

Plasma miRNA, an emerging biomarker for pancreatic cancer

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Abstract: Pancreatic cancer (PC) is one of the most dangerous types of cancer, much due to the lack of clinical symptoms in early stages. Early, noninvasive methods of detecting PC remain a great challenge in clinical practices. MicroRNAs (miRNAs), small and non-coding single-strand RNAs, emerge as potential biomarkers for PC. miRNAs are involved in PC progression and abnormal level of miRNAs in plasma has been observed in PC patients. A multi-center study recently conducted by Xu and colleagues demonstrated the potential value of using circulating miRNAs to distinguish PC from normal donors and other pancreas-related diseases.

Keywords: MicroRNA (miRNA); pancreatic cancer (PC); biomarker

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Pancreatic cancer (PC) represents the 4th leading cause of cancer-related death in the United States (1). In contrast to the stable or declining trends for most cancer types, the incidence rate of PC increases annually. It is estimated that approximately half a million new cases of PC will be diagnosed and that over 40,000 people are expected to die of PC in the United States in 2015. Worldwide, over a quarter million people die from PC each year (2). The incidence and death rates of PC are relatively associated with age, sex, and race. In contrast to the steady improvement in survival for major cancers, only 7% of patients diagnosed with PC survive over 5 years after their diagnosis, representing the lowest survival rate of all major cancers (1,2).

One of the main reasons PC is so deadly is that it is very difficult to detect in its early stages. Pancreatic ductal adenocarcinoma (PDA) that arises from ductal epithelium accounts for the majority of those malignant pancreatic tumors (85%). Most patients with early-stage PDA normally do not have symptoms until the cancer extends to more advanced stages. Imaging techniques have poor sensitivity and specificity for the diagnostic PC. Although in last several decades, greater experience of surgeons in high-volume hepatobiliary centers have improved the overall survival of PC, only 15% to 20% of patients are diagnosed when cancer of the pancreas is still surgically resectable. Even

among those who under surgical resection, the median overall survival after surgery is only about 27 months (3).

Currently, there were no tumor markers available and specific for the diagnosis of early PC. Antigen 19-9 (CA19-9) and carcinoembryonic antigen are the conventional useful tumor markers for PDA; however, these tests have limited sensitivity for the early PC diagnosis (4). In addition, the clinical and histological similarities between PC and chronic pancreatitis (CP) make early diagnosis of PC difficult. For instance, chromogranin A (CgA) is the most commonly secreted and measured tumor marker associated with pancreatic neuroendocrine tumors (PNETs). But false positive elevations of CgA can be present in a number of other conditions, such as inflammation or drug treatment.

Because of the poor prognosis and therefore the lethality of PC, novel low-cost noninvasive diagnostic tests with high sensitivity and specificity is greatly needed. Recently microRNAs (miRNAs) have been investigated as possible biomarkers and cellular targets for PC. miRNAs are a type of endogenous, small, noncoding single-stranded RNAs consisting of 18 to 24 nucleotides that regulate their target genes at the posttranscriptional level (5). miRNAs possess many features to make them an ideal candidate of biomarkers; their sequences are evolutionarily conserved, they are stable, and because they are detected by real-time

PCR assays can be highly sensitive and specific. miRNAs are involved in many stages and critical aspects of PC development including pathogenesis, progression, and metastasis (6). MiRNA profiles in PC tissues have been disclosed to be different from those of normal tissues, CP tissues, and tissues from other cancer types (7). Abnormal expression of miRNAs was observed during the progression of pre-invasive stages of PC. These studies indicate that miRNAs in cancer lesions might be useful markers for the early diagnosis of PC. The steady presence of miRNAs in the blood further advocates circulating miRNAs as a biomarker for PC in a noninvasive diagnostic approach. In addition, circulating miRNAs and their prognostic values in PC have been explored in recent studies (7,8). Although diagnostic value has been found in these studies, the findings are not consistent. In addition, these studies are limited to PC. Therefore, further studies are needed to clarify and determine the diagnostic value of circulating miRNAs in all PC types.

In their study recently published in *Annals of Surgery*, Xu and colleagues (9) sought to identify potential circular miRNA as a biomarker of PC. The authors conducted the first multicenter study that attempted to determine miRNA markers of PC by comparing differences in miRNAs in blood of healthy individuals to patients with CP, PDA, PNETs, and other pancreatic tumors (OPT). The authors used two preliminary studies to select 13 miRNAs for further evaluation in a larger sample size. In the first preliminary study, they used plasmid samples from seven patients with PC, six patients with CP, and five healthy volunteers. In the preliminary validation phase, plasma was collected from 29 patients with PC, 16 patients with CP, and 31 healthy volunteers. The main multicenter study was conducted with a larger sample size and with additional groups: 156 patients with PC, 65 healthy volunteers, 57 patients with CP, 27 patients with PNET, and 58 patients with OPT. The blood samples were all pretreatment samples collected before clinical intervention or surgery.

Results from the first preliminary study (discovery phase) showed that 29 miRNAs had the potential to differentiate PC cases from healthy volunteers. Those miRNAs selected from the discovery phase were validated by qRT-PCR in a second preliminary study. The authors selected 13 miRNAs for further validation in the final, large sample study. Of the 13 miRNAs selected for further validation in the final study, 5 miRNAs were found to be significantly up-regulated in PC samples *vs.* healthy volunteers, 3 of which were determined to exhibit diagnostic value. Eight miRNAs

in PC *vs.* CP were found to be significantly up-regulated, five of which were determined to exhibit diagnostic value. Four miRNAs were found to be significantly up-regulated in PC *vs.* PNETs, all of which were determined to exhibit diagnostic value. Four miRNAs were found to be significantly up-regulated in PC *vs.* OPT, only one of which was determined to exhibit diagnostic value.

To further test the diagnostic values of the identified miRNAs, the authors selected some of the miRNAs that they had determined to have diagnostic value and compared them to CA19-9 levels. The diagnostic value of miR-486-5p was not significantly different than CA19-9 in the patients with PC compared to healthy volunteers (compare the AUC values of miR-486-5p and CA19-9, $P=0.602$). The authors also found that the AUC values of miR-486-5p and miR-938 are comparable with the AUC values of CA19-9 in discriminating patients with PDA from the patients with CP.

The study by Xu *et al.* evaluated miRNAs in plasma extensively to identify the early stage of PDA from healthy donors or patients with OPTs. This was the first effort performed by multiple independent centers in a relatively large, well-conducted fashion, which further demonstrated the potential of circulating miRNAs as biomarkers for early PC. However, the nature of the investigation is still exploratory and further investigation into this topic is warranted. Despite the modest improvements in the area under the curve (AUC), the data presented in this study by Xu *et al.* did not demonstrate that the miRNA signatures provided better clinical implications over serum CA19-9 in PDA patients from healthy donors. In addition, the study was unable to evaluate the diagnostic value of the combination of CA19-9 with miRNAs in this large sample. It is understandable that matching the plasma samples to assess both miRNAs and CA19-9 becomes a challenge in a worldwide multicenter trial.

Another hurdle for using circulating miRNAs as biomarkers is how to quantify circulating miRNA from a small sample of total RNAs in plasma. Because of the low amount of total RNA in blood, it is virtually impossible to quantify the isolated RNA. As a consequence, it is of crucial importance to precisely normalize detected miRNA values for variances based on the amount of starting material and RNA extraction. This has been tried by seeking a "housekeeping" circulating RNA. Though still questionable, U6 or other miRNAs (for instance, miR-16) has been used as an internal control to normalize circulating miRNA. In addition, plasma volume has been suggested to standardize the amount of input miRNA (10). To minimize the risk of

false positive outcomes, Xu and colleagues utilized both U6 and miR-16 to normalize the level of miRNAs, and only use miRNAs with significant alterations in both normalized analyses for their further multicenter study. Future studies are needed to systematically characterize different normalization methods to find the best way to reproducibly measure miRNAs in plasma.

In conclusion, the studies by Xu and his colleagues successfully explore new diagnostic approach to detect the early stage of PC. Their results suggest that plasma miRNA signatures provide circulating biomarkers to reduce the misdiagnosis rate in early stage of PC. It would be interesting to explore the diagnostic values of the miRNAs in other gastrointestinal cancers in the future. However, additional technical and clinical work will be necessary to extend and develop these interesting observations, in terms of miRNAs quantification and their application with the combination of CA-19-9.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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