Global MicroRNA Profiling of Small Intestinal Neuroendocrine Carcinomas and Establishment of a Method to Study Serum MicroRNA Expression from the Same Malignancies

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Conclusions

The small intestinal neuroendocrine carcinoma (SI-NEC) microRNA (miRNAs) profile provides potential pivotal miRNAs. They may be involved in tumor progression and have a role in novel therapeutic targets development. One of the major goals is to extend this study to blood samples to overtake patient's tissue specimens collection.

Aims

To provide an exclusive miRNAs profile on SI-NECs at different stage of disease and identify miRNA expression from patient blood samples.

Background

MiRNAs are posttranscriptional regulators and function either as tumor suppressors or oncogenes in a variety of cells. MiRNAs may play a critical role in development, diagnosis and progression of SI-NECs.

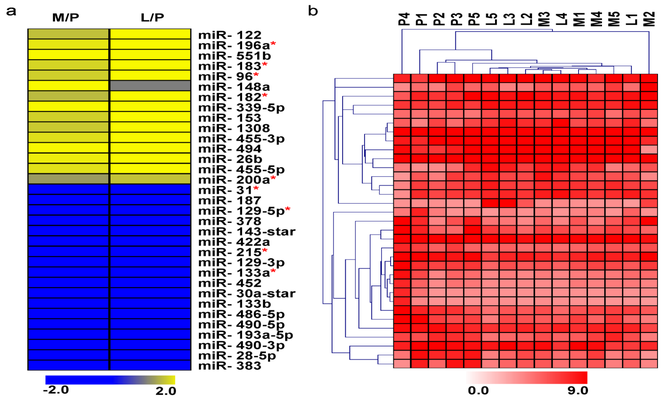

Figure 1. Thirty three differentially expressed miRNAs from frozen SI-NEC specimens. (a) Detection of 33 differentially expressed miRNAs from mesentery (M) and liver (L) metastases compared to primary tumors (P). Nine out of 33 miRNAs were selected for further investigation and they are marked by a red asterisk. Deregulated miRNAs expression is plotted in yellow (up) and in blue (down). **(b)** Cluster of 33 differentially expressed miRNAs from 5 P (P1-P5), 5 M(M1-M5) and 5 L (L1-L5) is shown. The 5 primary tumors cluster together differently from the 10 metastases .

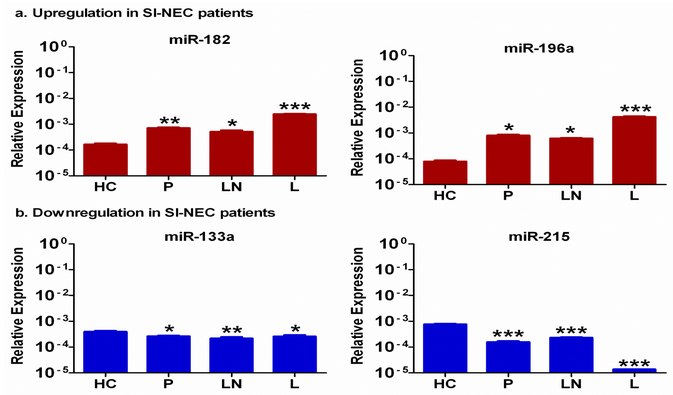

Figure 3. QRT-PCR analysis detected the expression of miR-133a, -182, -196a and -215 in serum. Total RNA was isolated from healthy control (HC), primary SI-NETs (P), lymph node metastases (LN) and liver metastases (L) to run the analysis. **(a)** Upregulated miRNA expression in tumor patients compared to HC. **(b)** Downregulated miRNA expression in tumor patients compared to HC. Results were plotted using the $2^{-\Delta\Delta Ct}$ method with miR-16 expression (set to 1) from each individual sample for normalization. Plotted results are mean ±SD for triplicate wells. Significance was calculated by One-Way ANOVA followed by Bonferroni test. * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$.

Results

The Global miRNA profile shows that nine miRNAs significantly altered their expression between primary tumors and metastases. QRT-PCR analysis from laser capture microdissected (LCM) tumor cells detected five upregulated miRNA expression in LCM tumor cells versus immuno LCM normal enterochromaffin (EC) cells. Whereas, four miRNAs were downregulated in LCM tumor cells. We also investigated miRNA expresssion from serum samples by QRT-PCR analysis. In addition, the nine deregulated miRNAs are expressed at different level on five human NET cell lines.

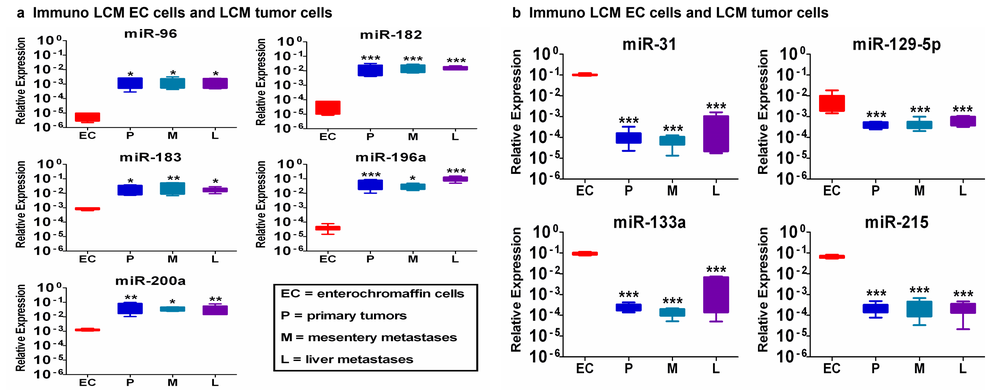

Figure 2. QRT-PCR analysis validated the expression of nine selected miRNAs from the second group of specimens. Total RNA was isolated from LCM tumor cells and immuno LCM normal EC cells. Analysis was run using 3 normal EC, 3 P, 3 M and 3 L samples. **(a)** Upregulated miRNA expression in tumor cells compared to normal EC cells. **(b)** Downregulated miRNA expression in tumor cells compared to normal EC cells. Results were plotted using the $2^{-\Delta\Delta Ct}$ method with RNU48 expression (set to 1) from each individual sample for normalization. Plotted results are mean ±SD for triplicate wells. Significance was calculated by One-Way ANOVA followed by Bonferroni test. * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$.

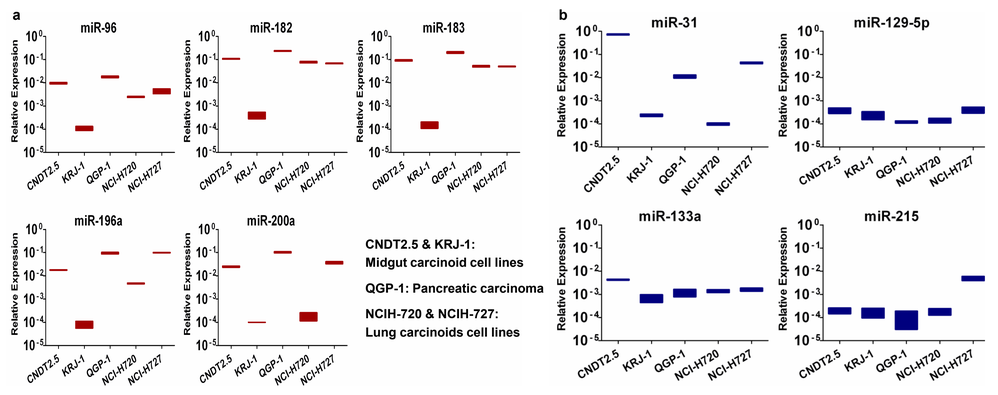

Figure 4. QRT-PCR analysis detected the expression of nine miRNAs in five human NET cell lines. Total RNA was isolated from CNDT2.5, KRJ-1, QGP-1, NCI-H720 and NCI-H727 cells to run QRT-PCR. **(a)** Upregulated miRNA expression in 5 NET cell lines. **(b)** Downregulated miRNA expression in 5 NET cell lines. Results were plotted using the $2^{-\Delta\Delta Ct}$ method with RNU48 expression (set to 1) from each individual sample for normalization. Plotted results are mean ±SD for triplicate wells.

Material & Methods

1. Total RNA from 15 SI-NEC specimens (5 primary tumors, 5 mesentery metastases and 5 liver metastases) to perform genome-wide Affymetrix GeneChip® miRNA arrays.

***2. In silico* data have been validated by QRT-PCR analysis from LCM normal EC cells and LCM tumor cells.**

3. SI-NEC serum samples were also used to validate our findings.

4. Five human NET cell lines were profiled as potential cellular models for further functional studies.