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Using FTIR to Support Accurate Tissue Assessment in Colorectal Surgery

Ashley Baker:

You're listening to *Clinician's Roundtable* on ReachMD. I'm psychiatric nurse practitioner Ashley Baker, and joining me to discuss their recent research on Fourier Transform Infrared Spectroscopy, or FTIR, biomarkers in colorectal cancer surgery are Drs. Heather Allen and Ran Li. Dr. Allen is a Full Professor in the Department of Chemistry and Biochemistry, as well as the Department of Pathology, at Ohio State University in Columbus.

Dr. Allen, welcome to the program.

Dr. Allen:

Good morning.

Ashley Baker:

Also joining us is Dr. Ran Li, a researcher who completed a PhD in Analytical Chemistry at The Ohio State University in 2015.

Dr. Li, thanks for being here.

Dr. Li:

Thank you, Ashley.

Ashley Baker:

So, Dr. Allen, starting with some background, what prompted you to look at FTIR as a diagnostic tool for colorectal cancer during surgery?

Dr. Allen:

Well, originally, our work started with Raman spectroscopy as a diagnostic tool for colorectal and other cancers, but particularly for intraoperative identification of clean and cancerous surgical margins. And this started with Dr. Li's PhD dissertation some 15 years ago. So we moved to infrared analysis when I began a collaboration with Professor James Coe. Dr. Coe is also in the department here at Ohio State in the Department of Chemistry and Biochemistry, and at that time, he was using metal grids to enhance infrared intensities, and he was looking at basic science of bilayers that he would make to mimic membranes, and I thought that the infrared focus and the way that he was enhancing signals could maybe apply to things in pathology.

We applied for NIH funding for the infrared and received a two years through an R21, and with that idea we were using microtome slices, and we were going to do the plasma enhancement from these grids. But the grant money ended up moving us, really, into infrared microscopy, so FTIR spectroscopy, but in microscopy, so images, and so infrared as well as Raman. And so both of these types of vibrational spectroscopies really capture molecular vibrations, so you look at the molecular signatures to categorize what you see, and it gathers the vibrational fingerprint of molecules that you're probing. And so this molecular fingerprint is also highly sensitive to its environment, so meaning that if the molecules we're detecting are in a cancerous, perturbed environment we can also see changes or shifts in energies from what we would normally see in, not human tissue, we're not a cancerous perturbed environment. These signatures, though, are somewhat difficult to discern in that you're really looking at small changes. It's a very complex signature, and so it's kind of like looking for a needle in a haystack in many ways.

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Ashley Baker:

And turning to you Dr. Li, can you walk us through how you gathered and standardized the spectral data and why pulling from a range of sources was so important to your approach?

Dr. Li:

Absolutely. The data was collected from different colorectal cancer cases in the literature, so we also had some criteria to guarantee the data quality, so only the data that passed this criteria were used in the subsequent analysis. And because the data was collected from different sources—so they have different X values—we have to standardize them to make sure they all have the same set of X values, which are actually the right numbers. So this is achieved through a linear interpolation. So we estimated the value between like two data points using a straight line. We wanted to see how generalizable these biomarkers are. So, these biomarkers have to be powerful in differentiating cancers and in cancerous tissues, but more importantly, their power has to be maintained across like various conditions. I mean like different settings. So, for example, some biomarkers may be effective in female patients, but they lose their accuracy when they apply to male patients, so by using data from a range of sources, we were able to see whether our biomarkers could remain effective and stay reliable across like a broad range of real-world cases.

Ashley Baker:

Now, Dr. Allen, I understand that one of your key findings was a biomarker called B1. What makes it so effective at distinguishing cancerous from noncancerous tissue?

Dr. Allen:

So mapping the B1 biomarker showed the highest correlation against the gold standard H&E stain. But why? So I would say it's because it probes the ratio of the fatty acid changes to changes in the phosphate environment of the lipids and nucleic acid, but this was actually quite a surprise because I had thought, and I know Ran had thought, for some time that it would be subtle changes in the protein amide bands against the fatty lipid bands that would really be the mainstay or the target, and that's what we had thought prior to this, and so I was certainly surprised with the nucleic acid and the phosphate part as well as the glycogen, that's not the B1 but the B1 against that phosphate and the lipid was certainly a surprise. You know, we suspected that the phosphate region was important, but the intensities are lower, and the region can be very convoluted, so this is why you use machine learning and AI. So you can't just take a spectrum and say, "Oh, this is cancer, or not." It's not as simple as maybe an H&E stain in that way, because H&E staining has been developed over years and years, but it takes time. And even an H&E stain, by the way, is quite a bit subjective, and you will get different answers from different pathologists many times in that area as to where the margins are, etc. But with the molecular signatures, we knew the power of it, but it also took in particular, Ran's approach. This was her novel approach of really taking the lasso and then the elastic net and taking somewhat of a simple approach: taking the power of many different sources from many different libraries, not just our own lab, but gathering large amounts of information and then testing against our data. But then the B1 came out on top.

Ashley Baker:

For those just tuning in, you're listening to *Clinician's Roundtable* on ReachMD. I'm psychiatric nurse practitioner Ashley Baker, and I'm speaking with Drs. Heather Allen and Ran Li about how we can apply FTIR spectroscopy to enhance surgical detection of colorectal cancer tissue.

So, Dr. Li, if we continue to look at the results, what did you discover about the impact of combining biomarkers on accuracy?

Dr. Li:

So combining biomarkers definitely improved the accuracy, but after including two or three biomarkers, adding more does not really help, so it can't even introduce some redundant information without significantly improving the accuracy, so it's kind of like a balance between the number of the biomarkers and the accuracy.

Ashley Baker:

Coming back to you, Dr. Allen, as you analyzed tissue beyond the tumor itself, were there any patterns that hinted FTIR might be detecting changes earlier than we typically see with standard pathology?

Dr. Allen:

Well, in analyzing the images after obtaining this data, the full integrated IR image showed areas that indeed correlated with the best biomarkers, but moreover, we always knew that providing the molecular signatures that are inherently sensitive to the actual microenvironment of the cancer, the microenvironment of the tissue, such as FTIR, the FTIR itself should outperform standard

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pathology, and we always do that just because again it's giving information about all of the vibrations, so all of the molecules and all of its environment. There is certainly more work to be done, in particular for developing instrumentation that can be used in real time during surgical resection, but in this paper, it's an amazing step forward given that it takes a very different approach in developing the biomarkers. It takes data from an entire published database, not just from our lab.

So to kind of get back to your actual question though, as Ran analyzed the tissue and I analyzed the data from her analysis, beyond the tumor itself, the patterns looked complex, and it wasn't clear because every single person has variation; every single cancer has variation in infrared signatures. There's variability everywhere, and that's where, again, the approaches with machine learning and AI and taking samples from across the literature or infrared data from across the literature was so critical and so important to this work and I think advancing the whole area of spectroscopy and molecular vibration, molecular vibrational spectroscopy to the next level for colorectal cancer and beyond.

Ashley Baker:

Before we wrap up our program, Dr. Li and Dr. Allen, let's look ahead for a moment. How do you both envision this approach being adapted for real-time use during surgery?

Dr. Li:

We are very positive about its ultimate implementation during cancer surgery in the near future. So in fact, there are already some products developed by IR-Medtek that bring FTIR into the operating room with an ATR probe. So, as a next step, I think these biomarkers need to be further validated with additional cases, so like around like 200 would be ideal. So if the results are satisfactory, then they can be implemented in the surgical center.

Dr. Allen

I was thinking about, how do you take this into the operating room? And it's really with—I think it's like the da Vinci machines with like robotics and so forth. I guess something like da Vinci wouldn't work, but also, coming in with fiber optics, you could think of this well beyond colorectal though because you really just need a fiber optic probe that you would have two fiber optic cables and an ATR, attenuated total reflection spectroscopy with the FTIR technology. And like I said, this is already being implemented to some extent with IR Medtek in dermatology, but really the next step is the intraoperative, but also it's truly about coming up with these biomarkers because the biomarkers, the instrumentation is out there. It's a little bit expensive right now, but really, I think compared to other medical areas, it's really not that expensive, but it's really I think about more clinical studies and convincing surgeons, to utilize this more.

Truly, this was Ran Li's idea in taking the literature and being able to do this, so I was just stunned, years later—because we've been keeping in contact—that we could go back and use some of our old data, which was the image in the paper for the testing, but take all the other, you know, data that's out there with infrared on just colorectal and be able to develop that biomarker.

Ashley Baker:

With those key takeaways in mind, I want to thank my guests, Drs. Heather Allen and Ran Li, for joining me to discuss the use of FTIR spectroscopy for intraoperative colorectal cancer detection. Dr. Allen, Dr. Li, it was great having you both on the program.

Dr. Allen:

Great. Thanks very much, Ashley. Appreciate it.

Dr. Li:

Thank you, Ashley. It's a pleasure to be part of this discussion and to share our work.

Ashley Baker:

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