Circulating Tumor Cells in Pancreatic Cancer

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Introduction

Pancreatic cancer has a very dismal prognosis with a 5-year survival rate of approximately 5% and a high rate of recurrence following attempted curative resection surgery (1). The vast majority of pancreatic cancers are adenocarcinomas of the ductal epithelium, and they represent the fourth most common cause of death in the US (2). Moreover, this trend shows no sign of abating. Pancreatic cancer is projected to be the second most common cause of death by 2030 (3).

The poor prognosis in cases of pancreatic cancer is associated with three key factors: delayed diagnosis, anatomic location and aggressive biology (4). Resection surgery remains the only curative option for patients diagnosed with pancreatic cancer. However, only 16% of patients typically present with Stage I pancreatic cancer in which the tumor growth is confined to the pancreas only (5). As a result, the overwhelming majority of patients present with tumors that have spread beyond the pancreas in which surgical resection is typically not an effective option.

Due to the anatomical location of the pancreas, performing a biopsy in order to offer a concrete diagnosis of pancreatic cancer is a challenge. In the past, biopsies have been obtained by aspiration of pancreatic or duodenal fluid via Endoscopic Retrograde Cholangiopancreatography (ECRP), mechanical exfoliation of pancreatic cells via brushing techniques as well as Endoscopic Ultrasonography via Fine Needle Aspiration (EUS-FNA) of the lesion (6). Of these, EUS-FNA has been shown to have a high sensitivity, specificity and diagnostic accuracy with minimal complications (7). As a result, this modality has emerged as the most useful when diagnosing pancreatic cancer. However, EUS-FNA can be expensive, inconvenient and is ultimately an invasive procedure that does carry some risk to the patient.

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In the cases of pancreatic cancer when the disease is found early enough to allow for curative resection surgery, the aggressive nature of the cancer makes it such that more than 80% of the patients who successfully undergo the procedure will experience distant cancer recurrences (3). This is an indication that metastasis, which usually occurs in the liver and peritoneum, has already taken place at the time of surgery and should serve to emphasize the aggressive nature of the primary tumor.

In this context of late diagnoses, complex and invasive biopsy procedures and high probability of recurrence, circulating tumor cells (CTCs) may provide an efficient solution to allow for a less invasive, more detectable and more easily monitored indicator of the progression of the disease. CTCs can appear early in the disease's progression, which could aid in early detection and thus increase the number of patients that are candidates for curative tumor resection surgery (8). Additionally, the liquid biopsies performed to collect CTCs in the peripheral blood are far more practical for diagnosis and monitoring of pancreatic cancer in particular due to the anatomic position of the organ

in the body (9). Beyond the limitations of the typical methods of tissue acquisition referenced above, clinical staging based on cross-sectional imaging such as CT and MRI may not be sensitive enough to detect small-volume metastatic disease in the case of pancreatic cancer (3). Circulating tumor cells may thus be able to provide information about distant recurrences that typical imaging methods may render quite difficult. Many studies have shown that the presence of CTCs in peripheral blood can be a strong indicator of the likelihood of metastasis to occur (8). For example, in a recent examination of nine cohort studies that included 268 CTC-positive pancreatic cancer patients and 355 CTC-negative pancreatic cancer patients, the results showed that patients in the CTC-positive group showed significantly worse progression-free survival rates and overall survival rates with hazard ratios of 1.89 and 1.23 respectively (10). Moreover, beyond testing for the mere presence of CTCs in peripheral blood, additional studies have shown that the risk of metastasis formation is proportional to the amount of tumor cells in the blood (9). As such, CTCs could serve as a powerful indicator for the likelihood of a patient diagnosed with pancreatic cancer to suffer a recurrence and could then help clinicians tailor the therapeutic regimen accordingly.

Obstacles

Current methodologies of detection of pancreatic cancer CTCs are not adequate enough to allow CTCs to serve as an efficient indicator. The most well-known CTC platform, and the only one currently approved for use in the US by the Food and Drug Administration called CellSearch, identifies CTCs via the epithelial cell adhesion molecule (EpCAM), a glycoprotein on the cell surface (11). The platform parses through cells in the blood and allows for the counting of CTCs via immunomagnetic separation using EpCAM-specific antibodies conjugated to magnetic particles. However, current rates of detection via the platform for pancreatic adenocarcinomas are quite low. Gao et al. claim the platform is only able to attain an 11% detection rate of CTCs in peripheral blood in the case of localized advanced pancreatic cancer and 19% for metastatic pancreatic cancer (12). These low detection numbers may result from a number of factors including the localization of CTCs in the portal vein, a decrease in blood flow in malignant pancreatic tumors when compared with normal pancreatic tissue and the epithelial-to-mesenchyme transition (EMT), which decreases expression of the epithelial markers on CTCs that are essential for their identification.

Additional obstacles remain in the path of using CTCs as a better diagnostic and monitoring tool for pancreatic cancer. Firstly, there remains robust debate about the proper protocol to collect and identify CTCs in general and in the case of pancreatic cancer in particular. Many studies have stipulated that due to the fact that some CTCs may lose their epithelial characteristics because of the epithelia-to-mesenchyme transition (EMT) that occurs to the CTCs, the current practice of using anti-EpCAM antibodies to identify CTCs may not lead to precise results (13). Some studies have shown that coupling the collection of circulating free DNA (cfDNA) known to have been shed by the primary tumor alongside or instead of CTCs in peripheral blood may provide a more accurate set of biomarkers for managing patients with pancreatic cancer (14). It seems fair to say that in the case of pancreatic cancer, the current methodologies available for identifying CTCs may not be sensitive enough to collect, analyze and diagnose the status of the progression based on CTCs alone.

Secondly, a recent study showed that CTCs released from cancers of the pancreas were more likely to be found and were found in higher numbers in portal blood vis-à-vis peripheral blood (15). This finding may necessitate the somewhat less convenient practice of gathering blood from the portal vein when attempting to collect CTCs for diagnosis or monitoring of tumorigenesis in pancreatic cancer patients, thereby nullifying the potential advantage of using peripheral blood.

Lastly, when compared to other forms of cancer such as colorectal cancer, studies have shown that the frequency of CTC detection in peripheral blood was very low in pancreatic cancer. This may be due to the fact that pancreatic tumors tend to be poorly vascularized and the disease is more localized with metastasis likely in the liver or in the peritoneum (12). Moreover, as mentioned above, CTCs from pancreatic cancer seem to be localized in the portal vein and this may indicate that peripheral blood will not yield enough CTCs for material observations of the cancer progression to be made using currently existing protocols.

Current Research and Future Directions

Researchers continue to attempt to identify the optimal proportion of CTCs per unit of blood in order to develop a protocol for establishing the meaning of CTC levels in peripheral blood in the case of pancreatic cancer. Researchers Bidard et al. and Khoja et al. have used a detection cutoff point of 1 or more CTCs per 7.5 mL of blood to indicate a poorer prognosis when compared to patients without detectable CTCs (10,16-17). On the other hand, Maestro et al., have claimed that 2 or more CTCs per 7.5 mL could show a positive prognostic marker for patients with solid tumors (10,18).

Moreover, it appears that researchers continue to search for additional complementary biomarkers to CTCs also found in peripheral blood that could provide more specific and detailed information about the progression of cancer. In Earl et al.'s study on CTCs in pancreatic cancer, for example, researchers demonstrated that patients who tested positive for either CTCs in peripheral blood or KRAS mutations in circulating free DNA (cfDNA) in plasma had a significantly poorer survival rate. The researchers in this case tested pancreatic cancer patients for the specific presence of KRAS mutations, the most common genetic alteration in cancerous pancreatic tumors found in approximately 90% of tumors (14). They concluded that "the concentration of cfDNA may act as a surrogate marker of disease stage." However, they simultaneously qualified their conclusions by stating that more rigorous analysis of pancreatic cancer samples during the disease's progression and examination of larger patient cohorts would be necessary in order to explore the viability of CTC and cfDNA as prognostic and predictive biomarkers. Similarly, in He et al.'s overview of cancer biomarkers, the researchers provide evidence that the increased presence of specific free circulating micro RNAs (miRNA) in whole blood may indicate poorer prognosis for pancreatic cancer patients (19-20). Specific miRNA strands labeled miR-200a-3p and miR-200b-3p are posited to serve as an indicator of detection of previously undiagnosed cancerous tumors of the pancreas and another, miR-196a-5p, is posited to predict poorer survival for previously diagnosed pancreatic cancer patients (19,21-22). As researchers continue to parse through the cells and nucleic acids sloughed off by cancerous tumors in the blood, perhaps the most effective prognoses will be produced by using a combination of CTC, cfDNA and miRNA analysis together.

Given the nascent nature of CTC research in general and with regards to pancreatic cancer specifically, many studies on the matter make clear that more research with larger cohorts is necessary to further substantiate the strength of the claims that CTCs may help detect the disease, monitor its progression, track its response to curative surgery and ultimately improve patient prognoses. Additionally, it increasingly appears that diagnostic and prognostic accuracy may be boosted by complementing CTC analysis with information gleaned from circulating nucleic acids shed from cancerous tumors present in peripheral blood. Combining these methodologies may yield earlier diagnoses using less invasive methods and thus hopefully lead to higher overall survival rates and less recurrences for pancreatic cancer patients.

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