Diagnosing Cancer from a Drop of Blood

Kevin Pagel[1]

[1]Freie Universitat Berlin

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Abstract

This Research Highlight showcases the Research Paper entitled, Big cohort metabolomic profiling of serum for oral squamous cell carcinoma screening and diagnosis, https://doi.org/10.1002/ntls.20210071

RESEARCH HIGHLIGHTS

Diagnosing Cancer from a Drop of Blood

Kevin Pagel

Department of Biology, Chemistry, Pharmacy, Freie Universität Berlin, Berlin, Germany and Department of Molecular Physics, Fritz Haber Institute of the Max Planck Society, Berlin, Germany

Correspondence

Prof. Dr. Kevin Pagel, Freie Universität Berlin, Institute of Chemistry and Biochemistry, Altensteinstraße 23A, 14195 Berlin, Germany, Email: kevin.pagel@fu-berlin.de

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Cancer is the plague of our time. It is the leading cause of death worldwide, accounting for nearly one in six deaths.11**References**Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F (2020). Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. Available from: https://gco.iarc.fr/today, accessed [30 April 2022] The mortality of cancer is reduced significantly when diagnosed and treated early enough, ideally before or immediately after the first symptoms occur. The current gold standard in cancer diagnostics are biopsies from suspect tissue, which are examined using histology. However, the excision of specimen is often a very unpleasant and painful experience for the patient and potentially suffers from sampling inaccuracy due to tissue inhomogeneity. An alternative strategy are so-called liquid biopsies: the sampling of circulating or excreted body fluids such as blood or saliva, which are subsequently screened for specific DNA or protein-based tumor markers using immune recognition or sequencing techniques. A limiting factor of this approach is that each molecular probe is sensitive to only one particular feature, which may not be sufficient to come to a reliable conclusion. Multiplexing the detection of multiple DNA and protein-based features is generally possible, but causes further problems such as cross-reactivity and exploding costs.

Especially with the advent of easy-to-use chromatography and mass spectrometry instrumentation, another class of molecules got in focus for cancer detection: metabolites. As the name implies, metabolites are small molecular substrates or products of metabolism – a diverse potpourri ranging from amino acids, lipids, and sugars to hormones and reaction intermediates. Each individual has a characteristic metabolic profile that can be traced back in body fluids. This metabolic fingerprint varies depending on the state and

condition of the organism and therefore provides a snapshot of the underlying cellular processes22Johnson C, Ivanisevic J, Siuzdak G. Metabolomics: beyond biomarkers and towards mechanisms. *Nat Rev Mol Cell Biol*2016; **31:** 451–459. and their activity.33Rinschen MM, Ivanisevic J, Giera M, Siuzdak G. Identification of bioactive metabolites using activity metabolomics. *Nat Rev Mol Cell Biol*2019; **20:** 353–367. Cancer cells generally grow much faster than normal cells and consume vast amounts of nutrients. Not surprisingly, this leads to drastic changes in their metabolic profile – an effect that is often referred to as "metabolic reprogramming".44Hsu PP, Sabatini DM. Cancer Cell Metabolism: Warburg and Beyond. *Cell* 2008; **135:** 703–707. While the details of metabolic reprogramming are still not fully understood, it was shown for various examples that the resulting changes in the metabolic profile can be successfully used for the mechanistic elucidation55Jain M, Nilsson R, Sharma S, Madhusudhan N, Kitami T, Souza AL, Kafri R, Kirschner MW, Clish CB, Mootha VK.*Science* 2012; **336:** 1040-1044. and detection of cancer and other diseases.66Nagana Gowda GA, Zhang S, Gu H, Asiago V, Shanaiah N, Raftery D. Metabolomics-based methods for early disease diagnostics, *Expert Rev Mol Diagn* 2008, **8:** 617-633.·77Sinclair E, Trivedi DK, Sarkar D, Walton-Doyle C, Milne J, Kunath T, Rijs AM, de Bie RMA, Goodacre R, Silverdale M, Barran P. Metabolomics of sebum reveals lipid dysregulation in Parkinson's disease. *Nat Commun*2021; **12:** 1592.

Due to its exceptional sensitivity, speed, and the ability to obtain quantitative information, mass-spectrometry is a perfectly suited tool to determine metabolic fingerprints comprising hundreds of different components.88Alseekh S, Aharoni A, Brotman Y et al. Mass spectrometry-based metabolomics: a guide for annotation, quantification and best reporting practices. *Nat Methods* 2021;**18:** 747–756. However, the human metabolome database (HMDB) currently contains more than 200,000 entries.99Wishart DS, Guo AC, Oler E et al. HMDB 5.0: the Human Metabolome Database for 2022.*Nucleic Acids Res* 2022, **50:** D622–D631. Reliably monitoring only a fraction of these to identify diagnostic cancer biomarkers represents a formidable challenge, not only for the MS-based measurement itself but also for the underlying data analysis. In a Research Article recently published in Natural Sciences1010Yang, X, Song, X, Yang, X, et al. Big cohort metabolomic profiling of serum for oral squamous cell carcinoma screening and diagnosis. *Nat Sci* 2022; 2:e20210071. as well as a related study1111Song X, Yang X, Narayanan R, Shankar V, Ethiraj S, Wang X, Duan N, Ni Y-H, Hu Q, Zare RN Oral squamous cell carcinoma diagnosed from saliva metabolic profiling. *Proc Natl Acad Sci USA* 2020; **117:**16167-16173., Richard N. Zare, Qingang Hu and co-workers present an elegant way to drastically simplify the measurement as well as the analysis of metabolites for the detection of oral squamous cell carcinoma (OSCC).

The special beauty of the presented approach lies in the combination of multiple innovative techniques. First, instead of using off-line capillaries or a chromatography-based infusion system, the authors use the much simpler conductive polymer spray ionization. This technique utilizes small triangles of a conductive polymeric substrate1212Song X, Chen H, Zare RN. Conductive Polymer Spray Ionization Mass Spectrometry for Biofluid Analysis. *Anal Chem* 2018;**90:** 12878–12885. containing a solvent reservoir to which a few microliters of blood, serum or saliva are applied. Using a non-porous polymer rather than the often-employed paper1313Wang H, Liu J, Cooks R, Ouyang Z. Paper Spray for Direct Analysis of Complex Mixtures Using Mass Spectrometry. *Angew Chem Int Ed* 2010;**49:** 877-880. prevents unintended binding of apolar compounds such as lipids and significantly reduces the amount of background signal. Application of a high voltage to the emitter generates molecular ions by a mechanism similar to that of electrospray ionization (ESI),1414Yamashita M, Fenn JB. Electrospray ion source. Another variation on the free-jet theme. *J Phys Chem.* 1984;**88:** 4451–4459. which can subsequently be analyzed using the vast portfolio of available mass spectrometry and tandem mass spectrometry techniques. However, in contrast to ESI, virtually no sample work-up and no time-consuming purification steps are required as the sample is directly applied to the polymeric support. This prevents sample loss, makes the approach exceptionally fast and, most importantly for clinical applications, cheap. Zare, Hu and co-workers were able to measure an impressive 819 serum samples in 12 hours, which highlights the potential of conductive polymer spray ionization for high-throughput clinical applications. Second, the authors used a machine learning algorithm to identify diagnostic molecular markers that can be used to distinguish cancer from non-cancer cases. Usually, mass-spectrometry based metabolomics yields highly complex data, which are difficult to disentangle without applying a human bias.

The presented machine learning approach circumvents this step and identifies markers purely based on a mathematical algorithm. This not only impedes human error but also helps to pinpoint markers and the correlations between markers that would otherwise remain hidden. In total, 65 diagnostic metabolites, most of them lipids, were found to be significantly up- or downregulated in cancer cases. Monitoring these markers by mass spectrometry of serum can be used to quickly distinguish OSCC from healthy metabolites and even predict the stage of cancer, an aspect that is often crucial for the correct choice of treatment. Finally, the authors elegantly validate their findings by cross-correlating their measurements with analyses on other body fluids such as saliva and molecular imaging of cancerous tissue using desorption electrospray ionization. The imaging data nicely illustrate the spatial distribution of the identified markers in excised tumor tissue.

The present study is a perfect showcase for how mass spectrometry-based metabolomics workflows can be simplified to make them usable in clinical applications. Using body fluids in conjunction with conductive polymer spray ionization drastically reduces the discomfort for patients and significantly lowers the cost and time expenditure of the analysis. Recently, a comparable approach has been successfully employed for the metabolomics-based diagnosis of Parkinson's disease from sebum samples,1515Sarkar D, Sinclair E, Lim SH, et al. Paper Spray Ionisation Ion Mobility Mass Spectrometry of Sebum Classifies Biomarker Classes for the Diagnosis of Parkinson's Disease.*ChemRxiv.* Cambridge: Cambridge Open Engage; 2021; doi:10.26434/chemrxiv-2021-vsjwj-v2; This content is a preprint and has not been peer-reviewed. which further highlights its universal utility. Likewise, artificial intelligence has demonstrated its exceptional potential in aiding the identification of diagnostic markers in various other applications. The present paper therefore persuasively demonstrates the current transformation of mass spectrometry from an expensive research-only technique into a diagnostic tool that can be used in hospitals.