MicroRNAs As Early Gastric Cancer (GC) Biomarkers

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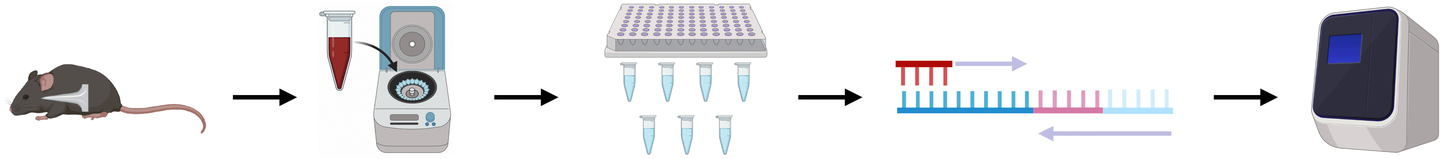
BACKGROUND

- Gastric cancer (GC) is the 3rd leading cause of cancer death among men and women & the 5th most commonly diagnosed cancer worldwide.[1]
- Current early detectors of GC (primarily endoscopies) are both expensive and invasive.[1]
- A need exists for a non-invasive biomarker to determine early detection for GC, which can significantly increase the chances for patient survival.[2]
- miRNAs: molecules that bind to the 3' untranslated regions of mRNAs to trigger changes in gene expression — have the potential to serve as an attractive biomarker.[1]
- The Singapore Gastric Cancer Consortium (SGCC) conducted a series of tests to identify the possibility of miRNAs as GC biomarkers.[1]
  - They unveiled a panel of 12-miRNA biomarkers that will help aid in early GC detection.[1]

ABSTRACT

Patients with gastric cancer have little to no options for early detection procedures aside from the traditional treatment options of endoscopies and photofluorography. However, these options have proved to be very limiting. Recent studies have focused on the utilization of microRNAs as biomarkers. These molecules circulate in the bloodstream and can indicate potential tumors due to up-regulated or down-regulated miRNA levels. Based on a previous study conducted by the Singapore Gastric Cancer Consortium, we chose to focus on three of the twelve miRNAs used in their biomarker panel, along with a fourth miRNA molecule of our choice — miRNA-93, miRNA-108, miRNA-140, and miRNA-183. Our study focuses on identifying the usability of these four molecules in our lab's TCON mouse model, a genetically engineered mouse model of gastric adenocarcinoma tumorigenesis based on $Kras^{G12D}$ expression plus inactivation of E-cadherin (Cdh1) and p53 in the gastric parietal cell lineage.[3] Using blood samples collected from the TCON and healthy mice, we isolated the miRNAs and converted the molecules into cDNA. We conducted qPCR to identify the levels of each molecule within the serum. The results were largely inconclusive and most likely statistically insignificant. Further tests must be conducted to validate the potential biomarker parallel between mice and humans.



METHODS

Submandibular Bleed: Puncture the vein in the area behind the mandible with a 25 gauge needle. As soon as the blood begins to flow, use a designated tube to collect the amount necessary for the protocol.[4]
- Tubes should be coated with an anticoagulant prior to blood collection to prevent clotting.

miRNA Isolation: mirVana isolation kit for miRNA isolation[5]

cDNA Synthesis: Use the Agilent 1st-Strand cDNA kit for cDNA synthesis[6]

qPCR: Use the Bio-Rad SYBR Green kit for PCR master mix[7]

RESULTS

Sample Name	Ct Mean	Ct SD
T1	27.149	5.898
T2	27.950	8.450
T3	25.924	0.891
T4	31.430	2.380
N1	25.436	1.573
N2	28.570	2.623
N3	28.534	2.018
H2O	28.775	1.648

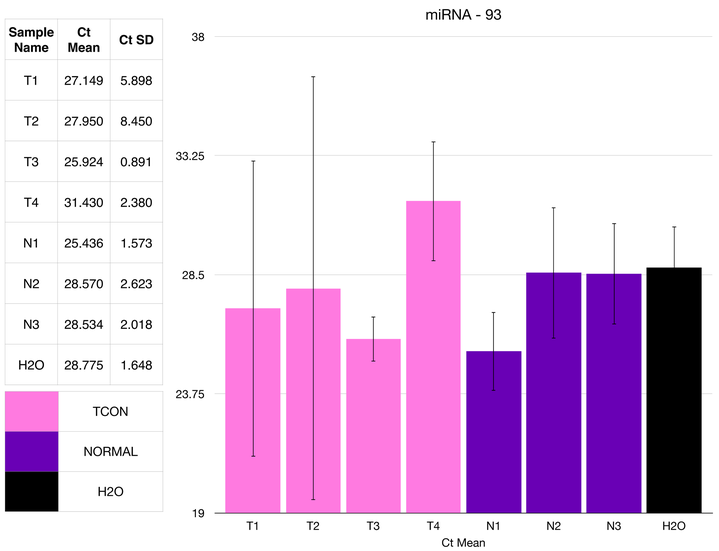


Figure 1: Analyzing the results for miRNA-93, a molecule responsible for the proliferation and metastasis of gastric cancer by targeting TIMP2.[8] that As represented by the key, the pink bars represent the TCON mice, the purple bars represent the normal mice, and the black bars represent the water sample used as a negative control. The results are most likely not statistically significant.

Sample Name	Ct Mean	Ct SD
T1	31.005	8.984
T2	29.447	4.021
T3	27.688	0.559
T4	24.981	2.228
N1	26.156	1.230
N2	28.714	1.760
N3	28.067	1.114
H2O	25.744	2.572

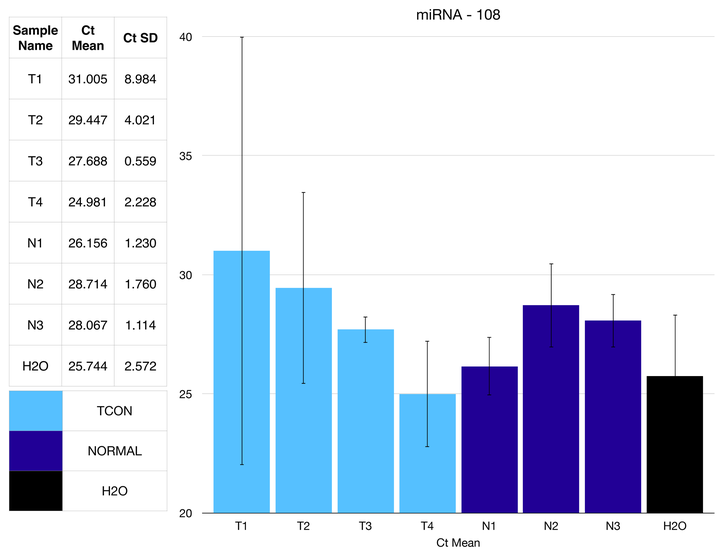


Figure 2: Analyzing the results for miRNA-108. As represented by the key, the light blue bars represent the TCON mice, the dark blue bars represent the normal mice, and the black bars represent the water sample used as a negative control. The results are most likely not statistically significant.

Sample Name	Ct Mean	Ct SD
T1	29.087	2.479
T2	30.244	4.760
T3	34.363	6.339
T4	32.635	7.339
N1	37.010	5.496
N2	33.736	2.272
N3	38.434	4.44497
H2O	38.575	4.201068

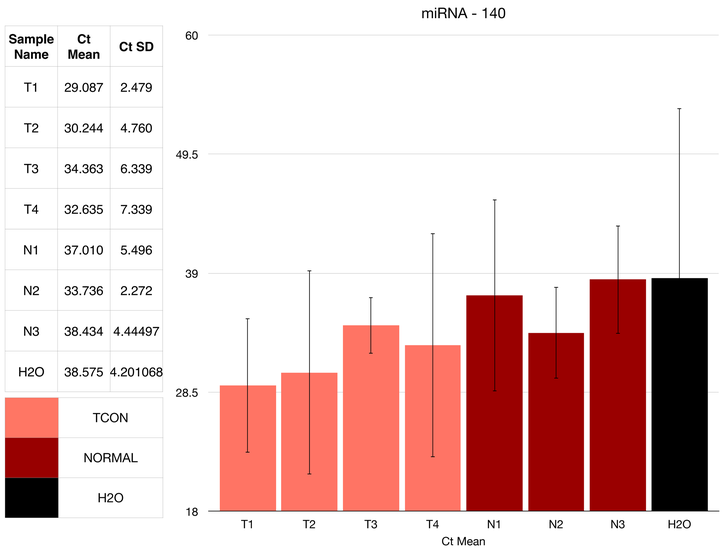


Figure 3: Analyzing the results for miRNA-140, a molecule that shows a reduced overall survival in gastric cancer patient as a result of downregulation.[9] As represented by the key, the peach bars represent the TCON mice, the red bars represent the normal mice, and the black bars represent the water sample used as a negative control. The results are most likely not statistically significant.

Sample Name	Ct Mean	Ct SD
T1	29.644	9.844
T2	27.788	14.963
T3	37.456	6.139115
T4	41	0
N1	31.621	16.24444
N2	37.970	5.248807
N3	35.545	9.448981
H2O	34.962	10.45848

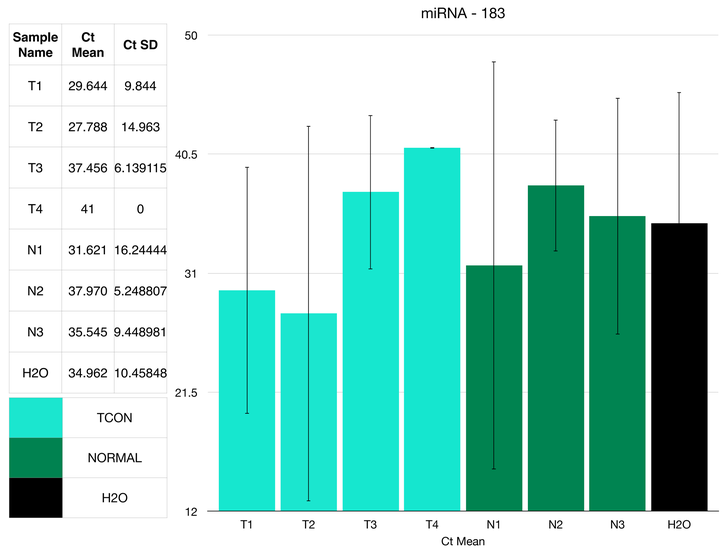


Figure 4: Analyzing the results for miRNA-183, a molecule that acts as a tumor suppressor in GC by partial regulation of Ezrin.[10] As represented by the key, the teal bars represent the TCON mice, the dark green bars represent the normal mice, and the black bars represent the water sample used as a negative control. The results are most likely not statistically significant.

CONCLUSION

1. Results likely aren't statistically significant but there may be biological relevance in some of the experimental samples.
2. Different species may have different miRNA biomarker potential — not as feasible in mice.
3. Future tests need to be conducted to confirm the usability of miRNAs as gastric cancer biomarkers. Another miRNA variant may produce more significant results.

FUTURE DIRECTIONS

1. Add a positive control

We can better identify the changes that are presented in the miRNA levels by comparing the experimental results to a known result.

2. Retest the samples used

Further validating the biomarker potential can provide insight to the possibilities of this experiment.

3. Conduct further research

Identifying if there are better matched miRNAs between humans and mice will allow us to establish a better parallel.

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