Rise of the Nanorobots

For centuries, people have relied on materials such as concrete, steel, and wood for the construction of buildings, bridges, and other structures. However, researchers have started exploring a far less conventional material for building therapeutics at the molecular level—DNA.

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In this episode, Charlene Lancaster from *The Scientist* spoke with Björn Högberg, a professor of biophysics at the Karolinska Institute, to learn how his team is developing DNA-based nanorobots to fight cancer.

<u>Introduction to DNA Nanotechnology</u>

Narrator:

Best known as the carrier of genetic information, DNA is now emerging as a powerful nanoscale building material. Traditional methods for building nanoscale devices, such as lithography and block copolymer self-assembly, often face limitations in precision, flexibility, and cost when working at molecular scales. DNA nanotechnology addresses these challenges by harnessing DNA's unique ability to self-assemble through predictable base-pairing, enabling scientists to design and construct highly organized nanostructures with molecular-level accuracy.

Researchers, such as Björn Högberg, are now using DNA nanostructures for various physical and biological applications. For example, scientists employ DNA nanostructures as precise scaffolds to arrange components in photonic crystals, which manipulate light in optical devices and are typically laborious to manufacture using traditional methods. Additionally, researchers design DNA-based drug carriers that can change shape or release drugs in response to specific biological cues, allowing for more controlled therapeutic delivery to target cells. DNA nanotechnology also enables scientists to examine nanoscale interactions on the cell surface by engineering highly specific molecular probes. Despite its remarkable potential, the DNA nanotechnology field is still relatively young.

Björn Högberg:

The field originates sometime in the 80's when Ned Seeman at New York University, he had problems making crystals of certain proteins. One of the ideas he had was to make a crystal out of DNA instead of the proteins and then put the proteins in that crystal. He was the first to think of DNA as a building

material, where you would design strands of DNA to attach to each other in a very specific way, and from that seed of an idea grew the field. Later on, Erik Winfree used DNA strands to assemble these large carpets, two-dimensional crystals basically, and they could program them really, really nicely. And then in 2006, Paul Rothemund, who was then in Erik Winfree's lab, discovered that you could do this in a very nice way that he called DNA origami.

Leveraging DNA Origami to Analyze Receptor-Ligand Interactions

Narrator:

DNA origami is a technique where scientists fold a long single strand of DNA into two-dimensional or three-dimensional shapes. They accomplish this feat by using hundreds of shorter oligonucleotides that hybridize to specific locations on the long scaffold strand and act like staples holding the structure's shape. You can envision this process by imagining your arm as the long strand, with distinct sequences at your shoulder and hand. If you were to design a smaller strand that binds to both locations, your hand would fold towards your shoulder. By strategically designing these staple strands, scientists can direct the DNA to fold into virtually any conceivable shape.

At the time that Rothemund published the original DNA origami paper, Högberg was a PhD student attempting to design small DNA fragments capable of self-assembly. After he learned of the breakthrough, he realized that he wanted to work with this remarkable technique. Now his team uses DNA origami as a tool to examine and tinker with receptor-ligand interactions. Cells in the body use these protein-protein interactions to communicate with each other. For example, pancreatic β-cells respond to rising glucose levels by synthesizing and secreting insulin into the bloodstream. Cells elsewhere in the body, such as fat and muscle cells, contain receptors that detect this hormone signal and respond by increasing glucose uptake. However, receptor-ligand interactions are not always as straightforward as the textbooks describe.

Björn Högberg:

There is evidence that it is not only binary interactions, like this ligand binds to that receptor. A lot of times, it is much more complicated. It is more like you need the ligands to cluster the receptors in a particular pattern on the nanoscale to give a certain type of receptor activation and a certain outcome in the cells. We try to understand how these more complicated receptor-ligand signaling systems work, and what makes the cells decide what to do depending on the nanoscale patterns of ligands that they encounter. We do that by using DNA origami to create artificial patterns of proteins. Then we can feed cells or tissues with these artificial patterns and gradually learn how cells react to different patterns. This would be useful for therapeutic applications because if we can really learn, for example, how cells are induced to commit suicide, then we can use those patterns to treat cancer.

Narrator:

A particularly important group of transmembrane proteins in the development of new cancer treatments is the death receptor family. These surface proteins, which include DR4 and DR5, detect signaling ligands, such as TRAIL, released by other cells. This triggers the cell to undergo programmed cell death or apoptosis. Although scientists have already engineered antibody- and peptide-based therapies to activate death receptors on tumor cells, these compounds often show limited efficacy in clinical trials. Högberg and other researchers hypothesize that these anticancer agents fail to effectively trigger death receptor clustering, which reduces their therapeutic potential.

Björn Högberg:

We published one paper looking at the exact effect of inducing clustering. We used a peptide that mimics TRAIL ligand. If we put that peptide on an origami in a sufficiently small and concentrated pattern, where the radius would be less than 10 nanometers, then they were very efficient in inducing cell death. But if we made the pattern slightly bigger, like 15 nanometers or 20 nanometers in radius, then suddenly these nanostructures with these ligands did not do anything.

Designing a DNA Nanorobot That Selectively Reveals Its Weapon

Narrator:

These earlier experiments emphasized the importance of the receptors' spatial arrangement. However, Högberg and his team quickly realized a problem with using these DNA nanostructures as a therapy. Many cell types, such as kidney cells, T lymphocytes, and endothelial cells, have death receptors on their surface even when they are healthy, and targeted therapies with this nanoscale ligand pattern would kill the cells indiscriminately. The researchers needed a logic-gated nanodevice—one that they could program to sense specific molecular signals in the microenvironment and activate only when those conditions occur. In this case, the nanorobot should selectively display the ligand pattern in the tumor but keep the ligands hidden when the structure is in healthy tissue. His PhD student Yang Wang devised a solution to this conundrum.

Björn Högberg:

Yang came up with the idea that we should try to use pH as a switch. Cancer cells typically have a high metabolic turnover. They need lots of energy. This generates a lot of waste that turns the tumor environment slightly acidic. It is well known that in these solid tumors, the pH is usually a little bit lower than in surrounding tissue. We were aware of techniques, where you can make DNA form a triple helix. So instead of forming the classical double helix, you can add a third strand that would bind around the double helix and form a triple helix. But that will only occur in relatively low pH. So, Yang designed this barrel-like origami structure, almost like a cup, where you would have the peptides hiding in the cavity of the cup. The structure is almost the size of a virus, around 50 nanometers. Only when it entered acidic environments would it form the triple helices along the edges of the cup, and they would stick out in this small pattern that would kill cells.

Narrator:

From cell culture experiments, Högberg and his group determined that this DNA origami nanorobotic switch was highly efficient at killing cancer cells in acidic conditions. After seeing these results, they decided to move on to an in vivo cancer model. They implanted human breast cancer xenografts in immunodeficient mice and then administered the DNA-based nanorobot. The researchers observed a 70% reduction in the tumor volume in mice treated with this nanodevice compared to those treated with a vehicle control. To ensure that the origami switch was responsible for this result, Högberg and his team prepared a mutated DNA nanorobot that was almost chemically identical to the switchable version but was pH insensitive. As a result, the hexagonal ligand pattern is permanently hidden in the nanostructure. Tumors in mice treated with this mutated nanodevice continued to grow, showing that the switchable nanodevice's antitumor activity was dependent on pH. While these results are promising, this DNA nanorobot still requires many more experiments and years of research before it might become a cancer therapy. Högberg plans to improve the nanodevice and further test its efficacy.

Björn Högberg:

We can put many other things on pieces of the structure. For example, we could put other targeting ligands that specifically enriches these structures in the tumor by binding to markers found in these tumors. And that is definitely something that one would do moving on to enhance the delivery. Another thing we need to do is try more advanced cancer models and look more carefully into side effects. Our cancer model that we used is not a very realistic cancer model. I think DNA origami is an excellent research tool, but the actual therapeutic might look completely different. Maybe origami will not be used in the drug, but origami can certainly be a big part of how we reach the knowledge for the eventual drug.

<u>Advancing DNA Nanotechnology Through Shared Innovation</u>

Narrator:

Högberg hopes this work will inspire other researchers to explore the DNA nanotechnology field. While his team is actively developing these logic-gated nanodevices, he believes true progress will come when more scientists begin experimenting with and expanding on these ideas. By building on existing findings, the field can continue to evolve in exciting and unforeseen directions.

Björn Högberg:

George Church, Shawn Douglas, they made a nanorobot that opens and delivers therapeutic antibodies when it attaches to a particular cell type. That was the first logic-gated nanorobot made from DNA origami. Our work is in the same tradition, but we are using a completely different mechanism. And the more of these logic-gated activation mechanisms that we can put together, I think makes it easier for the next research team to make something even more impressive.

Outro

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