Sickle Cell Disease: Symptoms, Molecular Basis, and Treatments

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1 Introduction

Sickle Cell Disease (SCD) is a chronic, mostly recessive, genetic disease suffered by 3.1 million people world-wide with 460 million asymptomatic gene carriers [6]. In many developed countries, SCD is diagnosed during neonatal screenings. Traditionally, techniques like protein chromatography / electrophoreses are used to differentiate between mutant and wild-type haemoglobin. Technological advancements have made genetic, even prenatal, tests more accessible [10]. The symptoms of SCD are numerous and diverse, but most patients suffer from acute pain, anaemia, and an increased risk of infection. Other symptoms include ischaemia induced tissue damage and an increased risk of clotting disorders such as stroke, acute chest syndrome, and embolism conditions. Additionally, individuals heterozygous for the sickle-cell trait benefit from a curious resistance to malaria (see Figure 2) [1, 3, 10].

2 Molecular Basis

2.1 Genetic Origin

Sickle Cell Disease is caused by a missense mutation in the 17th base of the β-globin gene that swaps the 6th protein residue from a glutamic acid to valine [1, 10]. Valine, unlike glutamic acid, has a hydrophobic side-chain which subtly alters the chemistry of the β-globin chain. The mutated β-globin goes on to form a complex with one other β and two other α chains, becoming a mature haemoglobin S (HbS) protein [1]. The S serves to differentiate this mutated form of haemoglobin from the wild-type (called HbA).

2.2 Sickle Cell Formation

In oxyhaemoglobin (the oxygen-carrying conformation of the haemoglobin protein), this mutation is silent and leads to no ill-effects as the rogue valine residue is buried within the body of the protein — the issue arises when the oxyhaemoglobin releases its bound oxygen and becomes deoxyhaemoglobin. In the deoxyhaemoglobin conformation, the hydrophobic valine sits on the surface of the β-globin and is free to interact with other molecules. As it happens, there is a second hydrophobic patch naturally present on the β-chain formed by a phenylalanine and leucine in positions 85 and 88 respectively. When enough deoxyhaemoglobin is present within an erythrocyte, these sites can form hydrophobic bonds. It's these hydrophobic interactions between β-chains that allows the haemoglobin to polymerise [1, 10].

This polymerisation eventually results in the formation of stiff haemoglobin fibres within the erythrocyte [1]. As the haemoglobin is precipitated out of solution, the osmotic pressure within the erythrocyte drops and water begins to evacuate the cell. The erythrocyte then shrivels around the polymerised haemoglobin fibres, taking on SCD's characteristic sickle-shape [5, 10]. Figure 1 shows this process.

2.3 Molecular Basis of Symptoms

2.3.1 Acute Pain

The recurring pain experienced by patients with SCD is thought to be caused by vaso-occlusion. During vaso-occlusion, stiff, sickled cells become lodged in capillaries leading to ischaemia (a lack of oxygen in the tissues) which manifests as an intense pain [10].

2.3.2 Anaemia

Sickle Cell Anaemia is a form of haemolytic anaemia resulting from the destruction of erythrocytes by the immune system. When sickled cells become trapped in the capillaries, they often trigger an immune response in which the leukocytes lyse the sickle-cells in an attempt to restore circulation. This leads to a lack of erythrocytes in the blood [1].

2.3.3 Clotting Disorders

The lysis of sickle-cells releases large amounts of haemoglobin into the plasma where it can form reactive oxygen species (ROS) that rapidly scavenge nitric oxide (NO) — an important vasodilator and regulator of endothelial adhesion [7, 10]. In the absence of NO, the blood takes on a hypercoagulable state, increasing the risk of vasculopathies like acute chest syndrome and stroke [10].

2.3.4 Immune Suppression

The increased susceptibility to infection is a multifactorial symptom thought to be caused in part by impaired circulation and micronutrient deficiencies [3]. The impaired circulation is a product of the aforementioned vasculopathies, but the mechanism of micronutrient loss remains an area of active study. It has been proposed that a combination of ischaemic damage to intestinal mucosa, and an elevated rate of metabolism (to support the production of new erythrocytes) are to blame [2].

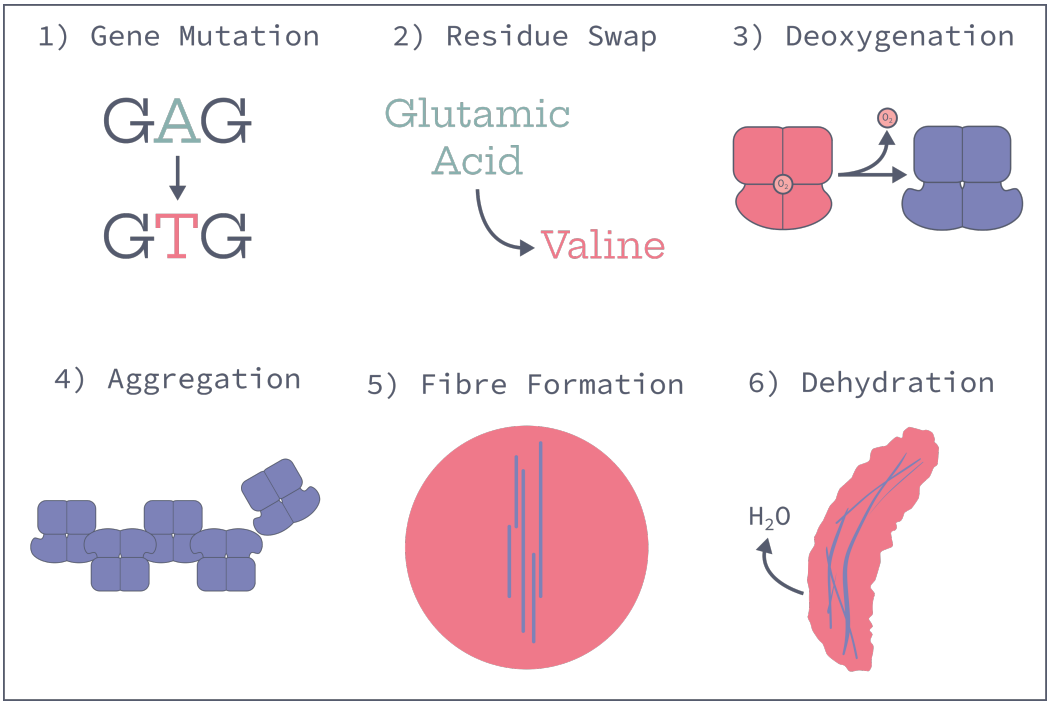

Figure 1: This figure presents an overview of the molecular processes responsible for sickle cell formation. **(1)** A missense mutation occurs in the 17th position of the β-globin gene, changing out an adenine for a thymine. **(2)** This codon change replaces the 6th residue in the chain, a glutamic acid, with a hydrophobic valine. **(3)** After the haemoglobin loses its oxygen, it undergoes a conformational change that results in the valine residue coming to the surface of the protein. **(4)** The newly exposed valine forms hydrophobic bonds with a second hydrophobic site on the β-chain, forming a polymer. **(5)** As polymerisation continues, large, stiff fibres form within the erythrocyte. **(6)** The precipitation of haemoglobin from the cytosol results in a drop in osmotic pressure. As a result, water flows out of the cell and the erythrocyte takes on a shrivelled, sickled appearance.

3 Treatments

3.1 Haemoglobin & Hydroxyurea

There are two forms of haemoglobin naturally present in humans: adult haemoglobin which is composed of two β and two α chains, and foetal haemoglobin (HbF) which is composed of two γ-chains and two α-chains. HbF is dominant during infancy, but mostly disappears a few months after birth. As HbF contains no faulty β-chains, fetal haemoglobin does not aggregate or lead to the formation of sickle-cells, thus preventing most SCD symptoms [1, 3, 4].

Hydroxyurea (HU) is a cytotoxic drug that induces the expression of HbF in adults. In small doses (∼20mg / day), HU shows little toxicity and substantially reduces HbS aggregation. While many mechanisms have been proposed to explain how HU induces the expression of HbF, a scientific consensus has yet to be reached [9].

In addition to increasing the production of HbF, HU has been found to generate NO in the blood plasma — further combating the excessive clotting exhibited by patients with SCD [10].

3.2 Blood Transfusions

Blood transfusions are a common, quick, and relatively safe treatment for SCD [3]. While this works well for a while, repeat transfusions can put the patient at risk of an iron overload. Excess iron can damage organs like the liver and the heart, but can be treated using iron chelating drugs that capture and flush iron from the body [3].

3.3 Bone Marrow Transplant

Currently, the only known cure for SCD is a bone-marrow transplant. By replacing the haematopoietic cells in the marrow, the patient's HbS can be replaced with the donor's HbA [3].

The biggest issue with this treatment is histocompatibility. While the treatment has a 82-86% cure rate, it also has a mortality rate of 7% and even if the transplant goes well, the patient often relies on a long-term regimen of immunosuppressants [3].

4 Conclusion

While SCD is one of the better studied heritable diseases, it's rarity in developed countries means that treatment options are limited. Fortunately, SCD treatment lends itself well to several emerging technologies: gene therapy and induced pluripotent stem cells (iPSCs). At the moment, as haematopoietic cells are difficult to culture, gene therapy tends to be carried out *in vivo* using lentivirus vectors [3]. While this works decently (there is even an ongoing clinical trial in France [10]), there is a risk of off-target mutations within the patient's genome. However, by culturing iPSCs from the patient and editing the genes *in vitro*, both the issue of histocompatibility and unintended mutations could be eliminated [8]. While SCD remains a complex condi-

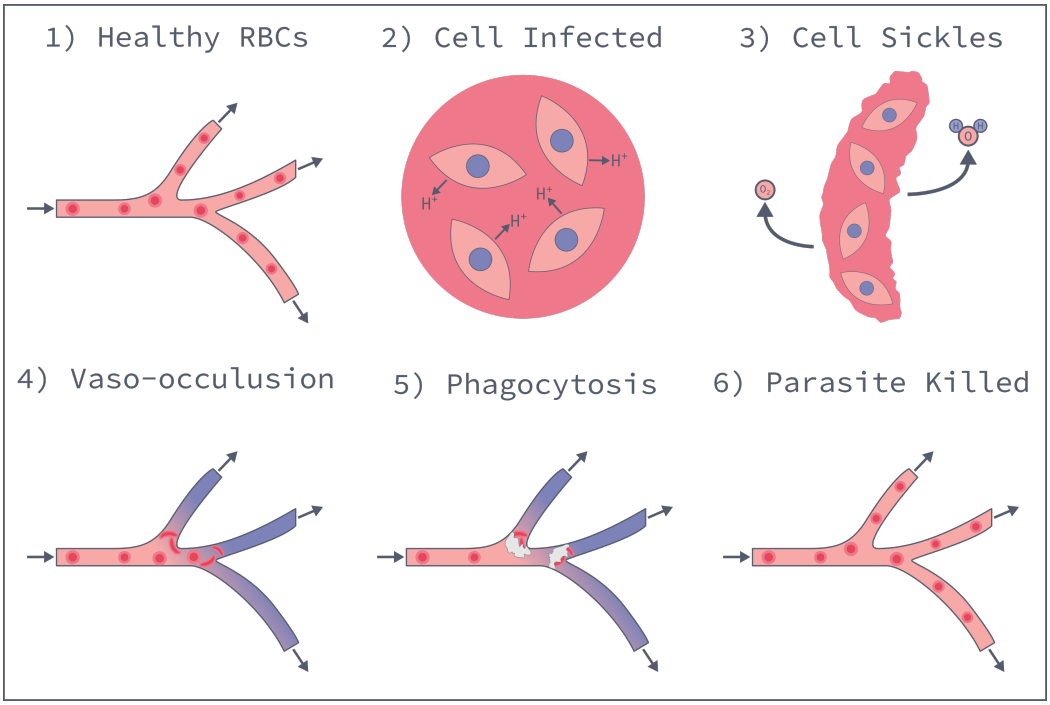

Figure 2: This figure depicts a proposed mechanism for malarial resistance in individuals heterozygous for the sickle-cell trait. **(1)** In humans with one copy of the mutant HbS gene and one copy of the healthy HbA gene, both forms of haemoglobin exist in a balance. The presence of non-aggregating HbA usually prevents haemoglobin fibres from forming and stops cells from sickling. **(2)** When the malarial parasite (*Plasmodium falciparum*) infects an erythrocyte and begins to metabolise, it releases lactic acid as a byproduct. This lowers the pH of the cytosol. **(3)** As the pH of the cytosol drops, haemoglobin loses much of its affinity for oxygen — this is called the Bohr effect [1]. As progressively more deoxyhaemoglobin is formed, a critical threshold is crossed and the HbS within the cell begins to aggregate despite the presence of HbA. This leads to the formation of a sickle-cell with the parasite trapped inside. **(4)** This sticky and inflexible sickle-cell can then become caught in capillary beds and lead to tissue ischaemia in a process called vaso-occlusion. **(5)** The lack of oxygen in the tissue then incites an immune response in which phagocytic cells lyse the sickle-cells blocking circulation. **(6)** When the sickle cells are destroyed, so too are the parasites trapped within them. This means that not only is circulation restored, but the malarial parasites are killed before they have the opportunity to spread.

tion, science continues to deepen our understanding of it and continues to show us how to better treat patients who are suffering.

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