Identifying molecular mechanisms and biomarkers of cancer using high-throughput methods

Antonia R. Sepulveda, MD, PhD is Professor and Vice Chair for Translational Research and Director of the Division of Gastrointestinal Pathology at Columbia University Medical Center. She is an expert in gastrointestinal pathology and molecular diagnostic pathology of cancer. Her clinical practice provides specialized gastrointestinal, biliary, and pancreas pathology diagnostic services with integration of molecular testing utilizing genomics, epigenomics, and specific tumor biomarkers for personalized cancer management and precision medicine of digestive organ cancers and pre-cancer conditions (esophageal cancer and Barrett's esophagus; gastric, colorectal, pancreas, and biliary cancers; and pre-cancer risk lesions).

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Dr. Sepulveda's research is focused on an innovative integromics cancer research program that is exploring computationally generated networks that integrate the molecular mechanisms and biomarkers of gastric, esophageal, and pancreatic cancers and pre-cancer lesions. Through these cutting-edge approaches, Dr. Sepulveda hopes to define novel tumor types and regulatory pathways of cancer development and progression, and biomarkers for their diagnosis and therapy. Dr. Sepulveda has over 120 publications (original research, reviews, chapters, and books).

Thermo Fisher Scientific: Please introduce yourself.

Antonia Sepulveda: I am a physician-scientist and professor of pathology at Columbia University Medical Center in New York City. As a physician, I practice gastrointestinal pathology and molecular oncology diagnostics. As a scientist, I lead a research laboratory with a focus on mechanisms and biomarkers of pre-cancer and cancer lesions in the gastrointestinal tract and in the pancreas.

Thermo Fisher: Tell us about your research.

Antonia Sepulveda: My research focus is on the molecular mechanisms that drive pre-cancer tissues to dysplasia and cancer, particularly in the gastrointestinal tract and pancreas. When a surgical resection is not feasible, cancers in these organs have a poor prognosis. Ideally, we should use biomarkers in pre-cancer stages to tell us which patients will develop advanced lesions. That way, we can closely follow these at-risk patients and eradicate the lesions before advanced cancer develops. One of my research goals is to discover tissue biomarkers that can be tested in patients' tissues that we use for routine diagnosis: namely, formalin-fixed, paraffin-embedded (FFPE) tissues.

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In the past several years, my research has focused on genomic biomarkers, and we have used next-generation sequencing (NGS) and SNP arrays for discovery and characterization of mutations and somatic copy number alterations in Barrett's esophagus, which affects over 3 million US patients and is the precursor lesion of esophageal adenocarcinoma (EAC).

Thermo Fisher: You also have a clinical practice focusing on gastrointestinal pathology and molecular diagnostics of cancer, correct? Can you tell us about it?

Antonia Sepulveda: Yes, my clinical practice integrates both novel genomic testing of cancers (to report cancer-predictive and prognostic biomarkers) and standard histological diagnosis. Using molecular diagnostics, I report mutational alterations in a range of cancers, providing critical information for precision oncology. Practicing both diagnostic and molecular genomic pathology is synergistic and intellectually stimulating to me. I feel I can best contribute to patient care as a member of a multidisciplinary team.

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Thermo Fisher: What are some of your recent research projects?

Antonia Sepulveda: Our recent studies aiming at the identification of molecular markers for early detection and risk stratification of Barrett's esophagus patients, dysplasia, and adenocarcinoma used NGS of routine FFPE specimens [1]. We found that patients who progress to dysplasia and EAC have frequent mutations in *TP53* and *CDKN2A* in their Barrett's biopsies.

Because these biomarkers are tested in pre-cancer tissues, we expect that only a few cells will carry genomic alterations, and detection assays with high resolution and high sensitivity are necessary. In a recently published study, we characterized somatic gene copy number alterations with high-resolution genome-wide arrays (Applied Biosystems™ OncoScan™ FFPE services and OncoScan™ CNV Assays) [2]. This longitudinal study interrogated large and focal copy number alterations in FFPE esophageal samples of patients with Barrett's intestinal metaplasia. This study uncovered frequent early genomic alterations in non-dysplastic Barrett's intestinal metaplasia of patients before progression to dysplasia and adenocarcinoma, involving *FHIT* exon 5 and/or *CDKN2A/B*, that suggest mechanistic and biomarker roles for these genes in Barrett's esophagus progression to dysplasia and cancer.

We are also pursuing a project titled "Genomics and Mechanisms of Esophageal Carcinogenesis" that is funded by the National Cancer Institute, and we are developing mouse models of esophageal dysplasia and cancer to better understand the biology underlying esophageal adenocarcinomas.

In another line of work in my laboratory, ongoing studies aim to identify biomarkers of progression to pancreatic adenocarcinoma, where we utilized the Applied Biosystems™ GeneChip™ Human Transcriptome Array 2.0 to establish gene expression profiles of normal ducts, pancreatic intraepithelial neoplasia (PanIN), and pancreatic ductal adenocarcinoma (PDAC) epithelium from laser-captured epithelial samples of pancreatic surgical specimens. We are exploring a number of coding genes that are upregulated in both PDAC and PanIN ductal epithelium as possible biomarkers of early progression to pancreatic cancer.

Thermo Fisher: What are some of your biggest challenges? How about your biggest accomplishments?

Antonia Sepulveda: There are many challenges to overcome in the discovery of early cancer detection biomarkers. First, there are technical issues we face when it comes to detecting alterations in small numbers of cells. It's like finding a needle in a haystack. This is particularly challenging for the detection of multiple targets, so high throughput and broad genome coverage is critical. So, we look for high-sensitivity and high-resolution approaches. Second, we often deal with limited amounts of tissue samples, often FFPE, which yield nucleic acids of lower quality and quantity.

We are very pleased with our results from NGS, high-resolution SNP arrays, and transcriptome arrays, which can utilize FFPE samples and low-nanogram amounts of DNA or RNA and still provide high sensitivity and high-resolution data, as reported in our publications on Barrett's esophagus [1,2].

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Thermo Fisher: How does Thermo Fisher fit into your workflow?

Antonia Sepulveda: I am using genomic and transcriptomic testing products and services from Thermo Fisher in my research program. So far, we have utilized OncoScan™ Assays and transcriptome arrays, either performing the assays in our institution or outsourcing them as a service provided by Thermo Fisher. The support teams are very helpful and the available software efficiently permits a streamlined analysis in our laboratory.

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Thermo Fisher: What is the current state of precision medicine?

Antonia Sepulveda: Precision medicine—precision oncology in particular—utilizes genomic mutational testing widely to look for biomarkers that are predictive of response to therapy or prognostic biomarkers. In 2017, the molecular testing guidelines for colorectal cancer were published, and I was the lead author and co-chair for the project—a combined initiative of the American Society for Clinical Pathology, the College of American Pathologists, the Association for Molecular Pathology, and the American Society of Clinical Oncology. This project illustrates how genomic mutational testing is a robust and well-established approach in precision oncology. However, limited progress has been made in defining clinical-grade transcriptional assays. Further, applications of genomic biomarkers in pre-cancer lesions are also lagging behind [the rest of the field].

Thermo Fisher: What advice would you give to a young woman considering a major in science or medicine?

Antonia Sepulveda: We need more women in science, especially in leadership roles. If you are lucky enough to find a mentor who champions you, grab the opportunity. General pointers are: Focus, focus, focus, be proactive, reach out to establish collaborations, leverage opportunities, and network.

References

1. Del Portillo A, Lagana SM, Yao Y et al. (2015) Evaluation of Mutational Testing of Preneoplastic Barrett's Mucosa by Next-Generation Sequencing of Formalin-Fixed, Paraffin-Embedded Endoscopic Samples for Detection of Concurrent Dysplasia and Adenocarcinoma in Barrett's Esophagus. *J Mol Diagn* 17(4):412–419.
2. Sepulveda JL, Komissarova EV, Kongkarnka S et al. (2019) High-resolution genomic alterations in Barrett's metaplasia of patients who progress to esophageal dysplasia and adenocarcinoma. *Int J Cancer* doi:10.1002/ijc.32351.

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