Sickle Cell Disease Case Study

Educator Document

**Teach time:** 30-minutes of class time, or broken up into two 25-30 minute segments.

**Overview:** This activity complements the 1-minute video *Sickle Cell Disease*. Students explore the biology of the disease as well as the mechanisms behind two therapeutics being tested phase 1 and 2 in clinical trials in 2019. There is an additional video on Sickle Cell Disease clinical trials that could be a nice way to introduce part II or conclude the activity that will premier in November 2019.

**Key Concepts:**

* Protein shape is intrinsically tied to protein function. Protein shape is influenced by ligand binding as well as environmental effects.
* Red blood cells are small cells mostly filled with hemoglobin
* Small blood vessels are only slightly larger than red blood cells, when red blood cells take on an irregular shape, they may not fit through smaller vessels
* Hemoglobin affinity for oxygen is influenced by environmental conditions

**Suggested audience:**

* Undergraduate biology students

**Student Learning Outcomes:**

* Explain the difference and connection between hemoglobin mutation (genetics) and the change in protein and cell shape
* Describe the connection between the concepts of ligand-binding-induced conformational change and the exacerbation of SCD symptoms during oxygen stress
* Describe how the risk factors, symptoms and complications of SCD have to do with protein and cell shapes
* Apply biology concepts to fundamentals of gene therapy and potential complications

**Prior knowledge:**

* Terms hydrophobic and hydrophilic and membrane transport dynamics
* Very basic idea of the immune system response (secondary responses)
* Basic understand of virus action (delivery of viral genes)

**Teaching tips:**

* This activity can work in a small classroom or a large lecture hall.
* This activity could be done as homework, but we recommend the students be able to discuss with each other either in person or online as some of these questions involve tying together complex concepts.
* We strongly recommend that you have checkpoints in which answers are given and misconceptions addressed. This can be done in a few ways:
	+ IF/AT immediate response cards (<http://www.epsteineducation.com/home/about/>)
	+ Make each question a clicker question and discuss after each one
* The two places for open response can be discussed in class or submitted as open response questions.
* In smaller classrooms, more of the questions could be converted to open response and the hemoglobin saturation graph could be drawn by the students, rather than presented as a multiple choice question

Answers:

1. B--The three-dimensional shape of a protein is determined by interactions among the protein’s amino acids. Environmental factors can change the likelihood of bonding among the amino acids and therefore influence the protein shape. In addition, when a ligand binds, it may draw or repel amino acids away from the binding site, causing a shape change as well. This is a good moment to remind your students of these biochemical basics, because (as we will see in question 5) ligand binding can influence the change in hemoglobin shape seen in sickle cell disease).
2. B--hydrophobic molecules are lonely in aqueous solutions and tend to clump to exclude water. The intracellular fluid is an aqueous solution and two exposed hydrophobic regions on nearby proteins will be drawn together. This leads to aggregation of hemoglobin in sickle cell disease and ties into question 4. You could show this image at this time (Image obtained from: Central Dogma and Genetic Medicine at <https://www.hhmi.org/biointeractive/central-dogma-and-genetic-medicine>):



1. C--Sickle cell hemoglobin clumps and healthy hemoglobin doesn’t because of the change from a polar/hydrophilic amino acid to a non-polar hydrophobic one.
2. A--The increased hydrophobicity of the mutated protein causes the proteins to aggregate together in fibers. It could be useful to show this TEM image (Image obtained from: The making of the fittest: natural selection in humans found here: <https://www.hhmi.org/biointeractive/how-do-fibers-form>). Courtesy of Thomas E. Wellems and Robert Josephs. University of Chicago.



1. B--Ligand binding can induce shape changes in proteins. In sickle cell disease, hypoxia exacerbates hemoglobin polymerization. The polymerization is reversible when oxygen conditions improve, but each time that significant polymerization occurs it puts strain on the RBC membrane. After several polymerization events, the membrane is permanently altered. Soon the red blood cell will be removed from circulation by the spleen or liver, leading to a reduction in hematocrit. This application makes for great extension of the knowledge or follow-up questions.
2. A--Here the video is useful in illustrating this phenomenon. You can follow up with this image if it is useful (Image obtained from: Central Dogma and Genetic Medicine at <https://www.hhmi.org/biointeractive/central-dogma-and-genetic-medicine>):



1. B--Here we bring it back to the good old central dogma. When we talk about a disease state in physiology we are often talking about symptoms that are far downstream of the cause, this question brings them back to the cause.
2. B--DNA cannot cross the membrane, if it could our cells would leak DNA all the time! Viruses are essentially genetic material delivery devices by nature and are often used in gene therapy approaches. One of the limits to gene therapy is getting a large amount of DNA into a cell, this is what makes Thomas’s trial so interesting, it was previously thought to be impossible to deliver an entire gene. This trial, by Bluebird Bio, uses a lentivirus to deliver the entire adult hemoglobin gene.

Open response: Because these two therapies are currently in clinical trial, we do not know the long-term outcomes of each approach (or even if they will work!). One often cited concern about Thomas’s clinical trial, run by a company called Bluebird Bio is that the hemoglobin gene is fairly large, and the lentivirus cannot control where in the genome the virus will insert the Hb gene. This means that the gene could be inserted into other genes, disrupting their function. One concern with Rhonda’s trial, run by the companies Biovertiv and Sanofi, is that if a female starts to express fetal hemoglobin, it could complicate oxygen transfer between mother and fetus if this woman ever becomes pregnant. This concept could make for a good extension activity. Again these trials are currently in early phases in children, it could be a long time before we get answers to these questions.

1. C--This question is designed to bring the students back to the concepts of hydrophobicity and molecular interaction. The fetal hemoglobin molecules will interrupt the hydrophobic attraction between the adult hemoglobin molecules. This occurs naturally in some individuals (called Hereditary Persistence of Fetal Hemoglobin) and predicts an improved clinical outcome (Wang and Thein, 2018).
2. C--This question targets the students’ understanding of the immune system. The first delivery of the virus/gene therapy is most successful because the immune system is mounting a primary response against the virus. Upon second delivery, the immune system will mount an efficient secondary response and it is unlikely that very much of the virus would be able to get to the hematopoietic stem cells.

Extensions:

* A natural extension or way to approach the topic of Sickle Cell Disease is through discussion of natural selection and fitness. HHMI has more resources on SCD and natural selection that can be found here: <https://www.hhmi.org/biointeractive/making-fittest>
* In an advanced biology courses, incorporation of some of the other SCD therapies and clinical trials could include examining hemoglobin polymer formation, antiadhesive therapies or pain management techniques (beautifully summarized in Kapoor et al., 2018).

References:

Kapoor, Little and Pecker, (2018). Advances in the Treatment of Sickle Cell Disease. *Mayo Clinic Proceedings* 93(12): 1810-1824.

Sankaran and Orkin (2013). The switch from Fetal to Adult Hemoglobin. *Cold Spring Harbor Laboratory Perspectives in Medicine* 3 a011643.

Wang and Thein (2018). Switching from fetal to adult hemoglobin. *Nature Genetics* 50: 477-482.

Kato, Steinberg and Gladwin (2017). Intravascular hemolysis and the pathophysiology of sickle cell disease. *Journal of Clinical Investigation* 127(3): 750-760.