TITLE: It's All in the Details: Molecular Markers in Cancer Diagnosis

AUTHORS: Anastasiya Vinokurtseva, Andrea Kassay

DEPARTMENT: Diagnostic Review

This article is a pre-print. The final version can be found in issue 89(1).

ABSTRACT

Cancer is estimated to directly affect approximately 1 in 2 Canadians in their lifetime and costs billions to the Canadian healthcare system annually. The diagnosis, prognosis and treatment of cancer all require the characterization of tumor cell morphology and genotype, which is conventionally done using invasive biopsy methods. However, less invasive modalities exist, such as liquid biopsy, which refers to the sampling of body fluids for cancer cells and cellular material. This method is less invasive compared to the majority of conventional practices and yields a variety of biomarkers for further laboratory analysis. Analytical techniques for imaging and diagnosis include aptamers, a synthetic analogue to antibodies with increased affinity to the target. Use of aptamers allows for robust analysis of molecular data. This article discusses directions of research and innovations in the use of molecular markers in diagnostics as well as their implications to clinical practice.

INTRODUCTION

Cancer is a disease that affects a large percentage of the population, impacting both individuals and their loved ones. The impacts of this disease are familiar to the majority of the population due to its overall burden and prevalence in the media. According to recent estimates, half of Canadians will develop during their lifetime, and a quarter of Canadian population will die of it.¹ Lung, colorectal, breast and prostate cancer are the most common types in Canada, accounting for half of all cancer diagnoses – hence the most well-known screening programs target some of these cancers.^{1,2} Cancer care in Canada has been estimated to cost around \$7.5 billion in 2012, and the expenditures have been steadily rising.³

Conventional diagnostic procedures vary depending on the type of cancer. In general, after a patient presents with a positive screening result or red flag symptoms, patient history guides the physical exam and relevant laboratory investigations.^{4,5} Following laboratory investigations, imaging allows for staging of the malignancy and examination of a tissue biopsy by a pathologist allows for grading of the cancer based on its cellular morphology.⁵ Such investigations currently guide treatment and prognosis.⁵ However, the molecular underpinnings of cancer allow for huge genetic and molecular diversity and these molecular characteristics determine the cancer's aggressiveness and susceptibility to therapy.^{5,6} Using molecular markers in cancer diagnosis allows for better understanding of cancer pathology, improved screening and diagnosis, and more precise targeting of therapeutics.^{6,7} In this article, we discuss advances in molecular diagnostics of cancer and how they could change cancer care.

MOLECULAR DIAGNOSTICS: CONCEPT AND EXAMPLES

One approach to tumor characterization is liquid biopsy.^{8,9} It uses physiological biofluids such as serum, saliva, sputum, and urine to detect markers of cancer.⁸ It is less invasive than direct tumour sampling, cuts procedural cost in half compared to conventional fine-needle aspiration biopsy, and reduces risks associated with the procedure.^{8–10} This investigation is comparable in invasiveness to a standard blood test for a majority of biofluids but, through a variety of analytical assays, it can test for a panel of oncogenic mutations and allow for specific characterization of cancer cells.⁸ Given the simplicity of the collection procedure, this test can be performed at initial stages of diagnosis and can expedite the process allowing for earlier intervention.^{8,11,12}

Liquid biopsy allows for the collection of *circulating biomarkers* from serum, which includes circulating tumor cells (CTC), circulating tumor DNA (ctDNA, a subtype of cell-free DNA, or cfDNA, of tumor cell origin), exosomes, miRNA and proteins.^{7,8,13–15} These biomarkers can vary with respect to the information they yield, and in addition to diagnostics, such as HCV RNA, can provide prognostic information revealing aggressiveness of particular cancer (eg. *HER-2* breast cancer), or predict susceptibility to particular treatments (*EGFR* lung cancer).¹⁵

Several target genes have been investigated and are currently undergoing validation. Detection of *KRAS* and *BRAF* gene mutations from ctDNA has been validated for clinical practice in investigation of metastatic colorectal cancer.¹¹ *KRAS* status is critical for determination of eligibility for targeted therapy, and provided the high sensitivity and specificity of this test, could replace current tumour section analyses.^{8,11,16}

Another molecular marker of interest is microRNA or miRNA.^{7,17,18} These short nucleic acid strands are speculated to play a huge role in gene expression regulation and can affect multiple processes in cell cycle.^{7,17,18} A five-miRNA signature has been found in serum of patients with

gastric cancer, and expression level of these miRNA was found to correlate with tumor progression.⁷

CTCs are tumor cells that are shed by the growing cancer and circulate in body fluids.^{8,19} They contain tumor genome, having potential to reveal all tumor characteristics, without the need to obtain the cells from the original tumor location.^{8,19} Current research is aimed at concentrating and isolating these cells from a biofluid sample, as they circulate at fairly low concentrations.⁸ Epithelial cell adhesion molecules, or *EpCAMs*, are some of the more widespread tumor markers and the FDA has approved a method for isolation of CTCs that uses *EpCAM* antibodies attached to magnetic beads to concentrate CTCs.¹⁹

Exosomes, or extracellular vesicles, are another target of liquid biopsy sampling.^{8,9,13} These are small vesicles containing a variety of molecules that are thought to be involved in cell signaling, protein clearance, and immune function.^{9,13,20} Circulating in the bloodstream, these small pieces of membranes enclose proteins, nucleic acids, and lipids, all of which can give cues into the behaviour of cancer cells from which these exosomes originate.^{8,9,13} Exosomal proteins include a variety of membrane and cytosolic proteins, such as tetraspanins, Rabs, annexins, flotillins, and heat shock proteins (HSP), which are used for cell-to-cell signalling and intercell transfer.¹³ Expression of some of these proteins has been linked to cancer progression or prognosis, such as tetraspanin CD81 in breast cancer, Rab7 in colorectal cancer, annexin A1 in glioblastoma multiforme and HSP70 in colon cancer.¹³ Nucleic acids, including both DNA, mRNA and miRNA, and exosomal lipids have also been implicated in cancer progression.^{9,13}

New technologies aim to identify intracellular signalling between the aforementioned molecules that mediate cancer growth, invasion and metastasis. One of them employs short synthetic nucleic acids – *aptamers* – that can bind other nucleic acids or proteins with high

affinity.^{21–23} Conjugation of aptamers to molecular tags detectable by fluorescence or colorimetry allows for the detection and visualization of molecular interactions of interest.^{13,24,25} Magnetism and electrochemical reactions can also be used to identify and isolate molecular targets.^{25,26} Aptamers are comparable to currently used antibodies in their applications, but have higher affinity and specificity to their targets than immunoglobulins.^{21,22} Use of aptamers for biomarkers of interest discussed above allows for high-throughput analysis of molecular data and is an innovation in laboratory analysis of molecular markers.²³ Some aptameric methods are currently entering clinical testing. Such methods include aptamers for Mucin-1, a glycoprotein abundantly expressed on a variety of malignant epithelial tumors derived from lung, colorectal, breast, and prostate cancers, among others.²³ Another critical part of diagnosis is imaging and aptamers hold a lot of potential in this field as well.^{21,23} They have low immunogenicity and high stability *in vivo* in animal studies, which may in the near future translate them to human use in imaging, including MRI, PET and SPECT scans.²³ Such properties of aptamers make them an appealing drug delivery system as well. Overall, aptamers have many applications for precision medicine in the field of oncology.²¹

IMPLICATIONS TO PRACTICE

Techniques described above are not yet widespread in clinical practice, and the detection methods discussed above require fine-tuning of biomarker isolation and analysis, in order to improve their efficiency and to reduce cost.²⁷ However, given their high sensitivity and specificity, low invasiveness, and low risk, they are a remarkable innovation in cancer diagnosis.⁹ Biomarkers obtained through liquid biopsy hold a lot of potential to facilitate diagnosis and to integrate precision medicine into clinical practice.⁹

Research on non-small lung cancer (NSCLC) is an excellent example of this integration.²⁸ In a study of NSCLC patients with malignant pleural effusion, CTCs were isolated from pleural fluid samples of NSCLC patients using aptamer-based technology.²⁸ Cancer cell DNA was analyzed for *EGFR* or *ALK/ROS1* mutations.²⁸ Determining patient status with respect to these mutations allowed for provision of targeted therapy with EGFR or ALK/ROS1 inhibitors.²⁸ Liquid biopsy has also shown great value in predicting response to radiation therapy with adjuvants in pediatric gliomas.^{29–31}

Conventional cancer diagnostics often involve doing a direct biopsy of the tumor, with fine-needle aspiration biopsy or more invasive surgical methods.^{5,29} Depending on the location, such a biopsy presents significant procedural risks, such as tumour cell seeding, such as in breast cancer, or bowel perforation, such as in colon cancer, and increases patient anxiety with respect to the procedure.^{29,32,33} Increased use of less invasive methods such as liquid biopsy would mitigate risks and reduce patient discomfort associated with the procedure.³²

CONCLUSION

In summary, cancer is a widespread disease affecting 1 in 2 Canadians over the lifespan.¹ It has a profound impact on individuals and families, leading to death in half of affected patients.¹ It costs billions of dollars to the Canadian healthcare system each year.³ These factors make it clear that innovation and improvement of cancer care are key issues in medicine.

Conventional diagnostic methods are often quite invasive but necessary in order to reveal specific cancer characteristics and allow for targeted treatment and predictions about prognosis.⁵ However, research in the field has developed less invasive and more precise diagnostic

modalities.^{8,9} Liquid biopsy, the circulating biomarkers it yields, and aptamers are some of the novel approaches that hold potential for improving cancer care.^{8,9}

Current translation of the associated basic science research into clinical practice is a key part of moving toward precision medicine.⁹ Basic science research is continuously unveiling new molecular pathways implicated in carcinogenesis and revealing new biomarkers for diagnosis and targets for treatment.^{7,14,18} Liquid biopsy is already used for diagnosis, but its applications are continuously expanding.^{28,29} Currently, over 40 companies are involved in medical applications of aptamers and their commercialization.²³ Awareness and facilitation of bench-to-bedside translation of knowledge are integral for innovation in healthcare and maximization of patient benefit.

REFERENCES

1. Canadian Cancer Statistics publication - Canadian Cancer Society [Internet]. [cited 2019 Dec 2]. Available from: <https://www.cancer.ca/en/cancer-information/cancer-101/canadian-cancer-statistics-publication/?region=on>.
2. Cancer Care Ontario. Get checked for cancer [Internet]. 2018 [cited 2019 Dec 2]. Available from: <https://www.cancercareontario.ca/en/get-checked-cancer>.
3. de Oliveira C, Weir S, Rangrej J, et al. The economic burden of cancer care in Canada: a population-based cost study. *C. Open*. 2018;6:E1–E10.
4. Diagnosis - Canadian Cancer Society [Internet]. [cited 2019 Dec 3]. Available from: <https://www.cancer.ca/en/cancer-information/diagnosis-and-treatment/diagnosis/?region=on>.
5. Cecil RL. Cecil Essentials of Medicine. 7th ed. Andreoli TE, Carpenter CJ, Griggs RC, et al., editors. Saunders Elsevier; 2007.
6. Yasui W, Oue N, Aung P, et al. Molecular-pathological prognostic factors of gastric cancer: a review. *Gastric Cancer*. 2005;8:86–94.
7. Liu R, Zhang C, Hu Z, et al. A five-microRNA signature identified from genome-wide serum microRNA expression profiling serves as a fingerprint for gastric cancer diagnosis. *Eur. J. Cancer*. 2011;47:784–791.
8. Tu M, Chia D, Wei F, et al. Liquid biopsy for detection of actionable oncogenic mutations in human cancers and electric field induced release and measurement liquid biopsy (eLB). *Analyst*. 2016;141:393–402.
9. Campos CDM, Jackson JM, Witek MA, et al. Molecular Profiling of Liquid Biopsy Samples for Precision Medicine Liquid Biopsy Biomarkers-CTCs, cfDNA, and EVs HHS

- Public Access. J. 2018;24:93–103.
10. Aggarwal C, Thompson JC, Black TA, et al. Clinical Implications of Plasma-Based Genotyping with the Delivery of Personalized Therapy in Metastatic Non-Small Cell Lung Cancer. *JAMA Oncol.* 2019;5:173–180.
  11. Thierry AR, Mouliere F, El Messaoudi S, et al. Clinical validation of the detection of KRAS and BRAF mutations from circulating tumor DNA. *Nat. Med.* 2014;20:430–435.
  12. Wei F, Lin C-C, Joon A, et al. Noninvasive Saliva-based EGFR Gene Mutation Detection in Patients with Lung Cancer. 2014 [cited 2019 Dec 3]; Available from: www.atsjournals.org.
  13. Jalalian SH, Ramezani M, Jalalian SA, et al. Exosomes, new biomarkers in early cancer detection [Internet]. *Anal. Biochem.* 2019 [cited 2019 Dec 3]. p. 1–13. Available from: <https://doi.org/10.1016/j.ab.2019.02.013>.
  14. Sunkara V, Woo HK, Cho YK. Emerging techniques in the isolation and characterization of extracellular vesicles and their roles in cancer diagnostics and prognostics. *Analyst.* 2016;141:371–381.
  15. European Commision. Stratification Biomarkers in Personalized Medicine. DG-Research. Brussels: European Commission Health Research Directorate; 2010. p. 1–12.
  16. Ohhara Y, Fukuda N, Takeuchi S, et al. Role of targeted therapy in metastatic colorectal cancer. *World J. Gastrointest. Oncol.* [Internet]. 2016 [cited 2019 Dec 4];8:642. Available from: <http://dx.doi.org/10.4251/wjgo.v8.i9.642>.
  17. Lee YS, Dutta A. MicroRNAs in Cancer. *Annu. Rev. Pathol. Mech. Dis.* 2009;4:199–227.
  18. Peng Y, Croce CM. The role of microRNAs in human cancer. *Signal Transduct. Target. Ther.* 2016;1.

19. Potdar P, Lotey N. Role of circulating tumor cells in future diagnosis and therapy of cancer. *J. Cancer Metastasis Treat.* 2015;1:44.
20. Iraci N, Leonardi T, Gessler F, et al. Focus on extracellular vesicles: Physiological role and signalling properties of extracellular membrane vesicles. *Int. J. Mol. Sci.* MDPI AG; 2016.
21. Dougherty CA, Cai W, Hong H. Applications of Aptamers in Targeted Imaging: State of the Art. 2015.
22. Song KM, Lee S, Ban C. Aptamers and their biological applications [Internet]. *Sensors.* 2012 [cited 2019 Dec 4]. p. 612–631. Available from: www.mdpi.com/journal/sensors.
23. Kaur H, Bruno JG, Kumar A, et al. Aptamers in the therapeutics and diagnostics pipelines. *Theranostics.* Ivyspring International Publisher; 2018. p. 4016–4032.
24. Zhang Y, Zhang T, Liu M, et al. Aptamer-Targeted Magnetic Resonance Imaging Contrast Agents and Their Applications. *J. Nanosci. Nanotechnol.* 2017;18:3759–3774.
25. Zhang Y, Lai BS, Juhas M. Recent advances in aptamer discovery and applications [Internet]. *Molecules.* 2019 [cited 2019 Dec 4]. Available from: www.mdpi.com/journal/molecules.
26. Li J, Zheng H, Bates PJ, et al. Aptamer imaging with Cu-64 labeled AS1411: Preliminary assessment in lung cancer ☆. 2014 [cited 2019 Dec 4]; Available from: <http://dx.doi.org/10.1016/j.nucmedbio.2013.10.008>.
27. Lakhin A V, Tarantul VZ, Gening L V. Aptamers: Problems, solutions and prospects [Internet]. *Acta Naturae.* 2013 [cited 2019 Dec 4]. p. 34–43. Available from: <http://aptamer.icmb.utexas.edu>.
28. Yao Y, Peng M, Shen Q, et al. Detecting EGFR mutations and ALK/ROS1

rearrangements in non-small cell lung cancer using malignant pleural effusion samples. 2019;

29. Panditharatna E, Kilburn LB, Aboian MS, et al. Cancer Therapy: Clinical Clinically Relevant and Minimally Invasive Tumor Surveillance of Pediatric Diffuse Midline Gliomas Using Patient-Derived Liquid Biopsy. 2018 [cited 2019 Dec 4]; Available from: <http://clincancerres.aacrjournals.org/>.
30. Gajjar A, Reaman GH, Racadio JM, et al. Brain Tumors in Children [Internet]. 2018 [cited 2020 Jan 26]. Available from: <https://books.google.ca/books?id=PuJwDwAAQBAJ&pg=PA203&lpg=PA203&dq=survival+benefit+or+managment+value+biomarker&source=bl&ots=6IMRVzz4ed&sig=ACfU3U27q217aWUic2dn90x39Qxjw-bhqQ&hl=en&sa=X&ved=2ahUKEwiO4Y3EwKLnAhVrS98KHRKVBYoQ6AEwAnoECAkQAQ#v=onepage&q=survival benefit or managment value biomarker&f=false>.
31. Ohno M, Miyakita Y, Takahashi M, et al. Survival benefits of hypofractionated radiotherapy combined with temozolomide or temozolomide plus bevacizumab in elderly patients with glioblastoma aged ≥ 75 years. Radiat. Oncol. [Internet]. 2019 [cited 2020 Jan 26];14:200. Available from: <https://ro-journal.biomedcentral.com/articles/10.1186/s13014-019-1389-7>.
32. Miller SJ, Schnur JB, Margolies L, et al. Pre-Biopsy Psychological Factors Predict Patient Biopsy Experience NIH Public Access. Int J Behav Med. 2014;21:144–148.
33. Shyamala K, Girish H, Murgod S. Risk of tumor cell seeding through biopsy and aspiration cytology. J. Int. Soc. Prev. Community Dent. 2014;4:5.