EVALUATING GASTROINTESTINAL NEUROENDOCRINE TUMORS THROUGH MICRORNA SEQUENCING

JUSTIN WONG[1], NICOLE PANARELLI[2], KATHRIN TYRYSHKIN[1], ADRIANNA MAJEWSKI[1], XIAOJING YANG[1], THERESA SCOGNOMAGLIO[2], MICHELLE KIM[3], KIMBERLEY BOGARDUS[4], THOMAS TUSCHL[4], YAO-TSENG CHEN[2], NEIL RENWICK[1,4]

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ABSTRACT

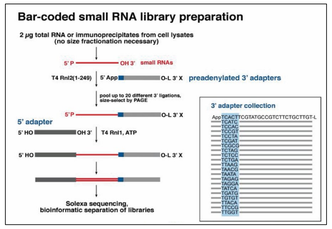
Gastrointestinal Neuroendocrine Tumors (GI-NETs) can be challenging to evaluate histologically. microRNAs (miRNAs) are small regulatory RNA molecules that are also excellent biomarkers. To evaluate miRNAs as adjunct tissue markers for classifying and grading well-differentiated GI-NETs, we generated and compared miRNA expression profiles from pancreatic, ileal, appendiceal, and rectal NETs using barcoded small RNA sequencing. Following data preprocessing, we manually assigned sample profiles to discovery (80%) and validation (20%) sets. Data mining using machine-learning techniques and leveraging prior knowledge that embryonic derivation influences GI-NET behavior, we developed and assessed the accuracy of a dual layer classifier for differentiating GI-NET types. We also investigated using miRNA expression to grade pancreatic NETs. Based on our results, we believe miRNA expression profiling has much potential for evaluating GI-NETs.

INTRODUCTION

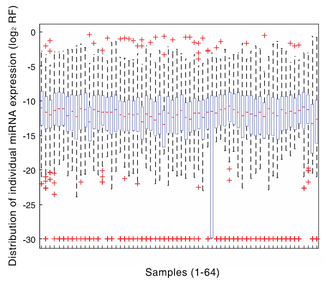
Gastrointestinal Neuroendocrine Tumors (GI-NETs) are increasingly common and clinically diverse neoplasms that are challenging to diagnose, classify, grade, and monitor. Occurring throughout the digestive system, these tumors arise more frequently in the pancreas, ileum, appendix, and rectum. Due to non- specific symptomatology, many GI-NETs are metastatic at diagnosis and the primary site is unknown in up to 20% of cases. Intriguingly, GI-NET behavior may be linked to site-of-origin in the embryonic fore-, mid-, or hindgut. Pathological evaluation of NET tissues is a key component of clinical management because tumor site-of-origin and grade are linked to treatment and overall survival. However, existing immunohistochemical markers and time-consuming and subjective mitotic counts or Ki67 immunostaining hamper accurate classification and grading. Novel approaches and tissue markers are needed to assist histologic evaluation. Here, we evaluated GI-NETs using miRNAs because these small RNA molecules are abundant, cell-type- and disease-stage-specific, and are stable in solid and liquid clinical samples.

METHODS (click to enlarge)

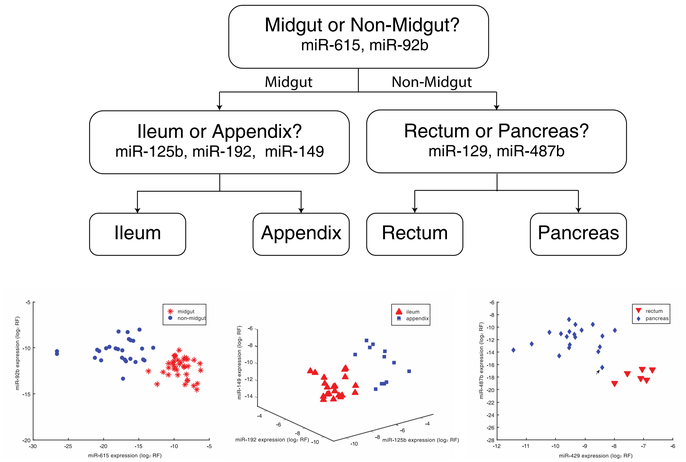
miRNA Grading and Sequencing



Data Preprocessing



RESULTS: TUMOR CLASSIFIER (click to enlarge)



CONCLUSIONS (click to enlarge)

(1) miRNAs are useful adjunct markers for classifying and potentially grading GI-NETs, complementing morphological and immunohistochemistry-based approaches to histological examination

(2) High expression analyses indicate that miR-375 is the most abundant individual miRNA and miRNA cistron in all GI-NET samples.

(3) Leveraging prior knowledge that embryonic derivation influences GI-NET behavior, we developed a dual-layer hierarchical classifier for differentiating four pathological types. Our classifier achieved overall accuracies of 98.5% and 94.4% in discovery and validation sets, respectively.

(4) We found provisional evidence that low- and intermediate-grade pancreatic NETs can be discriminated based on miR-328 expression.

RESULTS : CLASSIFIER ACCURACY
(click to enlarge)

RESULTS : TUMOR GRADING
(click to enlarge)

REFERENCES
(click to enlarge)

CONTACT
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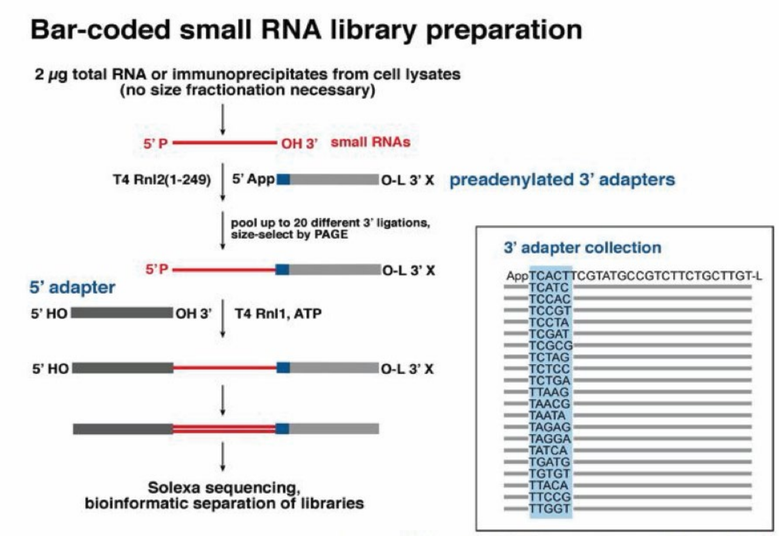
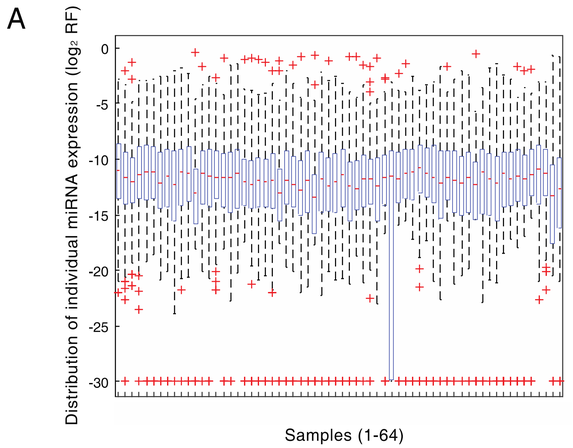
METHODS

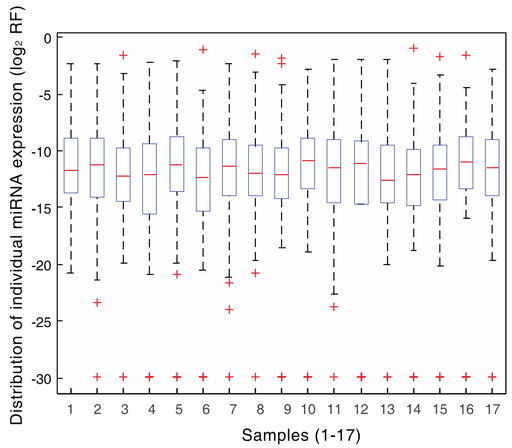
MIRNA GRADING AND SEQUENCING



Study overview: We obtained 84 formalin-fixed paraffin-embedded (FFPE) pancreatic, ileal, appendiceal, and rectal NET tissue blocks from the Department of Pathology, Weill Cornell Medical College. Hemotoxylin-eosin stained tissue sections from each case were reviewed and graded according to the 2010 WHO Classification of Tumors of the Digestive Tract. Total RNA was isolated from FFPE tissue blocks using the Qiagen RNeasy FFPE kit. miRNA expression profiles were generated using an advanced barcoded small RNA sequencing and annotation pipeline above (1, 2).

DATA PREPROCESSING





Data preprocessing: Individual and miRNA cistron expression profiles were preprocessed using MATLAB 2016b (Mathworks Inc). To identify sample outliers and/or sequencing batch effects, miRNA expression profiles were assessed through visual inspection and correlation analysis prior to data normalization, filtering, and mining. Using all miRNA sequence reads, we computed the mean Spearman correlation for each sample to all other samples. Sample outliers were detected using the interquartile range method with $\alpha = 2.2$; three samples were removed from subsequent analyses. Preprocessed profiles were assigned to discovery (A) and validation (B) sets above.

RESULTS: TUMOR CLASSIFIER

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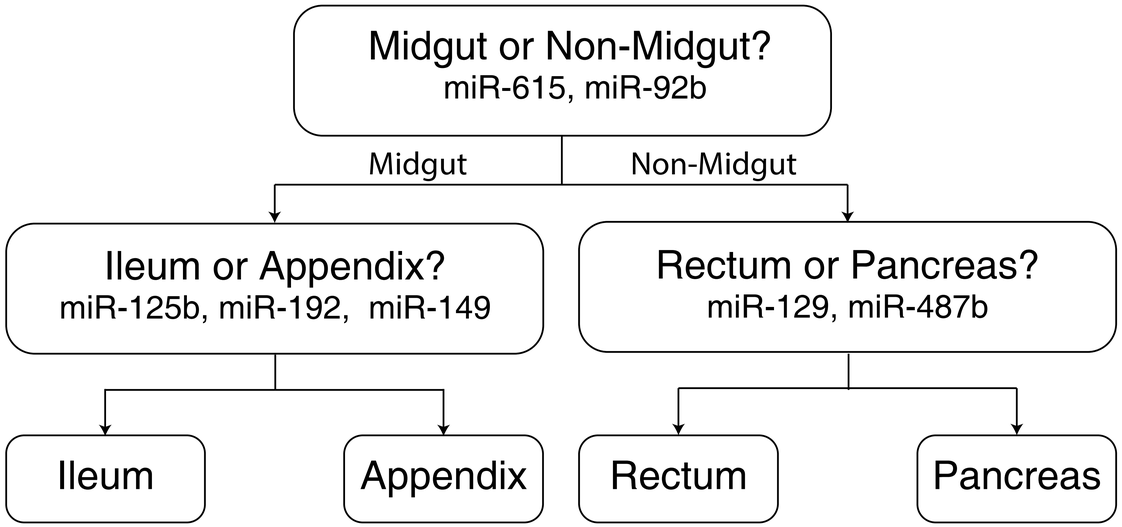
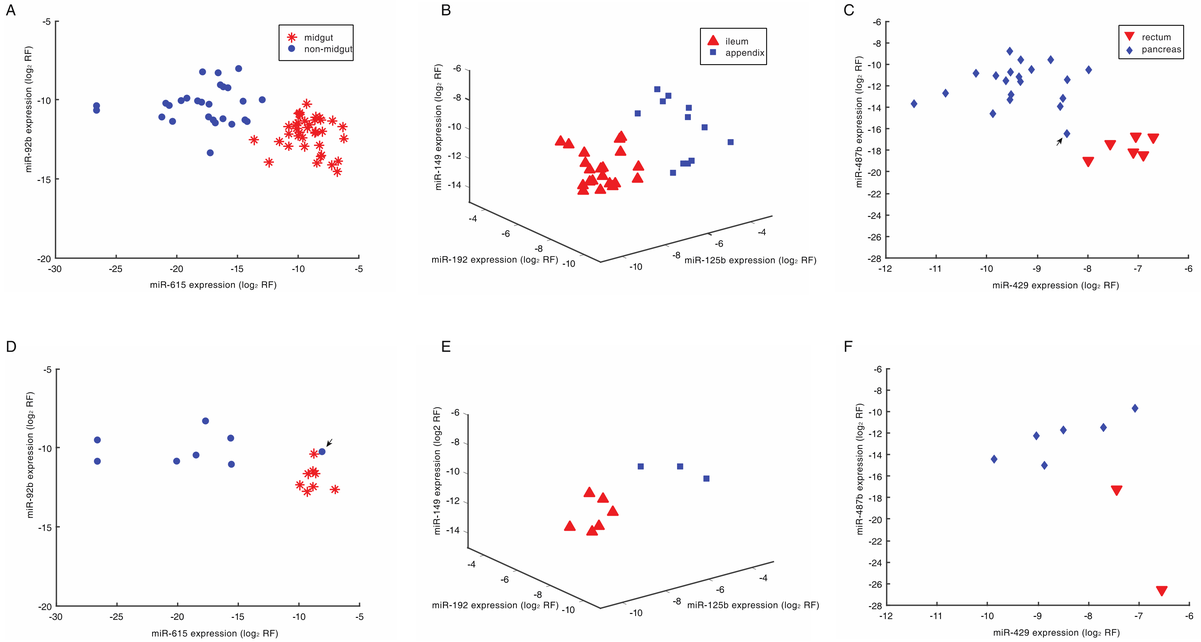
RESULTS: TUMOR CLASSIFIER

CLASSIFIER DEVELOPMENT



SCATTERPLOT ASSESSMENT OF CLASSIFIER



Classifier development: We identified the linear Support Vector Machine algorithm as the the most suitable classifier for our data. This classifier was applied iteratively with 10-fold validation to identify the smallest subset of individual miRNAs or miRNA cistrons for discriminating each comparison in our discovery set. Based on these subsets and the selected classifier, we constructed a dual-layer hierarchical classifier for differentiating GI-NETs.

Scatter plot assessment of select miRNAs for discriminating GI-NET types: Classificatory miRNAs used at each decision node in the hierarchical classifier were evaluated in discovery (A, B, C) and validation (D, E, F) set samples. Abbreviation: log2 normalized relative frequency (log2 RF).

METHODS

RESULTS: CLASSIFIER ACCURACY

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RESULTS : CLASSIFIER ACCURACY

		Established discovery set diagnosis				Established validation set diagnosis			
		Ileum	Appendix	Rectum	Pancreas	Ileum	Appendix	Rectum	Pancreas
Heirarchial Classifier Designation	Ileum	25	0	0	0	6	0	0	0
	Appendix	0	12	0	0	0	3	0	0
	Rectum	0	0	6	0	0	0	2	0
	Pancreas	0	0	1	20	1	0	0	5
Accuracy		63/64 (98.5%)				(16/17) 94.4%			

Overall accuracy of hierarchical classifier for discrminating GI-NETs: Using our hierarchical classifier, samples were assigned to one of four pathological types. Overall classifier accuracy was assessed by comparing these designations to established pathological diagnoses for the same samples in the discovery and validation sets.

RESULTS : TUMOR CLASSIFIER

RESULTS: TUMOR GRADING

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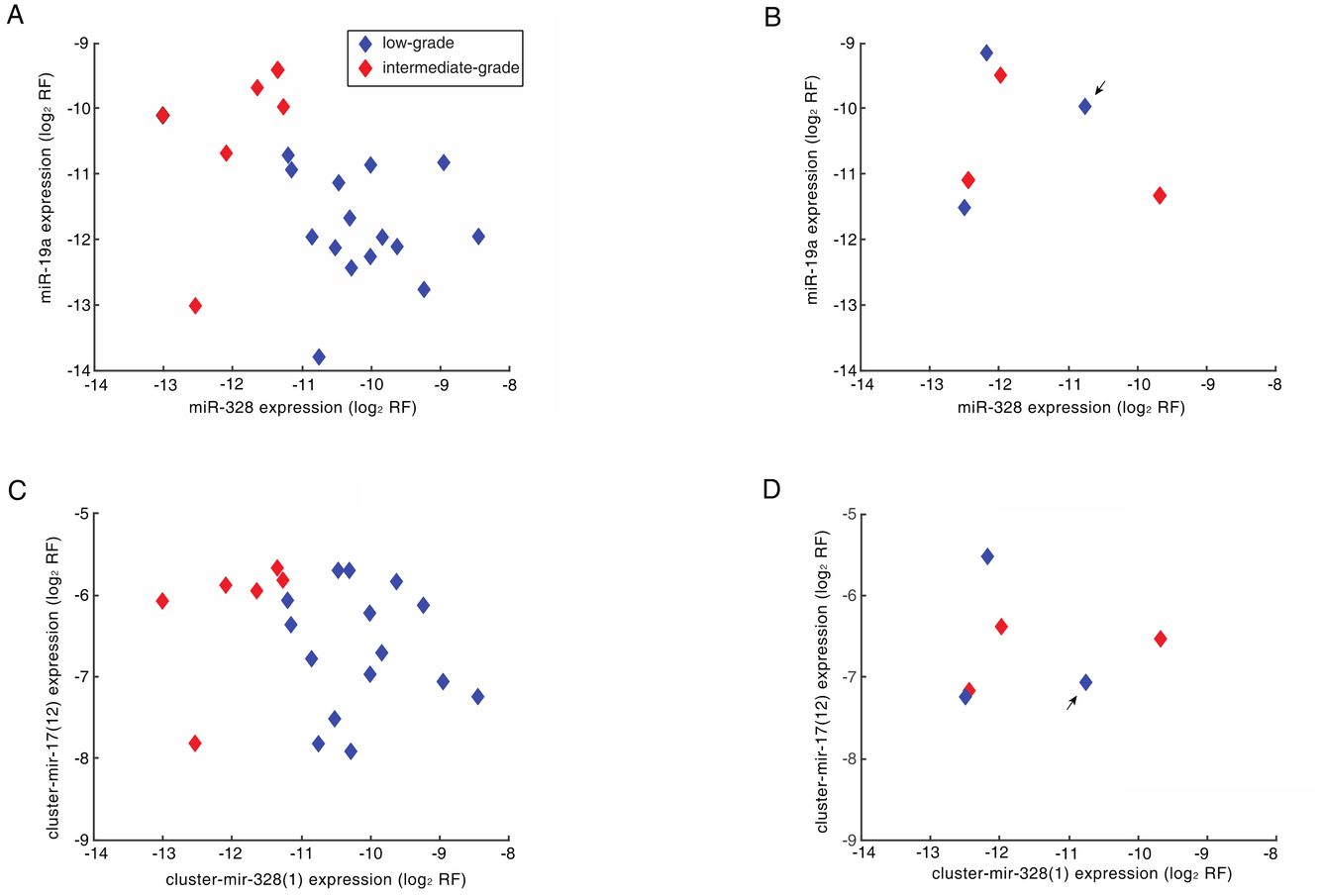

Scatter plot assessment of select miRNA for discriminating low- and intermediate-grade pancreatic NETs: Low- and intermediate-grade pancreatic NETs are effectively discriminated based on miR-328 expression and cistron-miR-328(1) expression in the discovery set (A,C) but not the validation set (B,D). The "misclassified" midgut NET (indicated by arrows) was included by random in the validation set. Highly ranked miR-19a and cistron-mir-17(12) expression is presented for comparison only. Abbreviation: log2 normalized relative frequency (log2 RF).

RESULTS : CLASSIFIER ACCURACY

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(1) Hafner M, Renwick N, Brown M, Mihailović A, Holoch D, Lin C, Pena JT, Nusbaum JD, Morozov P, Ludwig J, Ojo T, Luo S, Schroth G, Tuschl T. RNA-ligase-dependent biases in miRNA representation in deep-sequenced small RNA cDNA libraries. RNA. 2011 Sep;17(9):1697-712.

(2) Farazi TA, Brown M, Morozov P, Ten Hoeve JJ, Ben-Dov IZ, Hovestadt V, Hafner M, Renwick N, Mihailovic A, Wessels LF, Tuschl T. Bioinformatic analysis of barcoded cDNA libraries for small RNA profiling by next-generation sequencing. Methods. 2012 Oct;59(2):171-87.

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