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ANALYSIS OF miRNA EXPRESSION STRATIFIED BY SEX IN PATIENTS WITH LUNG ADENOCARCINOMA

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ABSTRACT: Introduction: Sex-related biological differences may contribute to the molecular heterogeneity of LUAD, including variations in miRNA expression. **Objective:** To identify sex-specific miRNAs associated with LUAD and evaluate their associations with patient survival and enriched biological pathways. **Methods:** miRNA expression and clinical data from *The Cancer Genome Atlas* were analyzed separately for male and female patients. Differential expression, survival, and pathway enrichment analyses were performed. **Results:** A total of 130 differentially expressed miRNAs were identified in males and 208 in females. Two miRNAs were associated with survival in the male group and eight in the female group. Pathway enrichment analysis revealed distinct functional profiles between the groups. **Conclusion:** These exploratory findings indicate sex-related differences in miRNA expression patterns and their associations with survival and biological pathways in LUAD, providing a basis for future studies investigating their potential prognostic relevance.

Keywords: Biostatistics; Lung cancer; Survival Analysis; Pathway enrichment; Biomarkers.

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Análise da Expressão de miRNAS Estratificada por Sexo em Pacientes com Adenocarcinoma Pulmonar

RESUMO: Introdução: Diferenças biológicas relacionadas ao sexo podem contribuir para a heterogeneidade molecular do LUAD, incluindo variações na expressão de miRNAs. **Objetivo**: Identificar miRNAs associados ao LUAD de acordo com o sexo e avaliar suas associações com a sobrevida e vias biológicas enriquecidas. **Métodos**: Dados de expressão de miRNAs e dados clínicos do *The Cancer Genome Atlas* foram analisados separadamente para homens e mulheres. Foram realizadas análises de expressão diferencial, sobrevida e enriquecimento de vias biológicas. **Resultados**: Foram identificados 130 miRNAs diferencialmente expressos em homens e 208 em mulheres. Dois miRNAs foram associados à sobrevida no grupo masculino e oito no feminino. A análise de enriquecimento revelou perfis funcionais distintos entre os grupos. **Conclusão**: Os achados exploratórios indicam diferenças relacionadas ao sexo nos perfis de miRNAs e em suas associações com a sobrevida e vias biológicas no LUAD, fornecendo subsídios para futuras investigações sobre seu potencial prognóstico.

Palavras-chave: Bioestatística; Câncer de pulmão; Análise de sobrevivência; Enriquecimento de vias biológicas; Biomarcadores.

Análisis de la Expresión de MicroARN Estratificada por Sexo en Pacientes con Adenocarcinoma Pulmonar

RESUMEN: Introducción: Las diferencias biológicas relacionadas con el sexo pueden contribuir a la heterogeneidad molecular del LUAD, incluidas las variaciones en la expresión de miARN. **Objetivo:** Identificar miARN asociados al LUAD según el sexo y evaluar su asociación con la supervivencia de los pacientes y las vías biológicas enriquecidas. **Métodos:** Se analizaron por separado datos de expresión de miARN y datos clínicos del *The Cancer Genome Atlas* (TCGA) para hombres y mujeres. Se realizaron análisis de expresión diferencial, supervivencia y enriquecimiento de vías biológicas. **Resultados:** Se identificaron 130 miARN diferencialmente expresados en hombres y 208 en mujeres. Dos miARN se asociaron con la supervivencia en el grupo masculino y ocho en el grupo femenino. El análisis de enriquecimiento reveló perfiles funcionales distintos entre ambos grupos. **Conclusión:** Estos hallazgos exploratorios indican diferencias relacionadas con el sexo en los patrones de expresión de miARN y en sus asociaciones con la supervivencia y las vías biológicas en el LUAD, proporcionando una base para futuras investigaciones sobre su posible relevancia pronóstica.

Palabras clave: Bioestadística; Cáncer de pulmón; Análisis de supervivencia; Enriquecimiento de vías biológicas; Biomarcadores.

INTRODUCTION

Lung adenocarcinoma (LUAD) is the most common histological subtype of non-small cell lung cancer (NSCLC), which accounts for approximately 85% of lung cancer cases, with LUAD representing approximately 40% of all lung cancers (Zappa et al., 2016; Myers et al., 2022). Despite advances in diagnosis and treatment, LUAD remains associated with high mortality, largely because nonspecific symptoms frequently delay diagnosis and limit therapeutic options (Imielinski et al., 2012). Consequently, the identification of molecular biomarkers capable of capturing tumor heterogeneity has become an important strategy for improving prognostic assessment and supporting personalized disease management.

Biological sex is an important source of variability in cancer and may influence disease incidence, prognosis, molecular mechanisms, and response to therapy through complex interactions involving genetic, epigenetic, hormonal, and immune-related factors (Lopes-Ramos et al., 2020). In lung cancer, men generally present higher incidence and mortality rates, whereas women tend to have more favorable clinical outcomes despite the increasing incidence observed in recent decades (May et al., 2023; Fu et al., 2023; Poleri, 2022; Ragavan and Patel, 2022; Stabellini et al., 2022).

Increasing evidence indicates that sex-related differences also extend to the molecular level. Distinct patterns of genetic alterations, gene expression, immune regulation, and gene regulatory networks have been reported in LUAD (Li et al., 2023; Saha et al., 2024). Furthermore, sex-specific transcriptomic models have shown improved performance over sex-independent approaches for identifying early-stage lung cancer biomarkers, suggesting that analyses combining male and female patients may overlook biologically relevant molecular signatures (Liu et al., 2021).

MicroRNAs (miRNAs) are small RNA molecules (~22 nucleotides) that regulate gene expression and participate in key cancer-related processes, including proliferation, metabolism, differentiation, and cell-cycle control (Griffiths-Jones, 2004; Jansson et al., 2012). Their expression may also differ between males and females due to interactions involving sex chromosomes, hormones, and epigenetic regulation (Caserta et al., 2023). Sex-associated miRNA expression patterns have been reported in cancer, supporting their potential contribution to biological differences between male and female patients (Haranguș et al., 2022; Caserta et al., 2023).

Despite growing interest in miRNAs as potential biomarkers in LUAD, the integration of sex-stratified differential expression, survival analysis, and biological pathway enrichment remains comparatively underexplored. Investigating sex-specific miRNA profiles may therefore contribute to a better understanding of the molecular heterogeneity of LUAD and provide a basis for future studies evaluating their potential prognostic relevance.

Therefore, this exploratory study aimed to identify sex-specific miRNAs associated with lung adenocarcinoma and evaluate their associations with patient survival and enriched biological pathways. By integrating differential expression, survival analysis, and functional enrichment separately in male and female patients, this study seeks to characterize sex-related molecular heterogeneity and generate hypotheses for future experimental and clinical investigations.

MATERIALS AND METHODS

Study design and TCGA dataset

This exploratory retrospective study used publicly available miRNA expression and clinical data from the TCGA Lung Adenocarcinoma (TCGA-LUAD) project, obtained from the Genomic Data Commons (GDC) Data Portal (https://portal.gdc.cancer.gov/; accessed in June 2022) (TCGA, 2018). The dataset comprised 553 patients and expression data for 709 miRNAs. Patients with available sex information and miRNA expression data were included and stratified into male (LUAD-M; n = 259) and female (LUAD-F; n = 294) groups.

Clinical variables included sex, tissue type, tumor stage, vital status, ethnicity, histological classification, tissue or organ of origin, age, and follow-up time. Tumor stage was grouped into early (I–IIB) and advanced (IIIA–IV) stages, whereas missing information was retained as not reported.

As analyses were based on processed TCGA/GDC data, no additional quality control of raw sequencing reads was performed. Data consistency and availability were verified before each analysis, which was conducted using the available observations. Analyses were performed in R (v. 4.1.1).

Differential expression, pathway enrichment and survival analyses

Differential expression analysis was performed separately for LUAD-M and LUAD-F by comparing tumor (T) and histologically normal adjacent tissues (N) using the Wilcoxon–Mann–Whitney test implemented through the wilcox.test() function in R. This nonparametric test was selected because it does not assume normality of miRNA expression distributions.

To account for multiple testing, the Bonferroni correction was applied (Weisstein, 2004), resulting in a significance threshold of $p < 0.00007$ (0.05/709). MiRNAs meeting this criterion were considered differentially expressed.

Functional enrichment analysis was performed using miEAA 2.1 (Aparicio-Puerta et al., 2023). Enriched KEGG pathways with $p < 0.05$ were retained for exploratory interpretation without additional multiple-testing correction.

Survival analysis was performed separately for LUAD-M and LUAD-F using follow-up time (days) and vital status. For each DE-miRNA, patients were dichotomized into high- and low-expression groups according to the sex-specific median expression value. The median was adopted as an exploratory, data-driven cutoff because no clinically established threshold was available. Survival curves were estimated using the Kaplan–Meier method (Kaplan and Meier, 1958) and compared using the log-rank test (Mantel, 1966), considering $p < 0.05$. Owing to the exploratory nature of the analyses, significant associations were interpreted as potentially prognostic rather than clinically validated biomarkers.

Analyses were performed in R (v. 4.1.1) using the survival (Therneau, 2022), survminer (Kassambara et al., 2021), stringi (Gagolewski, 2022), and ggplot2 (Wickham, 2009) packages.

RESULTS AND DISCUSSION

Clinical characteristics

The TCGA-LUAD cohort comprised 553 patients, including 294 females and 259 males. Most samples corresponded to tumor tissue, and early-stage disease predominated in both sex-stratified datasets. Mean age was similar between females (65.48 ± 10.23 years) and males (65.15 ± 9.74 years), with median follow-up times of 652 and 620.5 days, respectively. Significant associations with tissue type were observed for ethnicity in both datasets (LUAD-F, $p < 0.001$; LUAD-M, $p = 0.047$) and for vital status in LUAD-F ($p < 0.001$), whereas no significant associations were observed for organ of origin, tumor stage, or histological subtype (Table 1).

Table 1. Associations between clinical characteristics and tissue type in the sex-stratified TCGA-LUAD datasets.

Variable	LUAD-F p-value	LUAD-M p-value
Vital status	<0.001	0.78
Ethnicity	<0.001	0.047
Organ of origin	0.956	0.77
Tumor stage	0.566	0.70
Histological subtype	0.305	0.68

Pearson's chi-square test; Fisher's exact test was applied when appropriate.

Sex-stratified mirna analyses

Differential expression analysis identified 130 DE-miRNAs in LUAD-M, including 100 overexpressed and 30 underexpressed miRNAs, and 208 DE-miRNAs in LUAD-F, including 191 overexpressed and 17 underexpressed miRNAs. Among these, 98 DE-miRNAs were shared between sexes, whereas 32 were exclusive to LUAD-M and 110 were exclusive to LUAD-F (Figure 1A).

Among the ten DE-miRNAs with the lowest p-values in each dataset, five were shared between sexes (miR-21-5p, miR-21-3p, miR-210-3p, miR-301a-5p, and miR-409-5p), while five were sex-specific in each group (Figure 1B and Table 2). Most top-ranked DE-miRNAs were overexpressed in tumor tissue; miR-490-3p and miR-144-5p were the only underexpressed miRNAs among the top ten in LUAD-M.

Table 2. Top ten differentially expressed miRNAs according to statistical significance in LUAD-M and LUAD-F.

LUAD-M			LUAD-F		
DE-miRNAs	Medians		DE-miRNAs	Medians	
	N	T		N	T
miR-210-3p↑	68868	167346	miR-21-5p↑	492287	746722
miR-21-5p↑	21.3	740498	miR-183-5p↑	346410	632521
miR-1287-3p↑	1.5	41604	miR-21-3p↑	189753	375476
miR-301a-5p↑	1	36243	miR-210-3p↑	51215	170472
miR-21-3p↑	214660	386108	miR-182-5p↑	327483	581772
miR-409-5p↑	2	38986	miR-301a-5p↑	1	37113
miR-3607-3p↑	17630	119669	miR-33b-5p↑	1	37114
miR-616-5p↑	1	32540	miR-183-3p↑	0	19932
miR-490-3p↓	17628	0	miR-29b-1-5p↑	4	66913
miR-144-5p↓	242166	101718	miR-409-5p↑	1	36236

↑ and ↓ represent overexpression and underexpression, respectively.

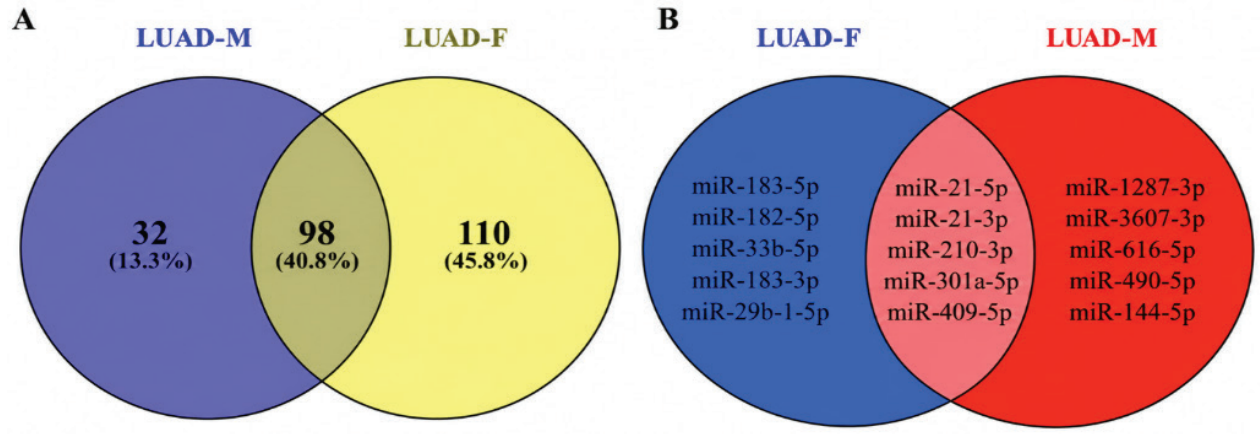

Figure 1. Comparison of differentially expressed miRNAs between LUAD-M and LUAD-F. (A) Venn diagram showing the overlap of DE-miRNAs identified in LUAD-M and LUAD-F. (B) Venn diagram showing the overlap among the ten DE-miRNAs with the lowest p-values in each sex-stratified dataset.

Pathway enrichment analysis identified 14 enriched pathways in LUAD-M and 26 in LUAD-F. Despite 98 DE-miRNAs being shared between the sex-stratified datasets, no enriched KEGG pathway was common to both groups, indicating distinct functional profiles.

Among the enriched pathways, representative cancer-related pathways were summarized according to their biological relevance to LUAD and the main functional processes identified in each sex-stratified dataset (Table 3). Complete enrichment results are provided in Supplementary Tables S1 and S2.

In LUAD-M, representative pathways included glycolysis/gluconeogenesis, the pentose phosphate pathway, proteoglycans in cancer, and HIF-1 signaling, highlighting processes associated with metabolic adaptation, extracellular matrix regulation, and hypoxia. In LUAD-F, representative pathways included Wnt signaling, ubiquitin-mediated proteolysis, focal adhesion, and NF-kappa B

signaling, involving cell signaling, protein regulation, cell–matrix interactions, and inflammatory responses.

Table 3. Summary of representative enriched pathways identified in LUAD-M and LUAD-F.

Sex-stratified dataset	Representative enriched pathway	Observed miRNAs	p-value	Main biological process
LUAD-M	Glycolysis / Gluconeogenesis	48	0.012	Tumor metabolism
LUAD-M	Pentose phosphate pathway	24	0.018	Metabolic adaptation
LUAD-M	Proteoglycans in cancer	95	0.020	Extracellular matrix
LUAD-M	HIF-1 signaling pathway	86	0.032	Hypoxia and angiogenesis
LUAD-F	Wnt signaling pathway	148	0.007	Cell growth and signaling
LUAD-F	Ubiquitin-mediated proteolysis	141	0.011	Protein regulation
LUAD-F	Focal adhesion	163	0.037	Cell–matrix interaction
LUAD-F	NF-kappa B signaling pathway	124	0.047	Inflammation and survival

Survival analysis identified distinct DE-miRNAs associated with patient outcomes in the sex-stratified datasets (Table 4). In LUAD-M, miR-30a-3p ($p = 0.023$) and miR-429 ($p = 0.011$) showed significant differences between expression groups (Figure 2A). Higher miR-30a-3p expression was associated with a slightly higher survival probability, whereas lower miR-429 expression was associated with higher survival probability.

In LUAD-F, eight DE-miRNAs were significantly associated with survival: let-7a-2-3p ($p = 0.045$), miR-19b-1-5p ($p = 0.037$), miR-154-3p ($p = 0.020$), miR-218-1-5p ($p = 0.033$), miR-3136-5p ($p = 0.029$), miR-503-5p ($p = 0.024$), miR-425-5p ($p = 0.037$), and miR-671-5p ($p = 0.047$) (Figure 2B). Higher survival probabilities were observed in the low-expression groups for seven of the eight miRNAs, whereas miR-19b-1-5p showed the opposite pattern.

Table 4. DE-miRNAs significantly associated with survival outcomes according to sex.

Dataset	miRNA	Log-rank p-value	Expression group with higher survival probability
LUAD-M	miR-30a-3p	0.023	High expression
LUAD-M	miR-429	0.011	Low expression
LUAD-F	let-7a-2-3p	0.045	Low expression
LUAD-F	miR-19b-1-5p	0.037	High expression
LUAD-F	miR-154-3p	0.020	Low expression
LUAD-F	miR-218-1-5p	0.033	Low expression
LUAD-F	miR-3136-5p	0.029	Low expression
LUAD-F	miR-503-5p	0.024	Low expression
LUAD-F	miR-425-5p	0.037	Low expression
LUAD-F	miR-671-5p	0.047	Low expression

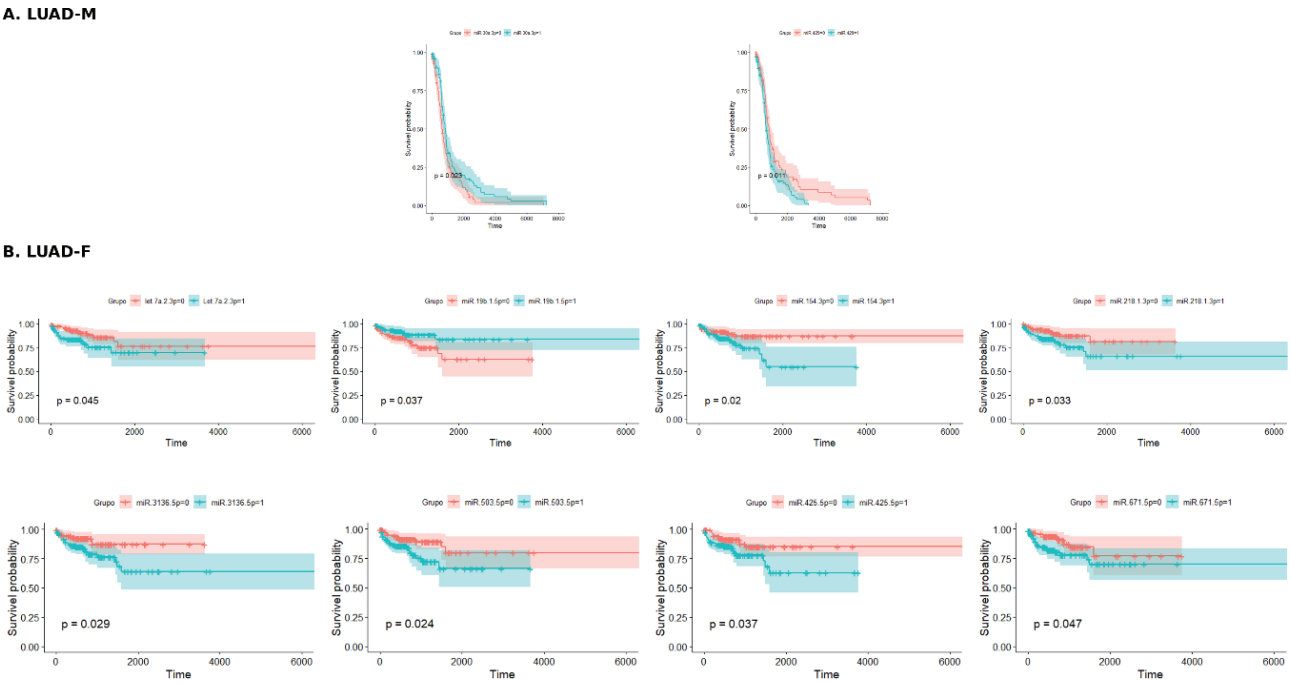

Figure 2. Kaplan–Meier curves showing sex-specific DE-miRNAs significantly associated with survival in LUAD-M (A) and LUAD-F (B). Source: Prepared by the authors (2026).

DISCUSSION

This exploratory study demonstrated that sex-stratified analyses reveal distinct molecular patterns in LUAD. Although several differentially expressed miRNAs were shared between male and female patients, their survival-associated miRNAs and enriched biological pathways differed substantially, supporting the concept that biological sex influences the molecular landscape of LUAD and reinforcing the importance of sex-aware biomarker discovery.

This interpretation is consistent with recent evidence demonstrating sex-related molecular differences in LUAD. Li et al. (2023) reported sex-biased patterns involving genetic alterations, gene expression, and immune characteristics, whereas Saha et al. (2024) identified differences in gene regulatory networks between male and female patients with LUAD. In addition, sex-specific transcriptomic models have shown improved performance compared with sex-independent approaches for identifying early-stage lung cancer biomarkers (Liu et al., 2021). Therefore, the greater number of female-specific DE-miRNAs identified in the present analysis should not be interpreted as evidence of greater molecular complexity in female tumors, but rather as an indication that the regulatory patterns captured by miRNA expression may differ between sex-stratified groups (Figure 3).

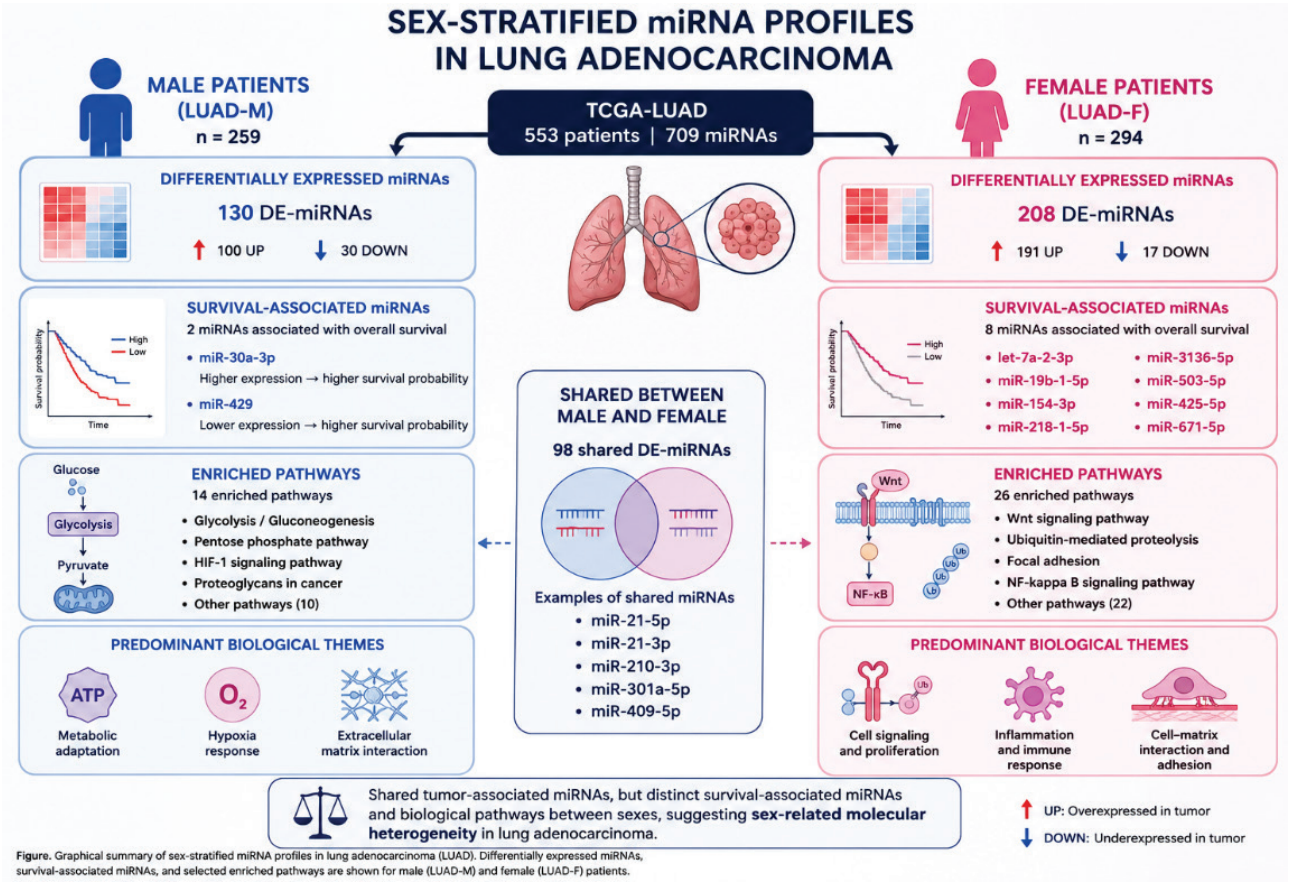

Figure 3. Graphical summary of sex-stratified miRNA profiles in lung adenocarcinoma. Differentially expressed miRNAs, survival-associated miRNAs, and selected enriched biological pathways are summarized for LUAD-M and LUAD-F. Source: Prepared by the authors using ChatGPT (OpenAI) as an AI-assisted graphical illustration.

Among the DE-miRNAs with the lowest p-values, miR-21-5p, miR-21-3p, miR-210-3p, miR-301a-5p, and miR-409-5p were shared between LUAD-M and LUAD-F. The presence of these miRNAs in both groups suggests that, despite sex-related differences, common regulatory mechanisms may participate in LUAD biology. In particular, miR-21 has been extensively associated with lung cancer progression. In the present study, miR-21-5p and miR-21-3p were overexpressed in tumor tissue in both sexes. Zhang et al. (2010) demonstrated that miR-21 can promote growth and invasion in NSCLC through regulation of the tumor suppressor PTEN, while Tang et al. (2021) described the involvement of the miR-21-5p/SMAD7 axis in lung cancer progression. More recently, Qin and Zhang (2025) reported significantly increased miR-21 expression in early-stage LUAD and observed an association between higher miR-21 expression and poorer survival. Their integrated analyses also implicated miR-21-related regulatory networks in proliferation, apoptosis, signal transduction, and tumor–immune interactions. Together, these findings reinforce the biological plausibility of miR-21 as one of the principal shared molecular signatures identified in both sex-stratified LUAD datasets.

Despite the overlap in individual DE-miRNAs, no enriched KEGG pathway was common to LUAD-M and LUAD-F. This apparent contrast is biologically relevant because individual miRNAs may regulate multiple target genes and participate in different regulatory contexts. Thus, partial overlap in miRNA expression does not necessarily imply equivalent functional consequences. Recent studies of sex-associated molecular regulation in LUAD have similarly suggested that

differences may emerge at the level of regulatory networks rather than through completely distinct sets of expressed molecules (Saha et al., 2024). Sex-related epigenetic differences have also been observed in lung tissue. Patel et al. (2024), for example, identified sex-specific DNA methylation patterns associated with gene expression and immune biomarkers and demonstrated that part of these alterations could also be detected in TCGA lung tumor datasets. These findings support the broader concept that sex-related tumor differences may reflect distinct regulatory architectures.

In LUAD-M, enriched pathways included glycolysis/gluconeogenesis, the pentose phosphate pathway, proteoglycans in cancer, and HIF-1 signaling. Metabolic reprogramming is a central characteristic of cancer cells and supports the energetic and biosynthetic demands required for tumor growth. Glycolytic and pentose phosphate pathways may contribute to energy production, nucleotide synthesis, and cellular adaptation to oxidative stress. The enrichment of HIF-1 signaling is also compatible with metabolic adaptation to hypoxic conditions, which may promote angiogenesis and tumor cell survival (Pezzuto and Carico, 2018). In parallel, proteoglycans can modulate extracellular matrix organization, cell adhesion, migration, and growth factor signaling (Iozzo and Sanderson, 2011). Together, these results suggest that the miRNA profile identified in male patients may be associated with metabolic adaptation, hypoxia, and tumor–microenvironment interactions. However, because enrichment analysis indicates statistical overrepresentation rather than direct pathway activation, these biological interpretations should be considered hypothesis-generating.

A different functional profile was observed in LUAD-F, with enrichment of pathways including Wnt signaling, ubiquitin-mediated proteolysis, focal adhesion, and NF-kappa B signaling. Wnt signaling participates in cell proliferation and differentiation and its dysregulation has been associated with tumor development (Teo and Kahn, 2010). Focal adhesion pathways regulate interactions between cells and the extracellular matrix and may influence migration and invasive behavior (Provenzano and Keely, 2009). NF-kappa B signaling integrates inflammatory and survival-related signals and can contribute to persistent cell proliferation and resistance to cell death (Hayden et al., 2006). The enrichment of these pathways in the female dataset suggests a regulatory profile involving cell signaling, protein regulation, inflammatory responses, and cell–matrix interactions. Importantly, these findings do not establish that such pathways are exclusively active in female LUAD; rather, they indicate that the DE-miRNA set identified in LUAD-F was preferentially associated with these biological processes.

The sex-stratified survival analysis further revealed different patterns between the two groups. In LUAD-M, miR-30a-3p and miR-429 were significantly associated with survival. Previous experimental evidence suggests that miR-30a-3p suppresses lung cancer progression through regulation of DNMT3A and the p38 MAPK pathway, supporting the biological plausibility of the association observed in male patients. (Wei et al., 2019). The present findings showed a slightly higher survival probability among male patients with higher miR-30a-3p expression, which is biologically compatible with a potential tumor-suppressive role. However, this association was based on Kaplan–Meier and log-rank analyses and should not be interpreted as evidence of an independent protective effect.

For miR-429, lower expression was associated with higher survival probability in LUAD-M. Xie et al. (2015) previously reported that a genetic variant in the miR-200b/miR-200a/miR-429 regulatory region was associated with NSCLC risk, while a variant related to miR-30a was associated with decreased disease risk. Although genetic variants and miRNA expression represent distinct

biological levels, the involvement of both miR-30a and the miR-429 cluster in previous lung cancer studies provides additional support for investigating these molecules. The present results extend this evidence by suggesting sex-stratified associations with survival in male LUAD patients.

In LUAD-F, eight miRNAs were associated with survival: let-7a-2-3p, miR-19b-1-5p, miR-154-3p, miR-218-1-5p, miR-3136-5p, miR-503-5p, miR-425-5p, and miR-671-5p. For seven of these miRNAs, lower expression was associated with higher survival probability, whereas miR-19b-1-5p showed the opposite pattern. The let-7 family is among the most established miRNA families in lung cancer and has been associated with regulation of oncogenic pathways and patient outcomes (Yanaihara et al., 2006). The association observed for let-7a-2-3p in the present study therefore reinforces the potential relevance of this family. Although its sex-specific role remains unclear, the present findings further support investigation of this miRNA family in LUAD.

The identification of miR-3136-5p is also noteworthy. Previous integrative analyses of LUAD have associated this miRNA with patient survival, and functional screening has suggested that modulation of miR-3136-5p may affect LUAD cell growth. In our analysis, lower miR-3136-5p expression was associated with higher survival probability in female patients. This result differs from simplistic classification of a miRNA as exclusively oncogenic or tumor suppressive and highlights the importance of biological context, including tissue characteristics, target availability, and potentially sex-related regulatory mechanisms. Accordingly, the present results should be viewed as evidence of association rather than direct demonstration of causality.

An important observation from this study is the numerical imbalance between survival-associated miRNAs identified in males and females. Two miRNAs were identified in LUAD-M compared with eight in LUAD-F. This difference may reflect distinct miRNA–target regulatory structures, but alternative explanations must also be considered, including differences in event frequency, follow-up patterns, clinical heterogeneity, and statistical power between the sex-stratified datasets. Therefore, the larger number of significant associations in female patients should not be interpreted as direct evidence of stronger prognostic regulation by miRNAs. Instead, it reinforces the need for sex-aware analytical designs and independent validation of the observed patterns.

From a biological perspective, the combination of differential expression, pathway enrichment, and survival analysis suggests that sex-related heterogeneity in LUAD may involve both shared tumor-associated miRNAs and distinct regulatory contexts. The shared overexpression of miRNAs such as miR-21-5p and miR-210-3p may reflect common components of LUAD biology, whereas the different enrichment profiles and survival-associated miRNAs suggest that their downstream biological consequences may vary between male and female patient groups. This interpretation is consistent with molecular studies indicating that sex-associated differences in LUAD can involve genetic, transcriptomic, immune, and regulatory network features (Li et al., 2023; Saha et al., 2024).

The potential clinical implications of these findings should be interpreted cautiously. The miRNAs identified in this study cannot be considered clinically validated diagnostic or prognostic biomarkers. However, the observation that different miRNAs were associated with survival in male and female patients suggests that analyses performed in combined populations may overlook potentially relevant subgroup-specific associations. After validation in independent cohorts, sex-stratified miRNA profiles could be investigated as complementary molecular features in prognostic

studies. Likewise, the distinct enriched pathways may guide future mechanistic studies exploring whether sex-related regulatory differences contribute to LUAD heterogeneity. These possibilities remain investigational and require substantial experimental and clinical validation.

Several limitations should be acknowledged. First, this retrospective exploratory study was based on data from a single public project, TCGA-LUAD, and the findings were not validated in an independent external cohort. Second, the analyses relied on processed TCGA/GDC data, and no additional quality control of raw sequencing reads or correction for potential batch effects was performed. Third, the survival analyses used median expression as a data-driven cutoff and log-rank comparisons; therefore, the results demonstrate unadjusted associations and do not establish the independent prognostic value of the identified miRNAs. Potential confounding factors, including age, tumor stage, smoking history, treatment, molecular alterations, and other clinical characteristics, were not incorporated into multivariable Cox regression models. The use of a single cohort and multiple survival comparisons may also increase the possibility of false-positive findings, particularly because no additional multiple-testing correction was applied to the exploratory survival analyses. In addition, pathway enrichment was based on statistical overrepresentation of miRNA-associated functional sets and does not demonstrate direct pathway activation or causal biological mechanisms. Finally, no experimental validation was performed in cell lines, tumor tissues, circulating samples, or animal models.

Future studies should validate the identified miRNAs in independent and preferably multicenter LUAD cohorts, maintaining sex-stratified analyses. Cox proportional hazards models incorporating relevant clinical covariates and external validation should be used to evaluate whether the identified miRNAs provide independent prognostic information. The reproducibility of the expression cutoffs should also be examined instead of assuming that cohort-specific median values are directly transferable to other populations. Integrative analyses combining miRNA expression with mRNA targets, genomic alterations, DNA methylation, immune profiles, and hormonal variables may help clarify the regulatory mechanisms underlying the observed sex-related patterns. Functional experiments should investigate the effects of selected miRNAs, particularly miR-30a-3p and miR-429 in male-derived LUAD models and the survival-associated miRNAs identified in female patients. Recent studies combining molecular, clinical, and single-cell approaches illustrate the value of multi-layer validation for characterizing miRNA-related mechanisms in LUAD.

In summary, this study identified sex-stratified differences in miRNA expression, survival associations, and enriched biological pathways in LUAD. The results suggest the coexistence of shared tumor-associated miRNAs and distinct sex-related regulatory profiles. Rather than establishing clinically applicable biomarkers, these findings provide a hypothesis-generating framework for further investigation of biological sex as a relevant variable in miRNA-based LUAD research.

CONCLUSION

This exploratory study demonstrated that sex-stratified analyses reveal distinct miRNA expression profiles, survival-associated miRNAs, and enriched biological pathways in lung adenocarcinoma. Although several miRNAs were shared between male and female patients, the observed differences in survival associations and functional enrichment suggest that biological

sex influences the molecular landscape of LUAD. These findings support the inclusion of sex as an important biological variable in miRNA-based studies and provide a hypothesis-generating framework for future investigations. Independent validation, multivariable prognostic analyses, and experimental studies will be essential to determine the biological and clinical relevance of the identified miRNAs and pathways.

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AI USE STATEMENT

No specific declaration regarding the use of artificial intelligence tools in the preparation of this manuscript was provided. Responsibility for the accuracy, integrity, originality, and entire content of the article, including any materials potentially produced or revised with the assistance of artificial intelligence tools, rests solely with the authors.

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Supplementary Table S1. Complete list of enriched KEGG pathways in LUAD-M.

KEGG pathway	Observed*	p-value
Glycolysis / Gluconeogenesis	48	0.012
Other types of O-glycan biosynthesis	34	0.014
Pentose phosphate pathway	24	0.018
Proteoglycans in cancer	95	0.020
Apelin signaling pathway	82	0.024
Protein processing in the endoplasmic reticulum	97	0.026
Pathways in cancer	117	0.029
B cell receptor signaling pathway	65	0.031
HIF-1 signaling pathway	86	0.032
Pyruvate metabolism	39	0.032
Cytokine–cytokine receptor interaction	88	0.039
Colorectal cancer	88	0.041
Valine, leucine and isoleucine biosynthesis	3	0.047
Primary immunodeficiency	22	0.048

*Observed: Number of miRNAs enriched in the pathway.

Supplementary Table S2. Complete list of enriched KEGG pathways in LUAD-F.

KEGG pathways	Observed*	p-value
Basal cell carcinoma	102	0.002
Axon guidance	149	0.006
Wnt signaling pathway	148	0.007
Alcoholic liver disease	137	0.007
Ubiquitin-mediated proteolysis	141	0.011
Tight junction	142	0.012
Virion – Adenovirus	8	0.015
Pancreatic câncer	150	0.020
Basic transcription factors	48	0.023
Inositol phosphate metabolismo	97	0.026
Cushing syndrome	154	0.027
Neomycin, kanamycin and gentamicin biosynthesis	6	0.028
Gastric câncer	157	0.028
Translation of flavor	38	0.033
Taurine and hypotaurine metabolismo	8	0.034
Focal adhesion	163	0.037
Virion – Lyssavirus	7	0.038
Longevity regulating pathway – multiple species	136	0.038
Thyroid hormone synthesis	90	0.038
Sulfur relay system	18	0.040
C-type lectin receptor signaling pathway	128	0.041
Signaling pathways regulating pluripotency of stem cells	151	0.043
NF-kappa B signaling pathway	124	0.047
Insulin resistance	142	0.047
Alcoholism	145	0.048
Glycosaminoglycan degradation	28	0.049

*Number of miRNAs enriched in the pathway