Abstract

Did you know that each of the trillions of cells in your body makes decisions at every moment of the day? While cells do not have brains, they do have molecular sensors that tell them what to do if a certain molecule or signal is present around them. This makes them similar to a computer program: input - in, response - out.

Unfortunately, just as a buggy software code can make a computer malfunction, sometimes bad genetic code inside our cells causes them to respond in a way that is bad for the body. A cancer cell, for example, has a broken code causing it to grow despite signals to shut down.

Scientists have long sought to develop gene therapy - a way to fix or replace a damaged or missing gene within a person’s genome. Our team approached this challenge in a new way: we engineered something called a protein circuit and it seemed to work!

Introduction

Imagine your school has a computer with a software bug that makes it open unauthorized pages and apps. You want to shut it off to keep the other computers in the system safe. To do that, you will need to figure out what kind of buggy code is affecting the faulty computer and then write some software code to stop it. However, the computer’s operating systems are written in dolphin’s language. A pretty hard job, right?

That’s the kind of situation a synthetic biologist is in. All living cells, just like all computers in the world, have roughly the same parts (proteins) and use the same software code (DNA) which tells them what to do. So in principle, it’s possible to create “software updates” which fix bugs in the code (i.e. genetic diseases). It’s just very hard.

Cancer cells have a bug in their genetic code causing them to keep their “grow and divide” systems turned on even when they are supposed to be turned off. So by editing their genetic code to make them stop growing, we could potentially cure cancer. But rewriting the code in exactly the right spot can be very difficult!

What is a protein circuit?

Each cell contains thousands of different proteins and each of them can change the behavior of other proteins - like a domino effect. For example, one protein called a receptor detects external signals and turns on other proteins, which turn other proteins on or off and so on. This network of interacting proteins is a protein circuit. There are many different kinds of protein circuits. Each one can generate different cellular programs causing the cell to do different things.

So we looked for a new way to protect the entire systems from the few buggy cells. We wanted to add a switch to turn off the cell with the bad program instead of fixing the bug in the code. To do this, we needed a protein circuit.
Cancer cells use the same circuits as healthy cells, but they turn their grow-and-divide programs on maximum. We decided to try to hijack part of that program to turn on a synthetic “shut-down” circuit.

The first part in our engineered circuit is a sensor (See Fig. 2). We designed a sensor that would only switch on in cancer cells by recognizing one of the enzymes from the cancer’s grow-and-divide circuit.

The other essential part of the circuit is an effector protein. The effector protein looks and acts just like the natural protein that flips on the shut down program, but we engineered it so that our sensor could turn it on.

We hooked the sensor to the effector to make a “detect-and-kill” circuit. This was our first circuit design - version 1.0.

We continued working on the design. In version 2.0, we added a threshold filter which ensured that the circuit stayed “off” unless the sensor got turned on beyond a certain level.

Then we had to give the new code to the cells. Finally, we checked if our protein circuits killed cancer cells but left healthy cells alone.

How do we transfer engineered DNA code into a cell?

One way to do it is to “load” the code onto plasmids. They are small circular pieces of DNA code. We coat them with lipids, which tricks cells into absorbing them. We use them to manipulate cells, because they can be inserted in the cell as a “packaged genetic message” without having to edit the cell’s own DNA. This is called transfection.

![Figure 2: Protein circuit designs.](image-url)

In the earlier design (1.0), any amount of signal turned on the shutdown program. This was a problem. (Why? Find out more in the Results!) However, for the 2.0 design, the input signal had to be above a certain threshold to kill the cells.
Results

Both the 1.0 and 2.0 circuits killed the model cancer cells we use in our testing. This proves that it is possible to hijack a cancer cell's own enzymes to start the shut-down program. And it only took two engineered switches!

There was a big problem though: for every ten cancer cells the 1.0 circuit killed, seven healthy cells also died.

The 2.0 circuit (the one with the threshold filter) did much better: for every ten cancer cells the it killed, fewer than two healthy cells died.

There are many types of cancer and they can use several different grow-and-divide pathways. We tried our protein circuit strategy using the same sensor on a different cancer enzyme. We got the same results!

Discussion

Think back to your school's computer network with the one buggy computer. Imagine if you wrote a program that shut down all the computers. This did turn off the faulty computer. But now you have even more serious problems!

Similarly, if we want to use synthetic biology to cure cancer, we need to make sure we only shut down cancer cells. Our results show that engineered protein circuits are effective at killing cancer cells. But the core circuit killed healthy cells, too.

The threshold filter was one way to help this problem. It made sure the effector turned on the shutdown program only if a cell was overusing its grow-and-divide circuit. This way it protected non-cancerous cells.

We still need to learn a lot about how to safely and effectively write these cellular software updates before we can use them in people. Individual cells grown in a petri dish are not the same as full living organisms. Even so, our protein circuit technique offers great promise for the future of human health.

Conclusion

Doctors have come a very long way in treating disease. Diabetes, heart disease, HIV and even some kinds of cancers that were once deadly are now treatable conditions.

With synthetic biology, doctors can make even more progress: directly reprogramming cells to fix them instead of treating a diseased organism with drugs.

Until recently, writing DNA code into cells was just in science fiction movies. But today young scientists in universities around the world are learning how to do this for the benefit of humanity. Is this a profession you might be interested in?

Check your understanding

1. Why does cancer make people sick?
2. Why did we use plasmids instead of adding our proteins directly?
3. Why was the threshold filter so important?
4. Can you think of any other diseases where an engineered protein circuit could provide a cure?
**Glossary of Key Terms**

- **Circuit** – a series of on/off switches which can interact and regulate each other's activity.
- **DNA** – the molecule found in each cell of a living organism. It contains all the instructions the cell needs - its genetic code. Because the genome is so large, DNA is tightly packaged up onto chromosomes in the nucleus.
- **Enzyme** – a type of protein that chemically modifies other molecules. You've probably heard that your stomach uses enzymes to chemically break down your food. They are also the proteins that act as switches inside a cell. Enzymes turn other proteins “on” or “off” by adding or breaking one of the protein's chemical bonds.
- **Gene** – a section of the DNA molecule that contain information about a specific task: e.g. producing a certain protein, or producing the pigment which determines eye color. Each gene is encoded (written) in hundreds (or thousands) of base pairs. We can read them in a letter sequence (e.g. ATCCGTTAAGC).
- **Genetic (or DNA) code** – all the instructions a cell needs throughout its life: blueprints for how to grow, which proteins to produce and how to act in different situations.
- **Genetic engineering (aka gene editing)** – modifying the code of a gene. This also alters its function.
- **Gene therapy** – a way to fix or replace a damaged or missing gene within a person's genome.
- **Lipids** – a type of molecule that makes up a cell's membrane. Lipids keep outside molecules (like plasmids, bacteria, or viruses) from coming inside the cell. This protects the cell from getting sick.
- **Molecular biology** – the branch of biology which studies both the structure and function of the molecules in living cells.
- **Plasmid** – a small circular piece of genetic material (similar to a super simple virus). Plasmids contain fewer genes than chromosomes and are only found in bacteria. They last longer in a cell than normal pieces of DNA or RNA because their ends are connected together to make a circle. This makes it harder for cells to degrade them (because proteins that break down DNA and RNA start at the ends).
- **Protein** – molecules encoded by the genes in DNA. Proteins are the molecules that make up a cell’s circuits. Proteins are made up of a chain of hundreds to thousands of amino acids linked together.
- **Protein circuit** – all the proteins involved in a cellular program. A circuit has at least one receptor to turn the circuit on and at least one enzyme to make a chemical change to activate the program. More complicated circuits can have dozens of proteins! Biology textbooks usually refer to such protein circuits as ‘signaling pathways.’
- **Receptor** – a type of protein that senses changes in its environment. There are thousands of different receptors in cells, and each one senses something different. When receptors sense their activation signal they change shape to turn their protein circuit on.
- **Ribosomes** – the molecules inside a cell that make proteins. Ribosomes read the RNA transcript of a gene and translate it to protein code (amino acids). A set of three base pairs encodes one amino acid.
- **RNA** – the molecule that gets translated into a protein. Cells write up an RNA transcript of the gene they want turned into a protein from its DNA. Just like DNA, RNA is written by base pairs, but RNA transcripts are much smaller - they only have the code for one gene. They are what the ribosome reads to make the proteins the cell needs.
- **Synthetic biologists** – scientists who try to design biological molecules which can perform new functions that did not exist in the cell before (e.g. a yeast that can make human insulin). They do that by inserting new genetic code in a cell's DNA which causes the cell to do something new to it (e.g. stop a cancer cell dividing).

**REFERENCES**


