

Bob Barrett:

This is the podcast from *Clinical Chemistry*. I am Bob Barrett.

Since the sequencing of the human genome and the development of high-throughput genotyping techniques, many genes that are associated with disease through single base and other mutations have been identified. Mutations in DNA circulating in the plasma can serve as biomarkers of early tumor development and potentially in monitoring the response to treatment.

The May issue of *Clinical Chemistry* reported that the use of snapback primers for enrichment and detection of minority alleles is simple, inexpensive to perform, and can be completed in a closed tube in less than 25 minutes.

Dr. Carl Wittwer, co-author of the article and a Pathology Professor at the University of Utah Medical School and Director of Flow Cytometry and the Advanced Technology Group at ARUP is our guest in this podcast.

So tell us, Dr. Wittwer, why is it so hard to detect early cancer?

Dr. Carl Wittwer:

Specifically because it is early. Early cancer is small cancer. It hasn't grown to a stage where the patient or the physician can actually recognize cancer as such. And medical progress over the past several decades has very much improved. For instance, imaging is a way to detect cancers and imaging is getting better and better in terms of being able to detect smaller and smaller tumors, but it still has limits.

The other alternative is some kind of nucleic acid testing to actually measure and detect the altered nucleic acid or DNA that's causing the cancer. The ways to retrieve or access the nucleic acid, you can try some sort of a biopsy. Those are getting smaller, like with fine needle aspirates or you can try to measure circulating DNA in the blood, which would be the most convenient kind of test.

The problem is that in early cancer, the amount of cells available and being able to harvest them are very difficult, because the amount of altered DNA is very, very small. So if you're going to target tumor itself with a needle, for instance, you have to be able to find it, and if you're looking for nucleic acid that the tumor has shed, particularly for very small tumors, so maybe a very, very small concentration.

So you're literally looking for a needle in a haystack, and it's a difficult process, but one that a lot of medical research is focused on, trying to improve the sensitivity of detecting earlier and end earlier cancer, because as you know, the

ability to cure cancer often is correlated with how early it can be detected.

Bob Barrett: Because cancer is caused by genetic mutations, how can these mutations be detected, and what exactly is the polymerase chain reaction?

Dr. Carl Wittwer: The polymerase chain reaction is one of these methods that has extraordinary sensitivity and is able to detect very small amounts of cancer DNA. It does this by replicating that DNA, targeting a specific area and exponentially replicating that DNA so they can be easily measured.

So the polymerase chain reaction is one of these nucleic acid testing reactions that if you're looking for a particular kind of cancer, you often know what genes and what DNA is mutated. So we can focus on a specific spot, amplify that region by the polymerase chain reaction so that you can relatively easily detect it in a test tube.

Bob Barrett: With that in mind, what are snapback primers?

Dr. Carl Wittwer: Snapback primers are a modification of the polymerase chain reaction that allows you to focus more on a particular cancer mutation. So the polymerase chain reaction itself, of course, requires two primers to identify the region that you want to amplify and the technique of snapback primers modifies one of those primers so that the primer can, in addition, serve as a probe for the specific mutation you want to detect.

So if you imagine focusing on one small fragment of DNA with two different primers and then having a tail on one of those primers that snaps back on the amplified product and forms a hairpin, forms a loop so that the end of one of those primers actually hybridizes or attaches by forming a three-dimensional hairpin onto the product that's been amplified.

So that means it can serve as an automatic probe and detect specific variance, even single base changes that are correlated with cancer.

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So, snapback primers are just primers, just PCR primers that incorporate a probe that enables the detection of specific variance.

Bob Barrett: Now, how does snapback primers enrich for mutations?

Dr. Carl Wittwer: This is another trick useful to snapback primers, because you'll usually make the snapback primer, the probe part of

that hybridized specifically to the normal or wild type fragment of DNA.

Now what that means is that it will be mismatched or less stable with variance, sequence variance that would be correlated with cancer. So what you have forming during the polymerase chain reaction is you have these hairpins particularly with the wild-type sequence, and those hairpins will stick stronger when wild-type is present and inhibit PCR amplification of the wild-type variant, of the wild-type allele, because the hairpin is stronger with the wild-type than it is with the variant.

So the polymerase in attempting to replicate through the region with the snapback primer is inhibited by the wild-type allele. The polymerase can't get through the hybridized area. It can't get through; it can't open up that hairpin. But the variant with a mismatch to the snapback structure is already destabilized, so the polymerase can displace and selectively amplify the mutations versus the wild type.

So it's a convenient way to selectively amplify variance and suppress or decrease the wild-type sequence.

Bob Barrett: Can snapback primer detection of cancer be more sensitive than sequencing?

Dr. Carl Wittwer: Interestingly enough, this snapback detection is more sensitive than sequencing, at least conventional sequencing, the sequencing that most people perform because that is limited to about 20%, or maybe 10%, of a cancer allele. Less than that, you'll miss it by sequencing.

And the attempt or effort to measure small amounts of variant DNA in early cancer detection requires being able to detect much smaller fractions of variant or mutant DNA.

So in the article about snapback primers, the sensitivity of detection in this case is approximately 0.02-0.1% of variant DNA that you are able to detect by this snapback primer amplification, blocking the wild-type sequence and detecting the mutant, variant or cancer DNA sequence.

Bob Barrett: So how does the cost of snapback primer detection compare to other methods of cancer detection?

Dr. Carl Wittwer: Good question. The molecular methods in general when they work effectively are usually less expensive than the imaging methods. Sequencing itself is still relatively expensive. The advantage of the snapback primers is that they are relatively simple to design to obtain at low cost and to implement.

So the snapback primers themselves just have a 5-prime tail of that probe element that forms the snapback structure and those are just simple primers. So they are inexpensive, and they are readily obtainable. Anyone doing this reaction, the polymerase chain reaction, and research are in clinical work knows that it's become a commodity now, to order primers you can do it and receive new primers by FedEx the next day from multiple houses.

So our hope is that this will enable more efficient development and execution of test probably because of the cost involved.

Bob Barrett: So how long does it take to detect cancer cells with snapback primers, and what is rapid-cycle PCR?

Dr. Carl Wittwer: Rapid-cycle PCR is a technique that's been around for quite a while developed in early 1990s. Now PCR goes back into the 1980s, and the general techniques that were developed, took usually about 2 to 3 hours to perform.

Now, in the early 90s, the rapid-cycle techniques cut that time down to more in the 10 to 15 minute range. So the polymerase chain reaction really can't be done in the 10 to 15 minute range and we found that this enrichment technique with snapback primers actually required or worked much better by this kind of rapid cycling.

So not only was it's a rapid cycling convenient in terms of getting answers back faster, but in the case of snapback primer enrichment, it was found to be absolutely necessary. Now the rapid cycling requires a particular kind of an instrument to perform the polymerase chain reaction, but they are becoming more common these days, and it certainly is nicer to get your answer out quickly rather than waiting a longer period of time.

Bob Barrett: Dr. Carl Wittwer is a Pathology Professor at the University of Utah Medical School and Director of Flow Cytometry and the Advanced Technology Group at ARUP, and he has been our guest in this podcast from *Clinical Chemistry*.

I am Bob Barrett. Thanks for listening.

Total Duration: 10 Minutes.