Survival of Patients with Gastrointestinal Cancers Can Be Predicted by a Surrogate microRNA Signature for Cancer Stem–like Cells Marked by DCLK1 Kinase

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Abstract

Doublecortin-like kinase 1 (DCLK1) is a gastrointestinal (GI) tuft cell kinase that has been investigated as a biomarker of cancer stem–like cells in colon and pancreatic cancers. However, its utility as a biomarker may be limited in principle by signal instability and dilution in heterogeneous tumors, where the proliferation of diverse tumor cell lineages obscures the direct measurement of DCLK1 activity. To address this issue, we explored the definition of a miRNA signature as a surrogate biomarker for DCLK1 in cancer stem–like cells. Utilizing RNA/miRNA-sequencing datasets from the Cancer Genome Atlas, we identified a surrogate 15-miRNA expression signature for DCLK1 activity across several GI cancers, including colon, pancreatic, and stomach cancers. Notably, Cox regression and Kaplan–Meier analysis demonstrated that this signature could predict the survival of patients with these cancers. Moreover, we identified patient subgroups that predicted the clinical utility of this DCLK1 surrogate biomarker. Our findings greatly strengthen the clinical significance for DCLK1 expression across GI cancers. Further, they provide an initial guidepost toward the development of improved prognostic biomarkers or companion biomarkers for DCLK1-targeted therapies to eradicate cancer stem–like cells in these malignancies. Cancer Res. 76(14); 4090–9. ©2016 AACR.

Introduction

Doublecortin-like kinase 1 (DCLK1) is a tuft cell and tumor stem cell marker that is important in colon and pancreatic carcinogenesis (1–5). Studies in murine models show that DCLK1 both specifically identifies tumor stem and stem-like cells and can serve as a potential therapeutic antitumor target with no apparent toxicity to normal cells or cellular homeostasis (1, 2, 5). Moreover, DCLK1 has been tightly linked to epithelial–mesenchymal transition (EMT), which is important in the metastatic processes of many tumors including those of the gastrointestinal (GI) tract (6, 7). However, although DCLK1 marks cells that initiate tumors and is expressed in the primary tumor, circulating tumor cells (8), and in metastases (9), expression levels of DCLK1 may be unstable. DCLK1 expression levels have a tendency to decrease with advancing disease status (3) possibly due to increased proliferation of tumor stem cell–derived progeny that make up the bulk of the tumor. Therefore, additional studies are necessary to determine whether direct measurements of DCLK1 levels in patients could be clinically useful as a biomarker (3).

miRNAs are a uniquely stable set of ubiquitously expressed small noncoding RNAs that regulate complex processes during both homeostasis and in disease. In cancer, miRNAs modulate stemness, EMT, expression of tumor suppressor genes, and oncogenes, and many other essential pathways that phenotypically affect cancer cells such as drug resistance, tumor growth, invasion, and metastasis (10–14). The Cancer Genome Atlas (TCGA) Project has collected and disseminated large multisite datasets that allow for assessment of the prognostic and diagnostic value of protein, RNA, and other markers, including miRNAs, in the setting of malignancy. In the present study, we utilized these datasets to identify a stable, surrogate miRNA signature for DCLK1 activity in tumors, and to study the prognostic significance of this signature in patients with cancers of the colon, pancreas, and stomach.
Materials and Methods

TCGA pan-GI cancer data

The miRNA and RNA-seq datasets from February 2015 data runs for colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), liver hepatocellular carcinoma (LIHC), pancreatic adenocarcinoma (PAAD), rectal adenocarcinoma (READ), and stomach adenocarcinoma (STAD) were downloaded from the University of California, Santa Cruz (UCSC) Cancer Genome Browser (15, 16).

Determination of DCLK1-associated miRNAs

Illumina HiSeq V2 RNA-seq and miRNA-seq data were loaded into R v3.2, and Pearson correlations were calculated for each miRNA against DCLK1 mRNA expression in the 5 cancers derived from organs that are thought to contain tuft cells (colon, esophagus, pancreas, rectum, and stomach; refs. 1, 5, 17). The resulting correlation P values were adjusted using the Bonferroni correction for each cancer correcting for multiple comparisons and reducing false discoveries. A Bonferroni-adjusted P < 0.05 was considered significant. Consensus miRNAs that were significantly correlated to DCLK1 expression in all cancer types were selected to create a DCLK1 miRNA-derived signature (Supplementary Fig. S1).

Kyoto Encyclopedia of Genes and Genomes pathway analysis

Kyoto Encyclopedia of Genes and Genomes (KEGG)-curated pathway analysis (pathways union) was performed using DIANA miRPath v2.0 (18) using Tarbase as a reference. All miRNAs with Tarbase references were included in the analysis, and a targeted pathway heatmap was generated with a P-value threshold of 0.05.

Statistical analysis

Basic statistical analyses were performed in R v3.2 and Graphpad Prism 6.0. Kaplan–Meier survival analyses were performed in Graphpad Prism 6.0. Cox regression analyses were performed using IBM SPSS Statistics 22. Circos plots for miRNAs across cancers were generated using the R Circos R package (19). Correlation plots were generated using the corrplot R package. Heatmaps were generated using Genesis. Receiver operating characteristic (ROC) curve predictions were generated using the PrognosticROC R package (20).

Clinical patient characteristics

Only publicly available, deidentified data were accessed from TCGA for the analyses reported here. Basic characteristics of the patients used in the survival analyses (colon, pancreas, and stomach) are provided in Supplementary Table S1. The average age was between 65 and 67 years for all three cancers. Gender was split approximately evenly between males and females for colon and pancreatic cancer, but the number of males in the stomach cancer group was significantly greater (286 males vs. 165 females). Cox regression analysis demonstrated that tumor burden, disease stage, and nodal invasion were important survival factors in all three cancers, whereas distant metastases were factors in colon and stomach cancers.

Cell lines

SW480 colon cancer and AsPC-1 pancreatic cancer cell lines were obtained directly from ATCC where they were tested and authenticated via morphology, karyotyping, and PCR to rule out interspecies and intraspecies contamination. Cells were cultured under standard conditions at 37°C in RPMI medium with 10% FBS.

Overexpression and siRNA-mediated knockdown of DCLK1

DCLK1 isoform 1 or vector control was expressed in AsPC-1 cells utilizing lentivirus as previously described (21). Overexpression was confirmed by Western blot. Knockdown of DCLK1 was achieved via transfecting SW480 cells with 50 nmol/L of DCLK1 specific siRNA (Santa Cruz Biotechnology; SC-456178) or scrambled siRNA confirmed not to target any human genes for 72 hours using Lipofectamine 3000 (Sigma). Efficient knockdown was confirmed by Western blot.

Western blot

Western blotting was performed as previously described (7) using specific primary antibodies against DCLK1 (Abcam; 88484) and Beta-actin (Santa Cruz Biotechnology; SC-1616). and IR dye 700 and 800 secondary antibodies (Licor). Results were visualized on a Licor Odyssey Infrared Imager and analyzed in ImageStudio (Licor).

miRNA-specific qPCR

Total miRNAs were isolated from treated cells using a mirNeasy Kit (Qiagen) according to the manufacturer's instructions. Mature miRNAs were amplified by polyadenylation followed by reverse transcription using an All-in-One miRNA First Strand cDNA Synthesis Kit (GeneCopoeia). Following reverse transcription, qPCR was performed using experimentally validated, specific commercial miRNA primers (GeneCopoeia). Results were calculated via the delta-delta CT method using U6 as a housekeeping miRNA.

Results

DCLK1 expression is correlated to EMT across GI cancers

Analysis of all six cancer types demonstrated a strong correlation between DCLK1 mRNA expression and epithelial–mesenchymal transition as determined by the EMT spectrum score previously described (Fig. 1A; ref. 22). DCLK1 was most strongly correlated to EMT in colon and rectal cancers followed by cancers of the pancreas, stomach, esophagus, and liver. Although DCLK1 was significantly correlated to EMT in liver cancer, the level of correlation was approximately 3-fold less when compared with colon and almost 2-fold less than the next least correlated cancer (Fig. 1A). It appears that in hepatocellular cancer, EMT transcription factors and mesenchymal markers are correlated with DCLK1, but the loss of epithelial marker expression is not. This finding suggests that EMT in GI tract cancers may be a process that is directly related to the presence of tuft cells that are known to be present in the esophagus, stomach, intestine, and pancreas but not the liver. Further studies will be necessary to determine if this relationship involves a hijacking of the tuft cell’s sensory and/or secretory function (17, 23, 24) during mutation and tumorigenesis.

Determination of a miRNA signature for DCLK1 tumor activity

Pearson correlations were performed to determine DCLK1’s association with miRNA expression across the 5 tuft cell–containing organ tumors. This analysis revealed a consensus of 15 significantly correlated miRNAs: miR-99a, Let-7c, miR-125b-1, miR-125b-2, miR-532, miR-200a, miR-200b, miR-429, miR-425, miR-218-1, miR-218-2, miR-192, miR-194-2, miR-100, and miR-141 (Fig. 1B). Comparison of this signature between low DCLK1-expressing (0–25th percentile) and high DCLK1-expressing (75–100th percentile)
tumors confirmed the veracity of these findings (Fig. 2). Moreover, high miRNA-signature tumors demonstrated greatly increased levels of EMT as well as DCLK1 expression when compared with low signature tumors (Fig. 3A and B).

The derived signature supports our previous finding that DCLK1 is both associated with and regulates miR-200 EMT suppressors (4, 25). In addition, we observed changes in expression of four key miRNA clusters including the miR-99a/125b-2/Let-7c stemness-associated cluster (upregulated); the miR-200a/200b/429 EMT-suppressor cluster (downregulated); the miR-192/194-2/200c tumor suppressor and p53-inducer cluster (downregulated); and the miR-100/125b-2 EMT-inducer cluster (upregulated). Interestingly, the expression of miRNAs that demonstrate shared sequence motifs but distant chromosomal locations was correlated to DCLK1 expression (e.g., miR-125b-1/2 and miR-281-1/2), suggesting targeted specificity for DCLK1 or vice versa. These findings, in consideration of our previously reported studies, suggest that DCLK1 is capable of inducing a stemness and EMT-supporting miRNA signature that may have significant implications in GI tumorigenesis.

To determine whether any of the miRNAs in the signature are directly regulated by DCLK1, we isolated mature miRNAs from SW480 cells, which express high endogenous levels of DCLK1, following transfection with scrambled or DCLK1-targeted siRNA, and from AsPC-1 cells, which express very low levels of DCLK1, stably expressing control vector or DCLK1. For both of these sets of cells, we isolated proteins to confirm the desired changes in DCLK1 expression. miRNA-specific reverse transcription and real-time PCR revealed that at least 5 of the miRNAs in the signature are directly regulated by DCLK1 in a binary fashion. Specifically, miR-141, miR-200a, miR-200b, miR-425, and miR-532 are all upregulated by DCLK1 knockdown (Fig. 3C and D) and downregulated by DCLK1 overexpression (Fig. 3E and F) in agreement with their correlation to DCLK1 in the TCGA datasets. These findings strongly suggest that the relationship between the derived miRNA signature and DCLK1 is not merely correlative, but that DCLK1 directly regulates at least one-third of the miRNAs that make up the signature.

To further assess the potential functional relevance of this DCLK1-specific miRNA signature, we subjected the 15-miRNA signature to KEGG pathway analysis using mirPath (DIANA Tools) with Tarbase as a reference for gene targets. Out of the 15 miRNAs, 11 had gene targets listed in Tarbase. Generation of KEGG pathways based on these targets revealed interesting enrichments for cancer-related pathways in which DCLK1 is known to have functional significance including colorectal, pancreatic, and renal cell cancers (3, 7, 21). In addition, important processes that affect tumor initiation and progression such as tight junction-regulating targets and TGF-beta signaling among others were also enriched (Fig. 4A).

A DCLK1-based 15-miRNA signature predicts survival in colon and pancreatic cancer

Following determination of the miRNA signature and its potential functional significance, we sought to determine if the signature could predict survival in any of the five studied cancers. An overall signature metric was calculated by summing values for upregulated miRNAs and subtracting values for downregulated miRNAs. Patients were grouped by level of signature expression into low (0–25th percentile), mid (25–75th percentile), and high (75–100th percentile) expression. The Kaplan–Meier survival analysis demonstrated that the DCLK1-derived miRNA signature
could be used to strongly predict both overall survival and recurrence-free survival in colon cancer. All of the colon cancer patients in the high signature expression group experienced a recurrence of disease by approximately 75 months, and no patient survived beyond month 100. In contrast, less than 20% of patients with a low expression signature experienced a recurrence by approximately 150 months, and overall survival remained at 70% for this time period (Fig. 4B).

In pancreatic cancer patients, the signature was able to significantly predict overall survival, but not recurrence-free survival. In patients with mid- to high-level signature expression, <15% of patients remained alive after approximately 73 months. However, approximately half of the patients with low signature expression survived to 90 months (Fig. 4C). Although analysis of recurrence-free survival did not reach statistical significance, there was a nearly 25% to 30% increase of recurrence observed among patients with high signature expression as compared with those with mid and low signature expression (Fig. 4C). Further studies utilizing a larger patient sample are required to determine if this signature can serve as a predictor of both overall survival and recurrence-free survival.

The DCLK1-derived miRNA signature did not predict overall or recurrence-free survival in patients with esophageal or rectal carcinoma, probably due to small sample size (data not shown). However, it was predictive of overall survival and recurrence-free survival in gastric adenocarcinoma (Fig. 4D). These data taken together suggest that the 15-miRNA signature presented here as a surrogate for DCLK1 activity in GI cancers may serve as a potential prognostic marker, especially those derived from organs with DCLK1-positive tuft cells.

**Subgroup analysis of the 15-miRNA survival signature**

In order to better understand the prognostic significance of the miRNA signature in patients with colon, pancreatic, or gastric cancer, we performed Cox regression analysis on clinical subgroups stratified by low- and high-risk miRNA signature and compared the resulting HRs. In colon cancer, early stage patients without signs of nodal or distant metastases who demonstrated high signature expression had a 2- to 4-fold higher HR when assessing overall survival (Fig. 5A). In those with pancreatic cancer, the high-risk signature appeared to be strongly predictive of overall survival in patients under the age of 65, but of limited use in older patients (Fig. 5A). Finally in gastric cancer patients, high signature expression was consistent for most subgroups assessed, but may be most useful for patients under the age of 65 (Fig. 5A). It also may have some prognostic value for patients undergoing radiotherapy (Fig. 5A), which may be related to DCLK1’s tumor stem cell role, as these cells are expected to be resistant to radiotherapy. We confirmed the significance of the miRNA signature to overall survival in the subgroups described above by the Kaplan–Meier analysis (Fig. 5B). Moreover, we performed further subgroup analyses to assess the value of the
miRNA signature in recurrence-free survival and found that the signature was mostly consistent across subgroups and that in pancreatic cancer the signature was again most valuable in patients under the age of 65 (Supplementary Fig. S2). These data suggest that the miRNA signature may have clinical value in specific subsets of patients.

Observed survival and ROCs of the 15-miRNA survival signature

To further assess the signature we divided the patients into groups with definite outcomes at 18 months, 3 years, and 5 years post-diagnosis and determined observed survival percentages. The signature performed well in colon, pancreas, and stomach cancer datasets both in terms of overall and recurrence-free survival. Patients with the low-risk signature demonstrated better actual survival while patients with the high-risk signature demonstrated poorer actual survival than total (Fig. 6A). The TCGA datasets utilized here are a work in progress, and it is not possible to define the specificity and sensitivity of the miRNA signature with current data. In order to estimate their probable value, we utilized the PrognosticROC R package to estimate the probable ROC area under the curve (AUC) for the signature (Fig. 6B). Values ranged from approximately 0.65 to 0.98 in colon cancer, 0.50 to 0.88 in pancreatic cancer, and 0.34 to 0.99 in stomach cancer. For the subgroups discussed in Fig. 5, values ranged from approximately 0.44 to 1. These findings suggest that this miRNA signature may have significant value as a prognostic tool in colon, pancreatic, and stomach cancers and demonstrate that the signature can be used in practice to predict patient risk of death and recurrence.

Discussion

GI cancers are commonly observed malignancies, and virtually all of these arise from normal tissue containing DCLK1+ tuft cells. These cells are thought to be involved in sensory functions and

Figure 3. DCLK1 directly regulates expression of the 15-miRNA signature. Boxplots demonstrating increased expression of DCLK1 (A) and increased EMT status (B) between miRNA signature low (miR Low) and miRNA signature high (miR High) tumors in all 5 tuft cell-containing GI cancers from the TCGA COAD, ESCA, PAAD, READ, and STAD datasets (P < 0.0001 for all comparisons). C and D, SW480 cells express high levels of DCLK1. Downregulation of DCLK1 in this cell line via DCLK1-targeted siRNA results in upregulation of miR-141, miR-200b, miR-425, and miR-532, whereas overexpression of DCLK1 in the AsPC-1 cell line, which expresses nearly undetectable levels of DCLK1, results in downregulation of these same markers (E–F; *, P < 0.05).
signaling during cellular homeostasis and in response to injury (17, 23, 24). Moreover, strongly increased expression of DCLK1 is observed in both precancerous lesions and cancers of these organs (1, 2, 5, 9, 26, 27), suggesting clonal expansion of DCLK1+ cells during tumor initiation and/or activation of downstream of oncogenic signaling. Recently, the presence of DCLK1 tumor stem and stem-like cells (27, 28) has been confirmed in models of colon and pancreatic cancers (1, 2, 5), elevating the importance of this marker. However, the development of prognostic biomarkers in GI cancers has been slow, but developing markers based on an essential target like DCLK1 may have the potential to improve treatment strategies and increase patient quality of life and survival.

DCLK1 has been measured in patient blood, on circulating tumor cells, and in tumor tissues, and functionally, it is highly related to cancer initiation and EMT (2–4). However, the use of DCLK1 expression as a biomarker remains controversial because of the complicated nature of its isoforms, which despite high homology may demonstrate altered expression in various tumors (7, 29). Also, DCLK1 expression may decrease in advanced stage tumors (3). We speculate that this apparent decrease in DCLK1 expression may result from a dilution effect caused by the proliferation of diverse tumor lineages. However, we hypothesize that the limitations to using DCLK1 directly as a biomarker could be overcome by developing a stable molecular signature indicative of DCLK1 activity.

MiRNAs demonstrate exceptional stability even in difficult biologic samples such as formalin-fixed paraffin-embedded tissue sections, blood, and urine (30, 31) and have been used as prognostic biomarkers in a number of cancers (32). Moreover, several recent reports suggest that DCLK1 regulates EMT through a miRNA-dependent mechanism, which has also been confirmed in pancreatic and colon cancer using tumor xenograft models (25, 33). In this study, we utilized miRNA- and RNA-seq datasets...
made available by TCGA Project to determine a stable surrogate 15-miRNA signature for DCLK1 activity in GI cancers. Furthermore, these miRNA signatures were subjected to KEGG pathway analysis confirming their functional relevance through their association with cancer initiation and progression-related pathways. Using Kaplan–Meier and Cox regression analysis, we found that the 15-miRNA signature was able to predict survival in colon, pancreas, and gastric cancers. The signature had the strongest predictive ability in colon cancer where strong data support a DCLK1⁺ cell origin for the APC mutant form of this cancer (2, 5).
and emerging data suggest a role in the KRAS-mutant counterpart (34). It is notable that the signature was particularly effective at predicting overall survival in patients with early stage (I–II) disease. Strikingly, when compared with early stage patients, high signature expression [HR, 2.751; 95% confidence interval (CI), 1.429–11.560] demonstrates poor survival consistent with advanced stage (III–IV) disease (HR, 2.851; 95% CI, 1.879–4.765) as confirmed by the Kaplan–Meier analysis (P = 0.314). This finding highlights the clinical potential of this miRNA signature, and with further validation, it may be used to identify high-risk early stage patients that might require more aggressive treatment and follow-up. In addition, in all stages of disease, the high-risk signature predicted a dramatically increased recurrence hazard (>7-fold compared with the low-risk profile). These findings support the role of the DCLK1-based miRNA signature in determining a prognosis for colon cancer patients. We note that a number of groups have demonstrated that DCLK1 expression can predict survival in colon cancer (9, 35). Although we found similar significant results using DCLK1 gene expression data from the TCGA colon cancer RNA-seq dataset (Fig. 7), the DCLK1-based miRNA signature was able to stratify risk with much greater efficiency.

As in colon cancer, the signature was able to predict overall and recurrence-free survival in gastric cancer. The signature demonstrated better predictive ability in younger (<65 years old) and female patients with an HR of approximately 3 for these groups. Because gastric cancer is characterized by high associated mortality, predictable biomarkers may greatly improve disease stratification, diagnosis, and treatment protocols. However, attempts to develop biomarkers based on alterations on the gene and protein level have so far failed to produce useful, stable assays for gastric cancer patients (35). Previous research has shown that female gender and diffuse histopathology are often seen in younger patients with gastric cancer (36), and that these tumors are molecularly unique and more aggressive than tumors in elderly patients (37, 38). Screening these patients with the DCLK1-based miRNA signature has the potential to allow clinicians to pursue different treatment strategies in high-risk gastric cancer patients.

<table>
<thead>
<tr>
<th>Disease/analysis type</th>
<th>Prognostic ROC model</th>
<th>Observed survival</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>AUC (95% CI)</td>
<td>18 MO Low</td>
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<tr>
<td>COAD (OS)</td>
<td>0.752 (0.653–0.855)</td>
<td>79.0%</td>
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<tr>
<td>COAD (RFS)</td>
<td>0.924 (0.840–0.984)</td>
<td>95.5%</td>
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<tr>
<td>PAAD (OS)</td>
<td>0.637 (0.498–0.770)</td>
<td>50.0%</td>
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<tr>
<td>PAAD (RFS)</td>
<td>0.738 (0.570–0.878)</td>
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<tr>
<td>STAD (OS)</td>
<td>0.600 (0.497–0.703)</td>
<td>48.0%</td>
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<tr>
<td>STAD (RFS)</td>
<td>0.768 (0.339–0.988)</td>
<td>88.9%</td>
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<tr>
<td>Early Stage COAD (OS)</td>
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<td>94.7%</td>
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<td>Radiation Therapy + STAD (OS)</td>
<td>0.768 (0.441–0.966)</td>
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<tr>
<td>65 y.o. &lt; PAAD (OS)</td>
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<td>73.3%</td>
</tr>
<tr>
<td>65 y.o. &lt; PAAD (RFS)</td>
<td>0.899 (0.708–1.000)</td>
<td>90.9%</td>
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Figure 6. The 15-miRNA signature delineates retrospective survival of colon, pancreatic, and stomach cancer patients at 18 months, 3 years, and 5 years. A, comparison of observed survival by miRNA-signature expression among patients with definite outcomes in the TCGA colon, pancreas, and stomach cancer datasets. B, predicted prognostic ROC data for colon (COAD), pancreas (PAAD), and stomach (STAD) cancer datasets as well as relevant subgroups as modeled by the prognosticROC statistical package and observed survival in patients with known outcomes at 18 months, 3 years, and 5 years after diagnosis.
Another interesting finding was the ability of the signature to predict overall survival in patients receiving radiotherapy. Although the confidence interval for this assessment was wide, this finding may support a stem-like role for DCLK1 in stomach cancer, as cancer stem cells are known to resist radiotherapy.

Finally, despite a small sample size \( (n = 163) \), the signature was able to predict overall survival in pancreatic cancer patients. In addition, there was a trend toward predicting recurrence-free survival among the stratified groups, but this did not reach statistical significance, likely due to the small sample size \( (n = 138) \). Pancreatic cancer carries a very poor prognosis, and the utility of biomarkers is unclear. However, there is emerging evidence to suggest that DCLK1 might serve as a new target for pancreatic cancer treatment \( (1, 3, 4, 21, 39, 40) \). To this end, we are currently pursuing DCLK1-targeted agents, and this signature may prove useful in determining patients who might benefit from anti-DCLK1 therapies.

Our findings presented here are novel in that we used the expression of the DCLK1 tumor stem cell marker as a guide to derive a unique, potentially stable miRNA signature that predicts survival in patients with colon, gastric, and pancreatic cancer. To our knowledge, this may be the first time this type of literature-informed technique has been used to determine a prognostic biomarker signature and may be worth exploring further with other known, important targets. Our results indicate that the prediction efficiency of the miRNA signature is associated with tumor pathology, stage, and treatment strategy, and we believe it will be important to take these clinical factors into consideration while further developing this signature and other markers.

Furthermore, these results lend support to a potential pan-GI role for the DCLK1+ tuft cell and tumor stem cell and the functional significance of DCLK1 in colon, pancreatic, and gastric cancer. Finally, our findings suggest that the DCLK1-based miRNA signature should be studied further and has the potential to define new therapies for colon, gastric, and pancreatic malignancies.

Disclosure of Potential Conflicts of Interest

M.S. Bronze is a consultant/advisory board member for Genentech. C.W. Houchen has ownership interest (including patents) in and is a consultant/advisory board member for COARE Biotechnology Inc. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): N. Weygant, J.J. Tomasek

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): N. Weygant, Y. Ge, J.S. Kaddis, P. Chandrakesan

Writing, review, and/or revision of the manuscript: N. Weygant, Y. Ge, D. Qu, J.S. Kaddis, P. Chandrakesan, E. Bannerman-Menson, K.J. Vega, M.S. Bronze, C.W. Houchen

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Y. Ge, W.L. Berry, R. May, E. Bannerman-Menson

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References


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