hsa-mir-335: Improving Diagnosis and Treatment in Uterine Cancer Patients

Elizabeth Miller, Amber Hoskins, Riyaz Basha PhD

University of North Texas Health Texas College of Osteopathic Medicine

Abstract/ Background Information

•MicroRNAs, miRNAs, are small non-coding RNAs that function in post-transcriptional gene regulation. Thus, their dysfunction can play a key role in cancer development and progression. MiRNAs can be used to evaluate prognosis in cancer types, including OV (Ovarian Serous Cystadenocarcinoma), UCEC (Uterine Corpus Endometrial Carcinoma) and UCS (Uterine Carcinosarcoma).

•Cancer of the uterine corpus represents the most common gynecologic cancer and is the fourth most common malignancy in women worldwide. Endometrial cancer (more precisely, uterine corpus endometrial carcinoma, or UCEC) is associated with unfavorable prognosis with incidence and mortality rates rising quickly (11).

•As of 2025, approximately 420,368 women are diagnosed with an estimated 97,723 deaths each year due to UCEC (11). Prognostic UCEC biomarkers are needed to improve pre- and postoperative risk stratification and therapeutic targets (12).

•MicroRNAs are gaining attention as circulating biomarkers for various malignancies, including ovarian cancer. [4]

Methods

- Data was obtained from The Cancer Genome Atlas (TCGA) via UALCAN: a portal for analyzing tumor genes and survival curves.
- A comparison was made between the common miRNA-coding genes in OV vs UCS, OV vs UCEC and UCS vs UCEC. Survival curves were obtained for OV vs UCEC.
- Clinical trials were identified using clinicaltrials.gov
- All gene data came from UALCAN and statistical significance was determined using student's t-test (fortwo-group comparisons) or one-way ANOVA (for multiple-group comparisons), and survival curves were analyzed using Kaplan–Meier method with log-rank test. A p-value of 0.05 was used.[3]

Comparison of Shared OV and UCEC Statistically Significant miRNA

OV and UCEC shared miRNA	OV P-value	UCEC P-value
hsa-mir-4709	0.0158075	0.0488707
hsa-mir-335	0.0265661	**7.40E-06**
hsa-mir-7110	0.0330461	0.037279
hsa-mir-149	0.0419972	0.0271775
hsa-mir-4674	0.0424127	0.0367826
hsa-mir-760	0.0435977	0.0092397

Figure 1: The lowest P-value, and therefore the most statistically significant miRNA for OV and UCEC was hsa-mir-335 at 7.40E-06.

Acknowledgements

- Data for this analysis was collected from The University of Alabama Cancer Data Analysis portal.
- This project was conducted under the guidance of Dr. Riyaz Basha at The University of North Texas Health

Results: Kaplan-Meier Survival Curve, Dataset and Tumor Grade Expression

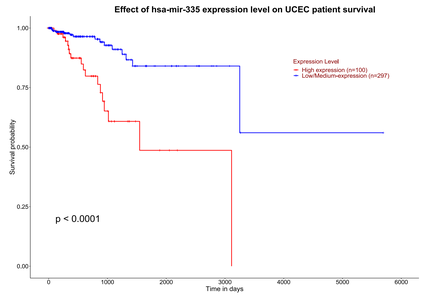

Figure 2: Kaplan-Meier Survival Curve depicts difference in patient survival based on High expression and Low/Medium expression of UCEC hsa-mir-335. High expression of hsa-mir-335 depicts a shorter survival period versus low/medium expression of hsa-mir-335 for UCEC patients.

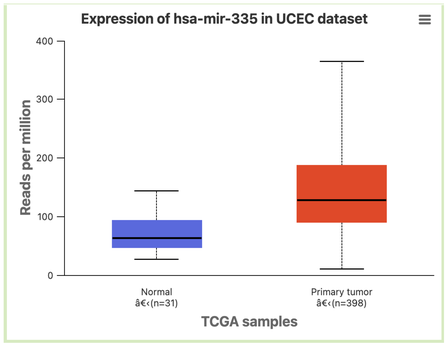

Figure 3: Box-and-whisker plot shows expression levels of hsa-mir-335 in UCEC dataset (normal versus primary tumor). The plot depicts higher expression of hsa-mir-335 in UCEC primary tumors versus normal UCEC with statistical significance of 1.62714286489063E-12.

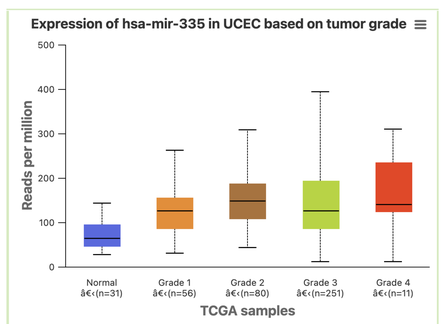

Figure 4: Box-and-whisker plot depict differences in expression of hsa-mir-335 in normal uterine corpus endometrial lining versus UCEC Grade 1-4. Expression of hsa-mir-335 increases as tumor grade increases, with the most statistically significant comparison between normal and Grade 3 at 3.18369774987559E-11

Relevance to Clinical Trials and Mechanism Target

- A current clinical trial (ClinicalTrials.gov ID NCT03776630) is exploring miRNAs to personalize therapy of gynecologic tumors such as ovarian and endometrial cancers. Estimated completion in 2027 is validating a five-miRNA signature measured in plasma to predict lymph node metastasis in endometrial and ovarian cancers. MicroRNAs are at the center of both the biomarker panel and the trial's primary goal (9).
- The mechanism for hsa-miR-335 is also of interest. Xenograft mouse model studies identified hsa-miR-335 targeting RBM10. High levels of hsa-miR-335 in tumors downregulates RBM10 and upregulates tumor-promoting Numb-L levels (10).

Conclusions/Discussions

- **Figure 1: Comparative miRNA analysis** identifies hsa-miR-335 as a shared prognostic miRNA between Ovarian Serous Cystadenocarcinoma (OV) and Uterine Endometrial Cancers (UCEC). The p-value for OV is 0.0265661 and p-value for UCEC is 7.40E-06. We chose to focus on UCEC hsa-miR-335 as a potential UCEC biomarker due to its strong statistical significance (p-value 7.40E-06) and additional statistically significant UALCAN GEx profile data. Kaplan-Meier Survival Curve, Sample type and Tumor grade were obtained from the UALCAN data set. The lowest P-value, and therefore the most statistically significant miRNA for OV and UCEC was hsa-mir-335 at 7.40E-06.
- **Figure 2: Effect of UCEC hsa-mir-335 expression on patient survival:** High expression of hsa-mir-335 depicts a shorter survival period for UCEC patients compared to low/medium expression. The steeper the curve, the fewer days these patients survive. The p-value of <0.0001 shows the difference in survival timelines are not due to chance. The step-down curve of the Kaplan-Meier curve is a patient event (death or endpoint). The step-down curve of UCEC patient survival shows a bigger visible drop due to smaller sample size (n=100 for high expression) vs a smoother curve due to higher sample size (n=297 for low/medium expression). Therefore, high expression of hsa-mir-335 suggests this miRNA may behave as an oncogenic miRNA in UCEC.
- **Figure 3: Box-and-whisker plot shows expression levels of hsa-mir-335 in UCEC dataset (normal versus primary tumor).** The plot depicts a statistically significant higher expression of hsa-mir-335 in UCEC primary tumors versus normal uterine corpus endometrial tissue (P-value=1.6E-12). These findings suggest hsa-mir-335 is a potential prognostic marker biomarker for UCEC.
- **Figure 4: Box-and-whisker plot depict differences in expression of hsa-mir-335 in normal uterine corpus endometrial lining versus UCEC Grade 1-4.** Expression of hsa-mir-335 increases as tumor grade increases, with the most statistically significant comparison between normal and Grade 3 at 3.2E-11. These findings suggest increasing hsa-mir-335 levels is potentially associated with increasing UCEC tumor aggressiveness.
- Overall, the mechanism of hsa-mir-335 suppressing RBM10, which increases tumor-promoting Numb-L, is a potential therapeutic target for UCEC. Continuing to monitor clinical trials and oncology/ laboratory data is important for therapeutic application.

References

1. American Cancer Society. Cancer facts and figures. In: Society AGAC; 2008.
2. Zografos E, Zagouri F, Kalapanida D, et al. Prognostic role of microRNAs in breast cancer: Asystematic review. *Oncotarget*. 2019;10(67):7156-7178. Published 2019 Dec 24.doi:10.18632/oncotarget.27327
3. Chandrashekar DS, Bashel B, Balasubramanya SAH, et al. UALCAN: A Portal for FacilitatingTumor Subgroup Gene Expression and Survival Analyses. *Neoplasia*. 2017;19(8):649-658.doi:10.1016/j.neo.2017.05.002
4. Dejene SB, Ohman AW, Du W, et al. Defining fallopian tube-derived miRNA cancer signatures. *Cancer Med*. 2019;8(15):6709-6716. doi:10.1002/cam4.2416
5. Non-coding RNA in the Exosome of the Epithelia Ovarian Cancer. ClinicalTrials.gov. NCT03738319. Accessed September 1, 2025. https://clinicaltrials.gov/study/NCT03738319
6. GYNO-MIR: Plasma 5-miRNA Profile Predicting Lymph Node Metastasis in Endometrial and Ovarian Cancer. ClinicalTrials.gov. Identifier: NCT03776630. Accessed September 1, 2025. https://clinicaltrials.gov/study/NCT03776630
7. Luo SS, Liao XW, Zhu XD. Genome-wide analysis to identify a novel microRNA signature that predicts survival in patients with stomach adenocarcinoma. J Cancer. 2019 Oct 17;10(25):6298-6313. doi: 10.7150/jca.33250. PMID: 31772663; PMCID: PMC6856753.
8. Wang C, Jiang T. MicroRNA-335 represents an independent prognostic marker in cervical cancer. *Tumour Biol*. 2015;36(8):5825-5830. doi:10.1007/s13277-015-3252-2
9. **ClinicalTrials.gov**. *Exploring the potential of novel biomarkers based on plasma miRNAs for a better management of pelvic gynecologic tumors (GYNO-MIR)*. ClinicalTrials.gov Identifier: NCT03776630. Updated May 23, 2019. Accessed January 26, 2026. https://clinicaltrials.gov/study/NCT03776630
10. Dou XQ, Chen XJ, Zhou Q, Wen MX, Zhang SZ, Zhang SQ. miR-335 modulates Numb alternative splicing via targeting RBM10 in endometrial cancer. *Kaohsiung J Med Sci*. 2020;36(3):171-177. doi:10.1002/kjm2.12149
11. An Y, Feng Q, Jia L, et al. Present progress in biomarker discovery of endometrial cancer by multi-omics approaches. *Clin Proteomics*. 2025;22(1):15. Published 2025 Apr 26. doi:10.1186/s12014-025-09528-6
12. Coll-de la Rubia E, Martinez-Garcia E, Dittmar G, Gil-Moreno A, Cabrera S, Colas E. Prognostic Biomarkers in Endometrial Cancer: A Systematic Review and Meta-Analysis. *J Clin Med*. 2020;9(6):1900. Published 2020 Jun 17. doi:10.3390/jcm9061900