MicroRNA-10b Expression Correlates with Response to Neoadjuvant Therapy and Survival in Pancreatic Ductal Adenocarcinoma

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Abstract

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy. Diagnosis and management of PDAC are hampered by the absence of sensitive and specific disease biomarkers. MicroRNAs (miRNA) are noncoding regulatory RNAs involved in initiation and progression of human cancers. In this study, we sought to determine whether miR-10b could serve as a biomarker for PDAC.

Experimental Design: miRNA expression was characterized by fluorescence-based in situ hybridization using locked nucleic acid–modified DNA probes against miR-10b, miR-21, miR-155, miR-196a, and miR-210, followed by codetection of proteins by immunohistochemistry on the same tissue sections. miRNA expression in surgically resected PDAC tissues and in endoscopic ultrasonography (EUS)-guided fine-needle aspirate (EUS-FNA) samples was analyzed in cytokeratin 19 (CK19)–positive epithelial cells using optical intensity analysis.

Results: In 10 resected PDAC samples, miR-10b was the most frequently and consistently overexpressed miRNA among characterized miRNAs, exhibiting a four-fold increase in the cancer cells ($P = 0.012$). Given this preferential overexpression of miR-10b, we sought to determine whether miR-10b expression was clinically relevant. Accordingly, miR-10b expression was examined in 106 EUS-FNA samples obtained from pancreatic lesions. miR-10b expression was increased in cancer cells compared with CK19-positive epithelial cells in benign lesions ($P = 0.0001$). In patients with PDACs, lower levels of miR-10b were associated with improved response to multimodality neoadjuvant therapy, likelihood of surgical resection, delayed time to metastasis, and increased survival.

Conclusion: miR-10b is a novel diagnostic biomarker for PDACs when assessing pancreatic lesions. Expression of miR-10b is predictive of response to neoadjuvant therapy and outcome in this disease.

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Translational Relevance

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy, with few biomarkers for guiding therapeutic options. MicroRNAs have been implicated as having a role in PDAC. In the present study, we evaluated the expression of miR-10b by in situ hybridization using endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) samples that were obtained from suspicious pancreatic lesions. We determined that miR-10b expression was increased in cancer cells compared with cells from benign lesions. Lower levels of miR-10b were associated with improved response to multimodality neoadjuvant therapy, longer time to metastasis, and improved overall survival. Our findings indicate that miR-10b is a novel marker for assessing suspicious pancreatic lesions, that miR-10b may also serve as a biomarker for response to gemcitabine-based neoadjuvant therapy, and that it may be a predictor of early metastasis formation. Thus, an miR-10b–based clinical assay could potentially improve management and treatment of PDAC patients.

thereby inducing mRNA degradation and/or repressing translation. These novel mechanisms for regulating gene expression and function have been shown to modulate cell proliferation, migration, differentiation, and apoptosis, as well as tissue differentiation and morphogenesis during development, and to participate in cancer development and progression (10).

The expression of several miRNAs, as determined by quantitative reverse-transcription PCR analysis (qRT-PCR), is altered in PDACs. Among others, miR-21, miR-10b, miR-155, miR-196a, miR-203, miR-210, and miR-221 levels are increased in PDACs in comparison with that in the normal pancreas (11–15) whereas miR-375 and miR-148 levels are decreased in PDACs (12, 16). However, some of these studies did not take into account the fact that in the normal pancreas (11–15) whereas miR-375 and miR-148 levels are decreased in PDACs (12, 16). However, some of these studies did not take into account the fact that PDAC is highly desmoplastic and that the pancreatic tumor mass may include variable amounts of inflammatory cells and mast cells, degenerating acinar cells, proliferating ductal cells, foci of acinar to ductal metaplasia and/or pancreatic intraepithelial neoplasia, as well as many cancer-associated fibroblasts and stellate cells (17). It is important, therefore, to assess the spatial expression of miRNAs in PDAC tissues and to determine whether changes in expression of particular miRNAs are specific to the cancer cells or involve other cell types in the pancreatic tumor mass.

We have previously reported on a highly sensitive fluorescence-based in situ hybridization (ISH) technique to assess miRNA expression within individual cells in formalin-fixed, paraffin-embedded (FFPE) clinical specimens of solid tumors (18). In the present study, we initially characterized by ISH the expression of miR-10b, miR-21, miR-155, miR-196a, and miR-210 in 10 resected FFPE PDAC tissue samples and in 3 noncancerous pancreatic tissues. Among these miRNAs, miR-10b was most frequently and consistently upregulated in the cancer cells.

Previously, miR-10b has been reported to be expressed at high levels in metastatic breast cancer cell lines (19) and to promote the migration and invasion of esophageal cancer cells (20). We reasoned, therefore, that characterization of miR-10b spatial expression by ISH in PDAC, in conjunction with our ability to assess miRNA expression specifically in the cancer cells (18), may yield clinically relevant information. Accordingly, we sought to determine whether miR-10b is differentially expressed between cancer cells and adjoining nonmalignant cells in resected PDAC samples, whether its expression can be assessed by ISH in EUS-FNA samples and whether its levels of expression in pancreatic cancer cells within the FNA samples could serve as a diagnostic and/or prognostic marker in PDACs, thereby potentially guiding therapeutic options. Evidence is provided for a strong positive correlation between increased miR-10b expression in the cancer cells and shorter time to metastasis, decreased overall patient survival and resistance to multimodality neoadjuvant chemotherapy and radiation. Thus, miR-10b could serve as a novel diagnostic and prognostic biomarker in PDAC and as a tool for predicting response to therapy.

Materials and Methods

Human tissues

The study was approved by the Dartmouth College Committee for the Protection of Human Subjects. Ten FFPE PDAC tissue blocks and 3 noncancerous pancreatic specimens were obtained from the Dartmouth-Hitchcock Medical Center Department of Pathology archival files. The benign pancreatic tissues were derived from a gun-shot victim, a patient with nesidioblastosis, and a patient who had a partial pancreatectomy whose pancreas only exhibited mild inflammatory changes. Nonmalignant control tissues, deriving from the adjoining normal pancreas that was at least 2 cm away from the resection margin, were available for 8 of the 10 PDAC tissues.

After getting informed consent, EUS-FNA samples were obtained from 155 patients who were initially evaluated for suspicious pancreatic lesions that were mostly detected by computed tomography scans and in some cases by abdominal ultrasound. FNA samples that were either benign (n = 11) or confirmed as PDACs (n = 95) were included in this study. The remaining 49 cases, which were not included in this study were diagnosed as intraductal papillary mucinous neoplasm (11 cases), neuroendocrine tumors (20 cases), and other nonpancreatic malignancies (11 cases). In 7 cases, the pathologic diagnosis was undetermined.

All FNA samples were fixed in 10% formalin or ethanol, embedded in paraffin, cut in 4-μm sections, and mounted on positively charged barrier frame slides. Clinical outcome data were obtained from medical records. Disease response to treatment was defined according to standard Response
Evaluation Criteria in Solid Tumors (RECIST; ref. 21). Categories of response included: disease progression (DP), stable disease (SD), partial response (PR), and complete response (CR).

**Combined ISH and immunohistochemical staining**

FFPE sections (4-μm thick) were subjected to combined ISH/immunohistochemistry (IHC), as previously described (18). For miRNA detection, we used locked nucleic acid (LNA)-modified DNA probes directed against the full length of the mature miRNA sequence. miR-10b probe: 5'-CA+CAA+ATT+CAG+TT+CTA+CAG+GGTA-3'; miR-21 probe: 5'-T+CAG+CAT+CA+CTG+ATA+AG+CTA-3'; miR-155 probe: 5'-T+TA+AT+GCT+AAT+GCT+GAT+AG+GG+GT-3'; miR-196a probe: 5'-CC+CAA+CAG+GA+AA+CT+AC+CTA-3' and miR-210 probe: 5'-TCA+CAG+GT+CTA+A+GCC+CAG+ACAG+CAG-3' (+N denotes LNA-modified nucleotide). Fatty acyl moieties were coupled to 5' and 3' terminal Ts, not part of the miRNA complementary sequence, as hapten for antibody detection. Tissue sections were also incubated with biotinylated DNA probe against U6 small nuclear RNA (snRNA): 5'-CGTGCATACCTGCAAGGGCCATGCTAATCTTCTCTCCTCCTCCTCCT179-3'. Then, expression of cytookeratin 19 (CK19) and amylase was assessed by sequential tyramide signal amplification (TSA) reactions, as previously described (18). Briefly, CK19 was detected using mouse anti-CK19 antibody (1:200; catalogue no. SC-46657; Santa Cruz) and goat anti-mouse antibody conjugated to horseradish peroxidase (HRP; 1:500; catalogue no. 170-6516; Bio-Rad), and TSA reaction with tyramine conjugated to rhodamine or DyLight 594. Following heat-induced epitope retrieval in buffered citrate, amylase expression was detected using mouse anti-amylase antibody (1:200; catalogue no. SC-46657; Santa Cruz) and goat anti-mouse antibody conjugated to HRP (1:500, catalogue no. 170-6516; Bio-Rad) and TSA reaction with tyramine conjugated to DyLight 680. Slides were washed with PBS with Tween (PBST) and mounted with antifading ProLong Gold solution (Invitrogen).

**Quantitative image analysis**

Fluorescent images were captured with a monochrome camera (Exi Blue, QImaging) mounted on an Olympus BX60 microscope. Exposure time was determined by an automated setting using the Image-Pro software (Media Cybernetics), and the same magnification (200×) was used for image acquisition. A monochromatic image was obtained and saved for further analysis. The same field was captured through different filter cubes to record the staining for U6 snRNA (AMCA, Chroma filter set 31000), miR-10b (Fluorescein, Olympus filter set U-MNIBA), CK19 (rhodamine, Chroma filter set SP102V1 or DyLight 594, Chroma filter set SP107), and amylase (DyLight 680, Chroma filter set SP105). Image files were later colorized as displayed in Figures 1, 2, and 4. CK19-positive cells were marked as areas of interest (AOI) using Image-Pro software.

![Image](image-url)

Figure 1. Spatial characterization of miRNA expression in resected pancreatic tissues. A, expression of miR-10b, snRNA U6, CK19, and amylase was determined in a single tissue section from normal pancreas (top) and a single tissue section (4-μm thick) from a PDAC sample (bottom). H&E staining was carried out on the respective consecutive tissue section to reveal tissue morphology. Original magnification, 200×; scale bar, 100 μm. B, heatmap displays fluorescence intensity of miR-10b, miR-21, miR-155, miR-196, and miR-210 signal in CK19-positive cells in a panel of 10 PDAC samples and 3 benign pancreatic lesions. C, bar graph displays mean fluorescence intensity of the miR-10b signal in the CK19-positive cells in a panel of 8 PDAC lesions and adjoining normal pancreas. *, P < 0.006 when compared with mean value in normal pancreas.
This AOI was applied on the matched miR-10b images, and the fluorescence intensity for miR-10b was calculated using the signal intensity tool in the CK19-positive cells. Background subtraction of autofluorescence was carried out on the respective consecutive tissue section to reveal tissue morphology. Original magnification, 200×; scale bar, 100 μm. B, box & whisker plot of miR-10b levels in FNA samples obtained from benign (n = 11) and PDAC (n = 95) lesions. *, P < 0.001 when compared with corresponding value in benign lesions.

Results

miRNA expression in PDACs and normal pancreatic tissue

To determine the predominant cell type responsible for deregulated expression of PDAC-associated miRNAs, we characterized the spatial distribution of miR-10b, miR-21, miR-155, miR-196a, and miR-210 by fluorescence-based ISH assay in PDAC tissues and adjacent normal tissues. The same tissue sections were immunostained for CK19, a ductal cell marker in the normal pancreas and a cancer cell marker in PDAC, and for amylase, an acinar cell marker (Fig. 1A). The fluorescence intensity of each miRNA was determined in the CK19-positive cells in resected PDACs (n = 10) and in benign pancreatic tissues (n = 3). In the normal pancreas, hematoxylin and eosin (H&E) staining revealed an abundance of amylase-positive acinar cells and a few CK19-positive ductal cells, and both cell types exhibited a weak miR-10b signal (Fig. 1A). In contrast, in PDACs, there was an abundance of CK19-positive cells cancer cells that expressed high levels of miR-10b (Fig. 1A). Among the 5 tested miRNAs, miR-10b was the most frequently and consistently overexpressed miRNA within cancer cells that expressed high levels of miR-10b (Fig. 1A). Among the 5 tested miRNAs, miR-10b was the most frequently and consistently overexpressed miRNA within cancer cells (Fig. 1B, Supplementary Fig. S1), exhibiting a 4-fold increase in the cancer cells when compared with miR-10b levels in CK19-positive cells from normal pancreatic tissues (P = 0.012); miR-21 exhibited the second highest level of selective expression in PDACs (Fig. 1B, Supplementary Fig. S2).
PDACs often harbor chronic pancreatitis–like changes with foci of atrophic acinar cells and proliferating ductal cells, and both cell types may overexpress signaling molecules (22). We therefore sought to determine whether there was differential expression of miR-10b in the cancer cells in comparison with the adjoining, nonmalignant CK19-positive cells (Supplementary Fig. S3). Accordingly, only samples for which adjoining normal pancreas (8 of 10 used to generate the heat map) was available were included in this analysis. The mean fluorescence intensity in the CK19-positive cancer cells was significantly higher when compared with CK19-positive cells from the adjoining normal appearing pancreas (3,642 ± 1,322 vs. 733 ± 394 a.u.; \( P = 0.006 \)), with only 1 PDAC sample not exhibiting high miR-10b levels (Fig. 1C).

### miR-10b expression in US-guided FNA from suspicious pancreatic lesions

ELIS-FNA sampling from a suspicious pancreatic mass is useful for diagnosing PDACs. To investigate whether miR-10b detection by ISH could be informative for guiding therapeutic intervention, EUS-FNA samples were obtained from 155 patients with suspicious pancreatic lesions. PDAC was diagnosed in 95 of 155 cases by light microscopy, and benign cytology was suggested in 11 of 155 cases. The histopathologic diagnosis of the remaining 49 cases is detailed in the Materials and Methods section.

In the benign lesions, the CK19-positive epithelial cells exhibited a weak miR-10b signal, which was also faintly present in the pancreatic acinar cells (Fig. 1A). In the FNA samples obtained from patients with PDACs, CK19-positive cells exhibited a strong and intense miR-10b signal (Fig. 1A). On the basis of the analysis of miR-10b expression in the CK19-positive cells, miR-10b expression was significantly elevated in the samples that were subsequently diagnosed as PDACs (Fig. 1B), when compared with miR-10b expression in samples from benign lesions (6,291 ± 2,251 vs. 1,249 ± 921 a.u.; \( P = 0.0001 \)).

### Clinical utility of miR-10b measurement in FNA samples

Correlation of miR-10b measurements with clinical outcome was assessed retrospectively following determination of the mean fluorescence intensity for miR-10b. In patients with resectable or locally advanced disease, relatively low

| Table 1. Baseline characteristics and treatment choices of PDAC patients diagnosed by EUS-guided FNA |
|---------|---------|---------|---------|---------|---------|
|         | Stage I (n = 16) | Stage II (n = 33) | Stage III (n = 18) | Stage IV (n = 28) | \( P \) |
| Age, y   | 72 ± 12   | 68 ± 12   | 68 ± 11   | 66 ± 10   | 0.28    |
| Mass size, mm | 24 ± 7   | 28 ± 7   | 42 ± 10   | 40 ± 17   | 0.244   |
| CA 19-9, u/mL (median, range) | 131 (5–2,498) | 230 (6–10,000) | 190 (5–10,000) | 473 (5–10,000) | 0.56    |
| Normal CA 19-9 (≤35 u/mL) | 12% (2/16) | 11% (3/27) | 30% (4/13) | 10% (2/20) |         |
| miR-10b (a.u.) | 5,759 ± 2,335 | 5,947 ± 2,248 | 6,672 ± 2,060 | 6,759 ± 2,306 | 0.33    |
| Low miR-10b (<5,000 a.u.) | 50% (8/16) | 45% (15/33) | 22% (4/18) | 36% (10/28) |         |
| Treatment choices |
| Surgery alone, % | 0 | 0 | 0 | 0 |         |
| Surgery + adjuvant chemotherapy | 1/16 | 2/33 | 0 | 0 |         |
| Chemoradiotherapy, no surgery | 4/16 | 11/33 | 10/18 | 0 |         |
| Chemoradiotherapy and surgery | 7/16 | 15/33 | 2/18 | 0 |         |
| Palliative chemotherapy | 0 | 0 | 4/18 | 19/28 |         |
| Best supportive care/unknown | 4/16 | 5/33 | 2/18 | 9/28 |         |
| Median survival, mo | 16 | 12 | 8 | 3 |         |

Clinical characteristics and treatment modality

The clinical characteristics of the patients diagnosed with PDACs are described in Table 1 (23). As expected, patients with stage I disease had the longest median survival, whereas patients with stage IV disease had the shortest median survival. At Dartmouth-Hitchcock Medical Center, patients with PDACs without evidence of metastatic disease are generally offered a multimodality approach with neoadjuvant gemcitabine-based chemoradiotherapy followed by attempted resection (8, 24, 25). Forty-nine patients were treated with a multimodality neoadjuvant approach with the intention to follow with surgery, but only 24 of these patients underwent tumor resection (Table 1). None of the patients had surgery alone as a treatment modality (Table 1), and only 3 patients had surgery followed by adjuvant chemotherapy (Table 1). Among patients with stage I disease who did not have surgical resection, 4 patients elected to have palliative care and 4 patients had DP including detection of previously undetectable metastatic disease or local DP. Similarly, of the 16 patients with stage II disease who did not have surgical resection, 4 patients elected to receive palliative care, 1 was lost to follow-up, and 11 were treated initially with multimodality neoadjuvant chemoradiotherapy. Of these 11 patients, 1 died of a myocardial infarction, 1 opted to discontinue therapy because of treatment-related toxicity, 5 had DP, and 4 had worsening performance status that precluded surgical intervention. Patients with metastatic disease or with advanced disease that were not candidates for surgical resection were preferentially treated with palliative gemcitabine and/or best supportive care.
miR-10b expression was highly predictive of response to multimodality neoadjuvant gemcitabine-based chemoradiotherapy (Fig. 3A, n = 44, P = 0.0012). In contrast, miR-10b levels did not correlate with response in patients with metastatic disease treated with palliative gemcitabine-based chemotherapy (Fig. 3B, n = 18, P = 0.26). By logistic regression, low miR-10b expression (<5,000 a.u.) predicted tumor resectability (P = 0.0356). Moreover, the mean fluorescence intensity of miR-10b was significantly lower in patients with stage I or II disease who were treated with neoadjuvant chemoradiation and underwent surgery, when compared with similarly treated stage I and II patients whose tumors were unresectable because of DP (Fig. 3C; 5,314 ± 2,039 vs. 7,561 ± 2,061 a.u.; P = 0.01). Two patients with locally advanced disease who were unresectable at baseline (stage III) and were treated with multimodality neoadjuvant approach were downstaged and underwent resection. Although both patients had lower levels of miR-10b compared with the 10 patients who remained unresectable, the numbers were too small to meaningfully assess statistical significance (Fig. 3C).

To assess correlation with metastasis-free survival, miR-10b expression was analyzed on the basis of 3 levels of expression (Fig. 4A). The median time for progression to metastatic disease at diagnosis, but not in stage III or IV, high levels of miR-10b expression (3.7 months) than in patients with intermediate (7.1 months) or low (8.1 months) levels of expression (Fig. 4B, P = 0.001). The difference was statistically significant in stages I and II (HR: 3.3 for high vs. low miR-10b levels; 95% CI: 1.4–7.78; P = 0.0055). All patients with high levels of miR-10b developed metastatic disease within 12 months whereas approximately one third of patients with low miR-10b levels were alive and free of metastasis after 24 months.

A comparison of survival between patients with low and high miR-10b levels showed that miR-10b significantly predicted survival (P = 0.0003, as a continuous marker). The median survival in the low, intermediate, and high miR-10b expression groups was 12.1, 10.2, and 4.04 months, respectively (Fig. 5A). The HR of miR-10b expression in the high versus low group was 3.1 (95% CI: 1.66–5.77; P = 0.0003). In contrast, there was no significant difference between the intermediate and low miR-10b–expressing groups (P = 0.11). In patients with stage I or II disease at diagnosis, but not in stage III or IV, high levels of miR-10b were associated with decreased survival compared with the patients with low miR-10b levels (Fig. 5A; HR: 3.86; 95% CI: 1.55–9.65; P = 0.0032). Moreover, in patients who received multimodality neoadjuvant treatment followed by complete surgical resection, low miR-10b levels at diagnosis were associated with a statistically significant increase in survival compared with patients who had high miR-10b levels at diagnosis (Fig. 5B; HR: 5.1; 95% CI: 1.25–20.7; P = 0.02).
Discussion

In patients presenting with localized PDAC, resection is usually followed by adjuvant chemotherapy or chemoradiotherapy (26). However, most patients treated in this fashion will eventually die of recurrent disease. For patients with locally advanced disease, no standard of care exists. Consequently, a number of centers have evaluated the usefulness of with neoadjuvant therapy to attempt to downsize/downstage tumors, improve rates of complete resection, and reduce local relapse rates (27). This approach provides the potential benefit of early treatment of local disease and allows for the identification of those patients with rapidly progressive metastatic disease (thereby avoiding futile surgery). A recent large population-based comparison showed a survival benefit for the neoadjuvant approach (28). However, prospective randomized data comparing primary resection with neoadjuvant therapy are lacking.

In this study, we initially evaluated the spatial expression of miR-10b, miR-21, miR-155, miR-196a, and miR-210 in 10 resected PDAC samples and determined that miR-10b was the most frequently overexpressed miRNA in the cancer cells within the tumor mass. We also showed for the first time the spatial expression of miRNAs in EUS-FNA samples by ISH. The colocalization of miR-10b with CK19 in the same FNA samples enabled us to reproducibly assess miR-10b expression in the cancer cells within the tumor mass and the ductal cells in the normal pancreas. We were thus able to show that miR-10b expression was increased in the pancreatic cancer cells within the FNA samples in comparison with miR-10b levels in pancreatic ductal cells within benign lesions. Moreover, we determined that lower levels of miR-10b in the cancer cells were associated with response to neoadjuvant gemcitabine-based chemoradiotherapy, surgical resectability, time to metastasis, and overall survival.

The 5-year survival rate of PDAC patients treated with either upfront surgery or neoadjuvant chemoradiotherapy is less than 30% despite complete resection (4, 28). Although our study focused principally on patients who underwent neoadjuvant chemoradiotherapy, it shows for the first time that miR-10b expression in FNA samples can be used to delineate a subgroup of patients that will truly benefit from subsequent surgery. Thus, those patients whose cancers express low levels of miR-10b are predicted to have greater than 50% survival rate after 2 years. In contrast, high levels of miR-10b project poor outcome and early DP even after surgical resection. Our study design does not allow us to assess the potential role of characterizing miR-10b expression in patients who undergo primary surgical resection but raises the possibility that such an analysis would also be useful in this group of patients.

It is noteworthy that miR-10b expression is elevated in several malignancies, including leukemia (29), hepatocellular carcinoma (30), melanoma (31), and malignant glioma (32), underscoring the potential importance of this miRNA in human cancers. Interestingly, the significance of miRNAs in tumor metastasis was first described by Ma and colleagues who showed high expression of miR-10b in the metastatic breast cancer cell lines (19). Moreover, silencing of miR-10b led to more than a 10-fold reduction in the invasive properties of these cells whereas miR-10b overexpression induced a 4- to 5-fold increase in cell motility and invasiveness (19). In vivo, miR-10b overexpression triggered a significant desmoplastic reaction and enhanced...
cell invasion (33). Conversely, the administration of a miR-10b antagonist to orthotopic breast cancer cell implantation in mice markedly suppressed the formation of distant lung metastases (33). Taken together, these reports underscore the importance of miR-10b in human malignancies and its potential to promote metastatic disease.

PDAC is a highly metastatic malignancy, and miR-10b has been linked to the prometastatic protein Twist1 (18). miR-10b also exerts posttranscriptional inhibition of the target gene HOXD10 (19) and represses the expression of genes that have significant role in modulating cell migration and remodeling the extracellular matrix (19). Recently, a cascade of activation has been described whereby the extracellular matrix component hyaluronan binds to CD44 and promotes c-Src kinase activation, which then activates the transcription factor Twist (34). Further analyses reveal that miR-10b is controlled by Twist, and this process results in the reduction of a tumor suppressor protein HOXD10 leading to RhoA/RhoC upregulation and Rho-kinase (ROK) activation promoting breast tumor cell invasion (34). CD44 was also found to be expressed in tumor stem cells that have the unique ability to act as tumor-initiating cells (35). Additional targets for miR-10b were identified in esophageal cancer cells where miR-10b directly targets Kruppel-like factor 4 (KLF-4), promoting cell migration initiated by miR-10b (20). In contrast, Tiam1 (T-cell lymphoma invasion and metastasis 1) was identified as an additional target gene for miR-10b causing inhibition of breast cancer cell migration (36). This finding underscores the importance of context-specific expression of miR-10b in cancer cells. It will be important, therefore, to conduct studies with pancreatic cancer cell lines to examine the mechanisms of action of miR-10b in PDACs, which may provide additional evidence for considering miR-10b as a potential therapeutic target in PDACs.

Figure 5. miR-10b levels negatively correlate with overall survival. miR-10b expression was characterized in 95 EUS-FNA samples from PDAC patients. A, Kaplan–Meier plots display overall survival curves based on different levels of miR-10b expression: low (<5,000 a.u.), intermediate (5,000–7,999 a.u.), and high (>8,000 a.u.) for all patients or for patients grouped by stage at diagnosis. In patients with stage I or II disease at diagnosis, high levels of miR-10b were associated with decreased survival compared with the patients with low levels of miR-10b (P = 0.0032). B, Kaplan–Meier plots display overall survival curves based on different levels of miR-10b expression for patients who received multimodality neoadjuvant chemoradiotherapy followed by surgery. Patients with high miR-10b expression in the FNA samples (5,000+) had significantly shorter survival in comparison with patients with low miR-10b levels (<5,000, P = 0.02).
diminish the utility of this procedure. Previous studies that have analyzed miRNA expression in FNA samples have used qRT-PCR, which does not provide information about the cell type present in the sample and the specific site of expression of a particular miRNA. For example, let-7a-1 precursor RNA expression was consistently detected at lower levels in PDAC FNA samples than in normal pancreatic tissue, but the small number of patients studied did not allow for the assessment of its diagnostic or prognostic value (38). Similarly, the ratio of miR-196a to miR-217, as determined by qRT-PCR, was proposed to distinguish PDAC FNA samples from chronic pancreatitis and benign lesions (13). However, it is not apparent from either of these studies whether a minimum content of cancer cells in these FNA samples was required to obtain an accurate diagnosis. This is of particular importance in light of recent findings that indicate that the accuracy of prognostic value of epithelial growth factor receptor and gemcitabine resistance–related genes in FNA samples from PDAC patients who received gemcitabine-based therapy was greatly improved when laser capture microdissection (LCM) was carried out to enrich for cancer cell content prior to qRT-PCR analysis (39, 40). Thus, the use of a combined ISH/IHC assay enables rapid and direct evaluation of changes of miRNA expression within the suspicious cells highlighted by CK19 staining and provides an innovative alternative approach to the use of the technically demanding LCM coupled with qRT-PCR analysis.

Our findings must be interpreted with caution because of the limitations common to retrospective studies. Nonetheless, the present data suggest that assessing miR-10b expression by this combined ISH/IHC assay in FNA samples from pancreatic lesions could serve as a diagnostic marker that could help distinguish malignant from benign pancreatic tissue and that miR-10b expression could potentially serve as a prognostic marker in PDACs. Analysis of additional miRNAs and proteins in the same FNA sample may further enhance the diagnostic accuracy of the EUS-FNA procedure and inform therapeutic options. Moreover, the same technology could be used in assessing biopsy material from a variety of solid tumors. Therefore, prospective studies are needed to determine whether this approach may ultimately serve as an important tool for clinicians to guide clinical decision making about neoadjuvant treatment and surgery.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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