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CRISPR Methods for Nucleic Acid Detection Herald the Future of Molecular Diagnostics

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Guest: Dr. Asma Hatoum-Aslan is assistant professor in the Department of Biological Sciences at the University of Alabama.

Bob Barrett: This is a podcast from *Clinical Chemistry,* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

The December 2018 issue of *Clinical Chemistry* published a Perspective article titled "CRISPR Methods for Nucleic Acid Detection Herald the Future of Molecular Diagnostics," which discusses the findings of Chen and colleagues who earlier this year were the first to create a diagnostic tool out of a CRISPR-Cas system and demonstrate its clinical utility. The author of this Perspective, Dr. Asma Hatoum-Aslan is assistant professor in the Department of Biological Sciences at the University of Alabama and she joins us in this podcast. Welcome Dr. Hatoum. Let's get a little background first, what are CRISPR-Cas systems?

Dr. Hatoum: Certainly. So, CRISPR-Cas systems are a class of immune systems found in prokaryotes, that is bacteria and archaea. One of the main functions of CRISPR-Cas systems is to protect these microorganisms against their own viruses. So, just as humans are susceptible to viruses, bacteria have their own specific viruses to contend with, and these are called bacteriophages or phages for short. So out in nature, when a bacterium with a CRISPR-Cas system encounters a phage, the system creates a genetic memory of the invader by capturing a small piece of the phage's DNA and recording it into the bacterial chromosomes.

So, it will actually pick up a snatch of the phage's DNA and insert it into the bacterial chromosome as sort of a molecular memory. And these snips of phage DNA are transcribed into small RNAs and the RNAs combine with Cas proteins. Cas stands for CRISPR Associated, and the Cas proteins plus the CRISPR RNA together form what is called the Cas complex. This complex literally patrols the inside of the cell. It carries the phage-derived RNA for comparison much like a detective would use a fingerprint to identify a previously convicted criminal. Once a match is found, the Cas proteins chop up the invading DNA or RNA and essentially destroy the phage.

So, there are currently six different CRISPR-Cas types. They all use the same basic mechanism to identify foreign invaders and destroy them. Some CRISPR systems specialize in targeting DNA, others recognize and cut RNA, and there are some systems that do both.

Research in my lab really focuses on understanding basic mechanisms. So, we ask questions about the details, how do these CRISPR systems work, how do they impact bacterial physiology, and we ask questions about how phages are able to fight back against these defenses. But there are many out there who are very much interested in applying CRISPR-Cas systems.

So, you can think of these systems as a pair of programmable scissors that are naturally coded by bacteria to go after a targeted phage. But once we figured out the basic mechanism—and this occurred like eight to ten years ago—once this mechanism was hammered out, scientists quickly realized that these systems can easily be artificially reprogrammed to cut any sequence of interest by simply changing out the sequence of the small RNA in the Cas complex. And this revelation has given rise to a number of very powerful applications such as genome editing and disease diagnosis.

Bob Barrett: So, I know we've heard a lot of buzz about CRISPR-Cas9 and genome editing, but you're describing something else here. How can CRISPR be used to diagnose disease?

Dr. Hatoum: So, it turns out that some CRISPR systems have a special feature that make them particularly suited for diagnostic applications. So, let's take CRISPR-Cas12 for example, which was the subject of a study by Chen and colleagues. This is a group from Jennifer Doudna's lab at Berkeley. And so, CRISPR-Cas12 is a type five CRISPR system and like other Cas complexes, Cas12 can be programed to recognize and cut a specific DNA sequence. But here is the trick, once Cas12 finds the targeted DNA that matches its CRISPR RNA, Cas12 cuts the target and it also cuts any other DNA in the vicinity regardless of the DNA sequence. This is called collateral cleavage.

This collateral cleavage is unleashed only when Cas12 finds a match to the CRISPR RNA that it's carrying. And so, Chen and colleagues started out with this basic mechanistic discovery about how Cas12 works, and then they turned it into a diagnostic tool that they called DETECTR. And so, the way that DETECTR works, it basically combines the collateral cleavage activity of Cas12 with a special DNA molecule called the reporter.

And so, this reporter DNA is normally not fluorescent, but once it gets cut, it can emit a fluorescent signal. The DNA reporter in the system is key because it will give a visual output of Cas12's collateral cleavage activity.

So, let's imagine that you have a human sample and you wanted to determine if a specific pathogen is present in that sample using DETECTR. So, what you would do is you would extract DNA from that human sample and add it into a single tube that has a few other items in that tube. You would have some enzymes in there that multiply the DNA and the sample would make many copies of the DNA. You would also add the reporter DNA molecule, and of course, you'll add Cas12 preprogramed to recognize the specific sequence of the pathogen of interest. So, as you incubate these components together, if Cas12 finds a match in that tube, it'll chop up the pathogen DNA along with the DNA reporter and that would allow the liquid in the tube to emit a fluorescent signal.

So, I know it sounds a little complicated, but it actually works. To prove that it works, Chen and colleagues used DETECTR to screen 25 human samples for the presence of HPV, Human Papilloma Virus. And not only were they able to pretty accurately detect whether or not each of the samples have the virus, but they were also able to use DETECTR to distinguish between closely related high risk types HPV 16 and 18. These viruses have slightly different DNA sequences and DETECTR was able to distinguish between them. And to me, what's astonishing is that they were able to screen all 25 samples within an hour.

Bob Barrett: That's pretty impressive. This was made possible by one type of CRISPR-Cas system, but you mentioned there are actually six unique types. Are any of the others potentially useful as diagnostics?

Dr. Hatoum: Yes. Type VI CRISPR-Cas system, also known as CRISPR-Cas13, also have this collateral cleavage activity. But instead of targeting DNA, Cas13 actually recognizes and cuts RNA. There are couple of papers by Gootenberg and colleagues and this is from Feng Zhang's group at the Broad Institute. They created a nucleic acid detection platform that they called SHERLOCK using Cas13. And similar to DETECTR, SHERLOCK relies upon collateral cleavage. This time it's cleavage of RNA instead of DNA. There's also an RNA reporter that fluoresces when it gets cut, and inside the tube, when Cas13 is able to recognize a complementary RNA to the CRISPR RNA it is carrying, the collateral cleavage activity will be activated, the reporter will get cut, and then you have a fluorescent signal.

And as proof of concept, SHERLOCK was used for example to detect different strains of Zika virus, Dengue virus, as well as pathogenic bacteria such as pseudomonas. I also have to mention, the latest edition to the diagnostic tools set, CRISPR-Cas14. This is a very new discovery. It came out this past October. Jennifer Doudna's group reported on a new CRISPR system, the smallest known CRISPR system to date. It came out in the journal *Science*. Cas14 is unique not only because it's the smallest of the CRISPR systems, but it also specializes in recognizing single-stranded DNA. And Cas14 also exhibits this collateral cleavage activity and it has been adapted with Doudna's DETECTR platform.

So, Cas12, Cas13, and now Cas14 can collectively detect double-stranded DNA, single-stranded RNA, and now single-stranded DNA. And this essentially enables the diagnosis of a diverse array of bacteria and viruses that have these different genomic configurations.

Bob Barrett: So, doctor, the applications you mentioned so far seem specific to infectious diseases. Do you think these systems can be more broadly applied to detect, say, disease-causing mutations in human DNA?

Dr. Hatoum: Yes, absolutely. It seems there's been a heavier focus on infectious disease in these papers, I think most likely because of the time-sensitive nature of getting an accurate diagnosis so treatment can begin immediately. But I know that both groups have written about also exploring, for example, the diagnosis of cancer-causing mutations, as well as detecting single nucleotide differences that are not necessarily connected to any particular disease for basic genotyping purposes.

Bob Barrett: Well, finally, doctor, it seems this CRISPR technology is moving pretty quickly. Practically speaking, do you think we'll be seeing CRISPR-based diagnostics on the shelves any time soon?

Dr. Hatoum: I think the likelihood is very high. I mean, it's a lot easier to gain approval for a non-invasive diagnostic test than for using CRISPR for editing human genes inside a person.

So, I know that Jennifer Doudna has launched a new startup company. It's called Mammoth Biosciences, and the purpose is to commercialize DETECTR-based diagnostics. From what I understand, the technology is designed to be inexpensive, easy to conduct, doesn't require any specialized training or equipment. For example, in the paper by Gootenberg and colleagues, they developed a paper-based format of SHERLOCK with an estimated cost of $0.61 per paper-based test.

So, you can imagine these tools being used even in remote locations like out on the battlefield or in resource poor countries.

Bob Barrett: Dr. Asma Hatoum-Aslan is Assistant Professor in the Department of Biological Sciences at the University of Alabama. She's been our guest in this podcast from *Clinical Chemistry*, looking at new CRISPR-based diagnostics. I am Bob Barrett. Thanks for listening.