the DNA nucleotide from an A to a T. Tell students that this is a mutation.

   Ask: What effect will this have on the amino acid sequence.
   The amino acid # 6 is changed from glutamic acid to valine. This is the mutation that causes the sickle cell trait.

Extension:
An excellent, but lengthy, additional student activity on sickle cell anemia, called The Mystery of the Crooked Cell can be found at http://www.mdbiolab.org/pdf/Mystery%20of%20the%20Crooked%20Cell.pdf

Answer Key:
DNA nucleotide sequence for amino acids 3 through 9 of Hemoglobin A. (normal beta chain of hemoglobin)

	3			6			9
DNA	C	T G	A	C T	C	C T	G A G G A G A A G T C T

Complementary DNA nucleotide sequence :

DNA	G	A C	T	G A	G	G A	C T C C T C T T C A G A

Messenger RNA sequence:

mRNA	C	U G	A	C U	C	C U	G A G G A G A A G U C U

Corresponding amino acids:

amino acids	leucine	threonine	proline	glutamic acid	glutamic acid	lysine	serine

DNA nucleotide sequence for amino acids 3 through 9 of Hemoglobin S. (abnormal beta chain of hemoglobin)

	3			6			9
DNA	C	T G	A	C T	C	C T	G T G G A G A A G T C T

Complementary DNA nucleotide sequence :

DNA	G	A C	T	G A	G	G A	C A C C T C T T C A G A

Messenger RNA sequence:

mRNA	C	U G	A	C U	C	C U	G U G G A G A A G U C U

Corresponding amino acids:

amino acids	leucine	threonine	proline	valine	glutamic acid	lysine	serine

Student Handout: The Mutated Sickle Cell Gene

Below is the DNA nucleotide sequence for amino acids 3 through 9 of hemoglobin A.
Complete:

- the complementary DNA nucleotide sequence
- the messenger RNA sequence that would be transcribed
- and the corresponding amino acids.

	3	6	9
DNA	C T G	A C T C C T G A G	G A G A A G T C T
Complementary DNA			
mRNA			
amino acids			

This is the DNA nucleotide sequence for amino acids 3 through 9 of hemoglobin S which contains a mutation.
Complete:

- the complementary DNA nucleotide sequence
- the messenger RNA sequence that would be transcribed
- and the corresponding amino acids.

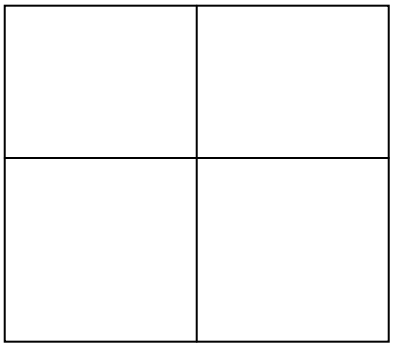
	3	6	9
DNA	C T G	A C T C C T G T G	G A G A A G T C T
Complementary DNA			
mRNA			
amino acids			

Question:
What is the difference between the two sequences and what effect will it have?

Appendix A

Introductory Activity:
Punnett Square Worksheet

1. Complete the Punnett square for a cross between a parent with 2 normal hemoglobin genes and a parent who carries the gene for sickle cell anemia.

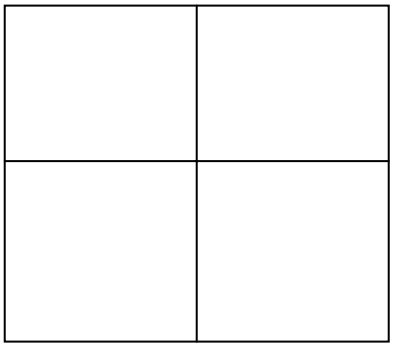


Genotypes

Phenotypes

Probability

2. Complete the Punnett square for a cross between two parents who carry the gene for sickle cell anemia.



Genotypes

Phenotypes

Probability

Appendix B

Answer Key to Introductory Activity: Punnett Square Worksheet

1. A cross between a parent with 2 normal hemoglobin genes and a parent who carries the gene for sickle cell anemia.

	A	A
A	AA	AA
S	AS	AS

Genotypes: AA, AS

Phenotypes: normal

Probability: 4/4 normal

2. A cross between two parents who carry the gene for sickle cell anemia.

	A	S
A	AA	AS
S	AS	SS

Genotypes: AA, AS, SS

Phenotypes: normal, sickle cell disease

Probability: ¾ normal
¼ sickle cell disease

Additional Resources

Because Web sites frequently change, some of these resources may no longer be available. Use a search engine and related key words to locate new Web sites.

http://www.scinfo.org/
Sickle Cell Information home page

http://www.nlm.nih.gov/medlineplus/sicklecellanemia.html
MedLine Plus

http://www.ornl.gov/sci/techresources/Human_Genome/posters/chromosome/hbb.shtml
gene structure

http://www-medlib.med.utah.edu/WebPath/HEMEHTML/HEME062.html
photo of sickled red blood cells

http://bioweb.uwlax.edu/GenWeb/Molecular/Theory/Translation/translation.htm
animation of translation

http://bioweb.uwlax.edu/GenWeb/Molecular/Theory/Translation/Translation_Lecture/translation_lecture.htm
interactive translation lecture

http://www.ncbi.nlm.nih.gov/Genbank/GenbankOverview.html
GenBank searchable database

http://www.ornl.gov/sci/techresources/Human_Genome/medicine/genetherapy.shtml
Human Genome Project Information page – doegenomes.org

http://www.artic.edu/~pgena/docs/CIMXI-gena-strom.pdf
musical synthesis of DNA sequences, including the hemoglobin sequence

http://www.biology.com/
the biology place (tutorials)

http://www.ornl.gov/sci/techresources/Human_Genome/education/education.shtml
The Web site to the governmentally-funded Human Genome Project, with links about genomics, the history of the project, and more.

Secrets of the Sequence Videos and Lessons
This video and 49 others with their accompanying lessons are available *at no charge* from www.vcu.edu/lifesci/sosq