Impact of Circadian Misalignment on Extracellular Vesicles in Inactive Ulcerative Colitis

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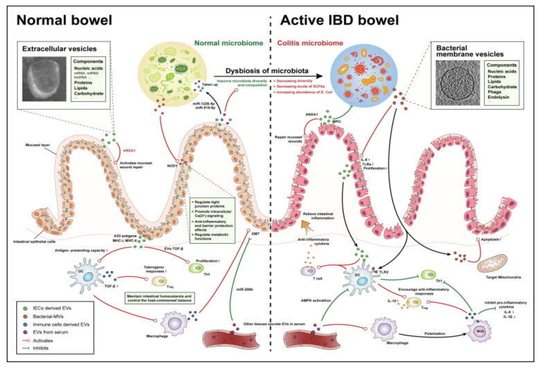
Introduction

Research Objective:

To investigate the relationship between exosomes and circadian misalignment in healthy controls and inactive ulcerative colitis subjects.

There has been recent evidence that patients with Inflammatory bowel disease (IBD), including ulcerative colitis (UC) have higher levels of circulating extracellular vesicles (EVs).[1]



Shen, Q., Huang, Z., Yao, J., & Jin, Y. (2022). Extracellular vesicles-mediated interaction within intestinal microenvironment in inflammatory bowel disease. *Journal of Advanced Research, 37*.

Figure 1: The EVs-mediated interaction in intestinal microenvironment.

To date, few studies have examined the relationship between EVs and circadian misalignment in subjects with UC.

Modern 24-hour society has made circadian misalignment extremely common, with higher incidences of night shift work.[2]

EV markers of interest:

- **GPA33:**
  - Gut specific to intestinal epithelium
  - High presence in CRC[3]
- **Tsg101:**
  - Total marker of endosomal sorting complex
  - High presence in CRC[4]

Central Hypothesis

Plasma EVs are regulated by the circadian clock.

Approach

To test this hypothesis, circulating EVs in UC subjects at baseline and after 3 days of a night shift simulated protocol were analyzed. Our goal is to establish if EVs are regulated by the circadian clock in UC.

Experimental Design

Disease Type	UC	HC
Number of participants	4	5
Age Range (years)	21-41	19-35
Gender		
Male	1/4, 25%	4/5, 80%
Female	3/4, 75%	1/5, 20%
Race		
African American	2/4, 50%	1/5, 20%
Hispanic or Latino	1/4, 25%	1/5, 20%
Caucasian	1/4, 25%	2/5, 40%
Other	0/4, 20%	1/5, 20%

UC, ulcerative colitis; HC, healthy control

Figure 2: Recruited subject demographics.

All subjects were screened with inclusion and exclusion criteria provided written informed consent at the time of recruitment.

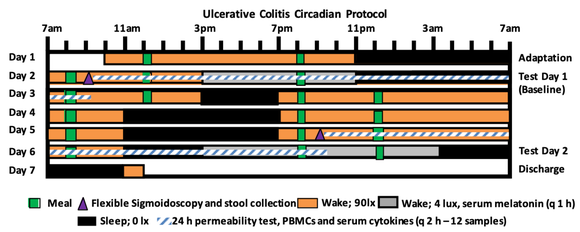


Figure 3: In lab 7-day circadian protocol.

The protocol includes a seven-lab day stay with an adaptation period, followed by a baseline blood draw every 2 hours over 24 hours (Test Day 1). This is followed by three days of simulated night shifts with a repeat blood draw every 2 hours over 24 hours (Test Day 2).

Specific Aim 1:

- **Hypothesis:** UC subjects with have increased disruption in exosome oscillation relative to healthy controls.
- **Approach:** Extract exosomes from blood samples taken during night shift protocol for use in western blots to determine oscillation of exosomes.

Specific Aim 2:

- **Hypothesis:** Both UC and HC subjects will demonstrate an increase in circulating exosomes after the simulated night shift when compared to baseline.
- **Approach:** Extract exosomes from blood samples taken during night shift protocol for use in western blots to determine GPA33 and tsg101 density.

Methods

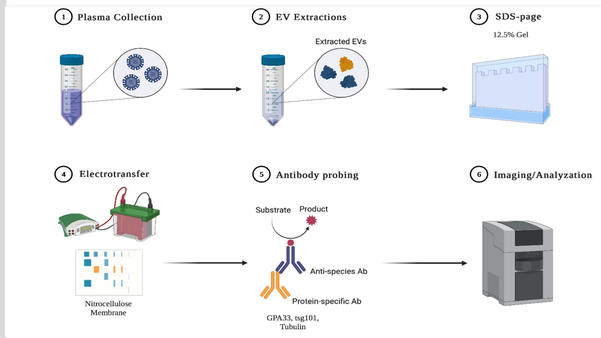


Figure 4: Overall project methodology.

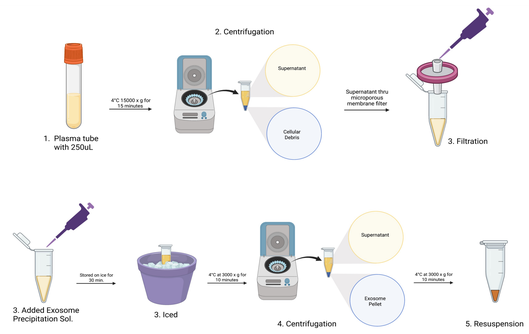


Figure 5: Methods for extracellular vesicle extractions.

Results

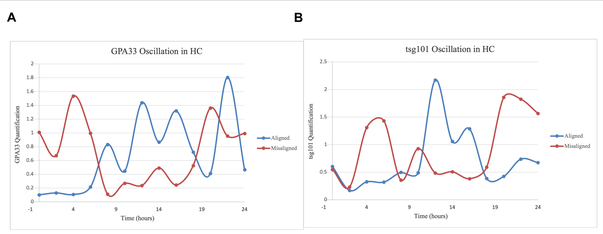


Figure 6: (A) Analysis of circadian oscillation in GPA33, **(B)** and tsg101 in a representative HC subject.

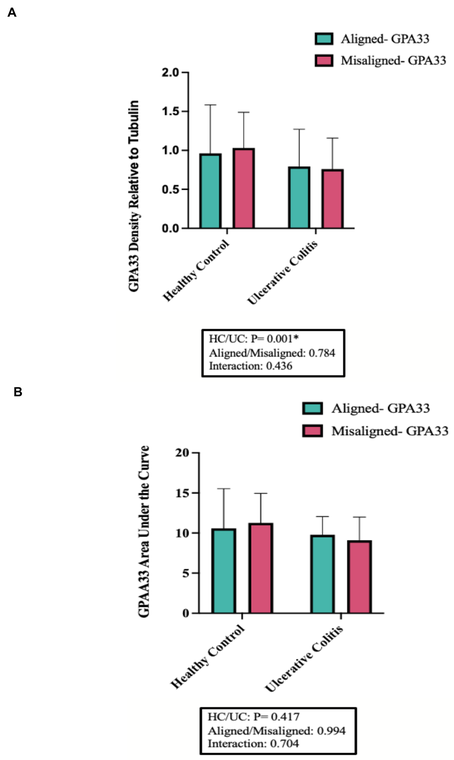


Figure 7: (A) Band density analysis of GPA33. **(B)** Area under the curve analysis of GPA33.

Results

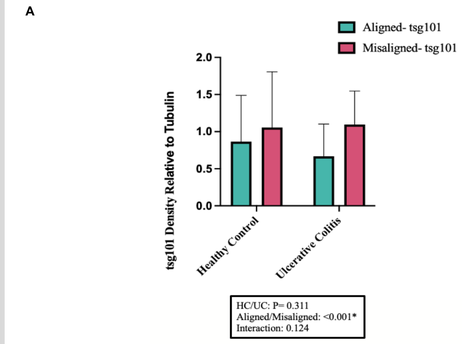


Figure 8: (A) Band density analysis of tsg101. **(B)** Area under the curve analysis of tsg101.

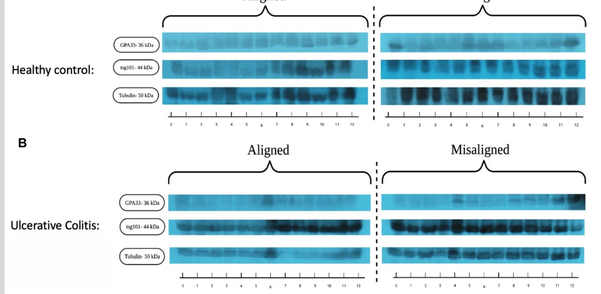


Figure 9: (A) Observed western blots obtained from a representative HC subject. **(B)** Observed western blots obtained from a representative UC subject.

Discussion

- EVs do not undergo circadian oscillation in HC and UC, regardless of condition
- UC subjects have less circulating GPA33 than HC
- Misalignment increases circulating tsg101 in both HC and UC

Future Directions

- Proteomics to further examine extracellular vesicle cargo and other components (bacterial, etc)
- Larger sample size
- Quality control runs

Acknowledgements

The CIMCR lab and lab faculty.

References

1. Shen, Q., Huang, Z., Yao, J., & Jin, Y. (2022). Extracellular vesicles-mediated interaction within intestinal microenvironment in inflammatory bowel disease. *Journal of Advanced Research, 37*.
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