The Exploration and Clinical Implications of Using Liquid Biopsy for Cancer Detection

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A Thesis Submitted to the Department of Physiology in the Partial Fulfillment of the Bachelor in Science Degree in Physiology

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Spring 2019

Abstract

Cancer is a disease the humans has been trying to eradicate for hundred of years. This disease, caused by the abnormal replication of various cells in our body, can cause a multitude of complications ranging anywhere from hormone imbalances all the way to physical obstructions of organs and organ systems. As technology begins to advance and the methods which determine how we detect, treat, and prevent cancer advance with it, it becomes possible to specify what kind of tumor the screening is being done for (benign versus cancerous) and the specific type of tumor it is in relation to its location and stage of maturation. One of the techniques which is making this degree of specificity possible is liquid biopsy which, if perfected, will provide a number of other innovate benefits for patients looking for tumor biopsies, specifically within the context of pancreatic cancer. If liquid biopsy were able to be used reliably in a clinical setting, patients would no longer have to undergo invasive procedures like surgical biopsy, and their progress through treatment would be more closely monitored and more accurate than ever before.

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Pancreatic cancer is a condition which has plagued the US since the 18th century. The American Cancer Society predicts that 56,770 people will be diagnosed with pancreatic cancer in 2019 alone, and of those 56,770 individuals, 45,750 of them will die. More than 7% of all cancer deaths are attributable to pancreatic cancer and there are a number of reasons as to why, specifically linked to its detection and diagnosis. Due to the pancreas being located deep within the abdominal cavity, it is hard to locate the tumor with the naked eye, and even during a physical exam. Additionally, symptoms of Pancreatic Cancer are not always obvious and slowly begin to manifest themselves over time. Lastly, there are no proven biomarkers to help doctors diagnose the disease in its early stages and as a result, most pancreatic cancers are not detected until stage IV, which in many cases may be too late. However, the issue does not stop there. In addition to the clinical difficulty associated with this particular form of cancer comes the difficulty experienced by the patient when it comes to traditional methods of pancreatic cancer biopsy and detection.

Traditionally, pancreatic cancer is diagnosed in what can be considered a three step process. First, various imaging is performed on the abdominal region of the patient to detect any sign of tumor formation. If there is evidence of a tumor present, many times the doctor will have the patient undergo a biopsy to determine whether or not the tumor is benign or cancerous. Traditional method of cancer biopsy, especially in a region located deep within the abdomen, is performed using one of three methods. The first of these methods is percutaneous biopsy, where a needle is inserted through the abdomen and into the pancreas, where a fine needle aspiration is performed. The second of these methods is an endoscopic biopsy, in which the physician passes

an endoscope down the throat of a patient and into the small intestine if the patient near the pancreas. From there, a fine needle aspiration or cell removal is performed on the tumor to obtain material for analysis. The last method used is a surgical biopsy. During a surgical biopsy, a laparoscopic surgery is performed, and the surgeon takes samples from all tissue on or around the pancreas so that they can be sent for biopsy. Each of these methods is not only expensive, ranging anywhere from $800 to a couple of thousand of dollars, but they are all considerably invasive and must be performed under anesthesia. However, there has been a new method of biopsy, known as liquid biopsy, which has been introduced to the scientific community. If made to be reliable, it may drastically change the way and the speed in which cancer, and pancreatic cancer in particular can be recognized and diagnosed.

Liquid biopsy is a test done to look for cancer cells or tiny fragments of circulating tumor DNA (ctDNA) that may be found in the blood of an at-risk patient.Through this process, it is possible to gather data about the cancer and its origins, and use this data to collect a variety of information that could be pivotal in the treatment of the cancer. First, once the ctDNA is obtained, it can be analyzed to specify the location mutation responsible for the appearance of the tumor. By doing this, physicians will be able to deduce which method of treatment will be most effective in the eradication of the cancer. Second, physicians can track the progression of the cancer and take note of how exactly it is responding to treatment. Not only will it be significantly cheaper and efficient to observe the cancer as often as needed, but monitoring the exact amount of ctDNA present will in the blood will also be a reliable indicator of whether or not the cancer is growing, shrinking or remaining the same. Lastly, this procedure will have a significant role in the early recognition of whether or not a cancer is in remission or recurrence.

In order to do this, patients may come in on a consistent basis to have their ctDNA levels checked, and if any trace of ctDNA is found in the patient's blood sample, their physician can be informed as soon as possible, increasing the chances of providing effective and timely care for these patients. Numerous studies have been performed to investigate the practicality and reliability of this new form of biopsy in a clinical setting, and there has been one type of cancer in particular for which the results appear to be especially significant - and that is pancreatic cancer.

The key to making liquid biopsy an effective tool in a clinical setting is not only the discovery of specific biomarkers which can serve as tumor identifiers, but also studying these biomarkers to figure out the specific information they give us regarding the nature and location of a tumor. Exosomes, which are membrane vesicles that are released from a wide range of cells including cancer cells, are one of such biomarkers which is proving to be a viable candidate in regards to the detection and analysis of pancreatic cancer. Due to their role in the formation of cellular environments and their wide array of possible interactions with target genes, miRNA expression profiles of these vesicles gives them tremendous potential to be biomarkers for cancers and their progression (). Another potential biomarker which is currently being investigated are circulating tumor cells, otherwise known as CTCs. While these cells are rare in the blood, the number of CTCs in the blood of a cancer patient is believed to be correlated with tumor development, treatment response, tumor recurrence, and long-term prognosis for many cancers. However, while this may prove to be in important component in liquid biopsy, its implications regarding pancreatic cancer in particular are unclear and thus require further evaluation. The last potential biomarkers which are being targeted in the investigation of liquid

biopsy are cell-free nucleic acids (cfNA). These cfNAs contain information that can be used to detect tumors, reflect tumor burden, and monitor response to therapies. cfNAs are also useful in the sense that they are found in abundance in the blood, making them easy to collect and analyze ().

When it comes to pancreatic cancer, all of these biomarkers have shown significant results in regards to the early and differential diagnosis of the disease. For example, in a study performed by Dr, Zi-Hao Qi and his team, it was observed that CTCs were located in the blood of subjects who had Pancreatic Ductal Adenocarcinomas, but no CTCs were detected in the blood of healthy donors. They also served as sufficient monitoring sources for treatment efficacy in pancreatic cancer patients. Additionally, they found that miRNA, a significant part of cfNAs, was a "sensitive and specific body fluid based biomarker" for pancreatic cancer. Not only could miRNAs be used as a method of early diagnosis for pancreatic cancer, but they could also help with differential diagnosis, indicating whether the tumor was benign or malignant, and the progressive extent of invasiveness. As for exosomes, the genomic DNA extracted from these vesicles has been used to determine DNA mutations for cancer diagnoses. They can also be used to measure a protein known as GPC1, which can distinguish between stage I-IV pancreatic cancer as compared to healthy individuals ().

In the future, liquid biopsy has an increasing potential to serve as a less invasive tool for cancer diagnosis, prognosis, and treatment markers. From using exosomes to evaluate the DNA composition of potential carcinomas to using CTCs to monitor and manage cancer treatment, this new form of biopsy could pave the way in terms of ease and efficiency for cancer diagnosis and treatment as we know it. However, the process is by no means perfect, and in the future, there are

many advancements which must be made before liquid biopsy will be an efficient tool in the clinical setting. For starters, liquid biopsy is still proving to have a lower sensitivity and specificity than when compared to its traditional alternatives. This is because when taking a blood sample for liquid biopsy, you are looking for significant chances in the composition of the bodily fluid, rather than taking a sample directly from the tumor itself. If the change in the bodily fluid composition is not significant enough to warn a physician of potential, growing, or resurfacing carcinomas, they may miss something that would not have been overlooked using traditional biopsy methods. The other big limitation of liquid biopsy is the reliability of the blood samples. As previously mentioned, CTCs are fairly rare in the blood, and there has not been a method of ctDNA isolation and analysis which has proven sufficiently reliable to date (). Once these issues have been adequately solved and implemented, there is no doubt that liquid biopsy will bring revolutionary change in the ways with which we detect, target, and eradicate cancer not only in the US, but around the world as well.

References

"Early Detection." *Pancreatic Cancer Action Network*, www.pancan.org/facing-pancreatic-cancer/diagnosis/early-detection/.

"Key Statistics for Pancreatic Cancer." *American Cancer Society*, 11 Feb. 2019, www.cancer.org/cancer/pancreatic-cancer/about/key-statistics.html.

Nuzhat, Zarin, et al. "Tumour-derived exosomes as a signature of pancreatic cancer-liquid biopsies as indicators of tumour progression." *Oncotarget* 8.10 (2017): 17279.

Qi, Zi-Hao, et al. "The Significance of Liquid Biopsy in Pancreatic Cancer." *Journal of Cancer* 9.18 (2018): 3417.

"Tests for Pancreatic Cancer." *American Cancer Society*, 11 Feb. 2019, www.cancer.org/cancer/pancreatic-cancer/detection-diagnosis-staging/how-diagnosed.html.