MicroRNA-10b – a new marker or the marker of Pancreatic Ductal Adenocarcinoma?

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miR-10b expression in pancreatic ductal adenocarcinoma (PDAC), as identified by in situ hybridization, is highly correlated with cancer diagnosis, therapy response, and prognosis. If these findings are further confirmed in prospective studies, miR-10b could be used to improve the management of PDAC and decrease the mortality rate of this deadly cancer.
In this issue of *Clinical Cancer Research*, Preis and colleagues (1) report that the level of microRNA-10b expression, as found using *in situ* hybridization (ISH) of endoscopic ultrasonography (EUS)-guided fine needle aspiration (EUS-FNA) biopsy samples, was significantly correlated with response to neoadjuvant therapy and outcome in pancreatic ductal adenocarcinoma (PDAC).

This study is important for several reasons. First, the authors wisely focused on a deadly type of cancer, PDAC, which is the 4th leading cause of cancer death and the second-most common gastrointestinal malignancy in the United States. The median survival duration of PDAC patients is 4.4 months, and the 5–year survival rate is around 4% for all PDAC patients (2). At present, no established biomarkers for early diagnosis and prognosis exist; therefore, it is imperative that we find new predictors for both patients and clinicians. CEA and CA19-9 are regarded worldwide as standard adjunctive markers, but their levels are also elevated in benign and other malignant conditions; thus, they are not recommended for diagnosis or prognosis by the American Society of Clinical Oncology (3).

Second, the identified gene, miR-10b, makes functional sense as being intimately linked to the mechanism of metastasis, the cause of most PDAC deaths. microRNAs (miRNAs) are 19- to 25-nt non-coding RNAs (ncRNAs) and are already “classic” examples of RNA molecules that do not codify for proteins. By degrading or blocking translation of mRNA targets, these miRNAs can regulate a large part of the mammalian genome, including genes involved in metastases (4). miRNAs are also involved in the initiation and progression of human cancers (5) and can behave as oncogenes or tumor suppressors (4). Among microRNAs, miR-10b was the first that was found, by Li Ma and colleagues (6), to be correlated with metastasis in breast cancer. It is induced by the epithelial-mesenchymal transition-related transcription factor Twist and
suppresses HOXD10, which represses several genes that contribute to cell migration and extracellular matrix remodeling in breast cancer; RhoC; urokinase plasminogen activator receptor; a3-integrin; and MT1-MMP (6). Another target of miR-10b is Kruppel-like factor 4 (KLF4), which acts as a transcriptional factor. In esophageal cancer cells, miR-10b was found to directly suppress KLF4, promoting cancer invasion (7). One target of miR-10b is the tumor suppressor neurofibromin; in neurofibromatosis malignant peripheral nerve sheath tumor cells, miR-10b directly suppresses neurofibromin’s mRNA, and RAS signaling in these cells is activated (8). In contrast, TIAM1 (T lymphoma invasion and metastasis 1) was identified as an additional target gene for miR-10b; silencing TIAM1 caused suppression of breast cancer cell migration (9) (Figure 1A).

Third, the authors used adequate material for the study. EUS-FNA is highly accurate at identifying patients with suspected PDAC, especially when other modalities have failed, and has rare complications (10). EUS-FNA biopsy specimens are as reliable as surgical tissues, with a reported positive predictive value, negative predictive value, and accuracy rate of 99%, 64%, and 84%, respectively. EUS-FNA biopsy is a sensitive method, but false-negative cases must also be evaluated (10). EUS-FNA biopsy is performed before therapy, allowing the clinician to predict response or outcome. Preis and colleagues used a highly sensitive fluorescence-based ISH technique combined with immunohistochemical analysis with cytokeratin 19 to identify epithelial cells in EUS-FNA biopsy specimens. This method was not time consuming and enabled them to evaluate miR-10b’s spatial cancer-specific expression. Another method that can select only cancer cells, laser capture microdissection (LCM) (11), is more time consuming and technically demanding than the combination of ISH and immunohistochemical analysis.
Finally, and most significantly, these authors’ findings have high translation potential. They initially determined the expression of miR-10b, miR-21, miR-155, miR-196a, and miR-210 using ISH in 10 resected PDAC formalin-fixed, paraffin-embedded clinical specimens and 3 non-cancerous tissues. The authors found that miR-10b was the most frequently and consistently upregulated in cancer cells. They then used ISH to determine miR-10b expression in 95 PDAC and 11 benign EUS-FNA biopsy samples. In cancer tissue, miR-10b expression (measured automatically as mean fluorescence intensity) was about 5 times higher than that in benign tissue. miR-10b expression was significantly useful for cancer diagnosis. Subsequently, they compared miR-10b expression and the response of neoadjuvant chemo-radiotherapy. They found significantly lower miR-10b levels in tumors that responded than in those that did not. In addition, they detected significantly lower miR-10b levels in resectable than in unresectable tumors. These findings indicate that miR-10b can be used to predict response to therapy.

Thereafter, they found a significantly longer time to metastasis in patients with low miR-10b expression. Moreover, patients with low miR-10b expression had longer overall survival durations than did patients with high expression, for all stages; durations were even higher for stages I and II. These results suggest that miR-10b could be predictive of prognosis. It is clear that further studies to identify the critical targets of miR-10b in pancreatic cancer will also identify other pathways to target, either alone or in combination with chemotherapy.

One of the most significant findings of this study is that of miR-10b’s therapy-decision value (Figure 1B). Preis and colleagues (1) found that patients with low miR-10b expression experienced relatively better responses to gemcitabine-based neoadjuvant therapy and better prognoses. The best treatment for patients with unfavorably high miR-10b expression will be the most difficult decision in clinical practice. New alternative therapies, including miR-10b-targeted therapy, are needed. Many miRNAs have been reported to be oncogenic or suppressive, but few in vivo therapeutic advances have been made. Researchers have evaluated the use of
antagomirs as *in vivo* miRNA antagonists; antagomirs are a type of chemically engineered, cholesterol-conjugated antisense RNA oligonucleotide (12). A miR-10b antagomir was evaluated in a 4T1 mouse mammary tumor metastasis model. Li Ma and colleagues found that antagomir-10b prevents metastatic dissemination of cancer cells from the primary tumor but does not affect late stages of the metastatic process, when tumor cells have disseminated (12).

An important question is whether detection of mir-10b expression by ISH is ready for use in clinical practice. This study was performed at a single institution and was retrospective; its findings must be confirmed in a multi-institutional prospective clinical trial. Clearly, cooperation and support among clinical surgeons, oncologists, pathologists, and molecular scientists is essential. microRNA detection with combined ISH and immunohistochemical staining could be challenging when the miRNAs are not well expressed. Furthermore, staining for the expression characterization of known target genes in addition to specific miRNAs could improve prediction accuracy ([Figure 1B](#)). If the findings of this study are confirmed, they will be of great help towards developing a clinical strategy to select PDAC patients who will experience a response to neoadjuvant therapy and may experience a better outcome.
Acknowledgements.

G.A.C. is supported as a Fellow of The University of Texas MD Anderson Research Trust, as a University of Texas System Regents Research Scholar, and by the CLL Global Research Foundation. Work in Dr. Calin's laboratory is supported in part by the National Institutes of Health; a Department of Defense Breast Cancer Idea Award; developmental research awards in Breast Cancer, Ovarian Cancer, Brain Cancer, Multiple Myeloma, and Leukemia SPOREs; a 2009 Seena Magowitz–Pancreatic Cancer Action Network AACR Pilot Grant; and the Arnold Foundation. We thank Ann Sutton (Department of Scientific Publications, MD Anderson Cancer Center) for her help with the editing of this manuscript.

Conflict of Interest.

The authors declare no conflict of interest.

Figure legend.

Possible future PDAC management decisions using ISH of miR-10b expression. (A) Molecular mechanism of miR-10b action, and (B) treatment decisions based on miR-10b and target expression.
1. References (12 refs.)


miR-10b low expression + high targets expression

miR-10b high expression + low targets expression

PDAC EUS-FNA

Surgical resection

Chemoradiotherapy

RNA inhibition therapy (miR-10b targeted therapy) +/- Surgical resection
+/- Chemotherapy (gemcitabine)
+/- Radiotherapy

Migration, invasion and metastasis or high tumorigenesis

Neurofibromin

miR-10b

RAS Tiam1 HOXD10 KLF4

RhoC

Twist
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Clin Cancer Res  Published OnlineFirst August 4, 2011.

Updated version  Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-11-1477

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