MiR-195-3p is a Novel Prognostic Biomarker Associated with Immune Infiltrates of Lung Adenocarcinoma

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Background: MicroRNA-195-3p (miR-195-3p) plays an important role in some tumors, but its role in LUAD is unclear. This study explored the expression of miR-195-3p in LUAD and the relationship between the expression of miR-195-3p and the clinical and prognostic characteristics of LUAD patients.

Methods: MiR-195-3p expression and clinical information of LUAD patients were obtained from The Cancer Genome Atlas (TCGA). Kruskal–Wallis test, Wilcoxon signed rank test, logistic regression, and Cox regression were used to assess the relationship between the expression level of miR-195-3p and clinical features in LUAD tissues. Kaplan–Meier survival curves were used to analyze the effect of miR-195-3p expression levels on the prognosis of LUAD patients. Target genes of miR-195-3p were predicted by several software. GO (Gene Ontology), KEGG (Kyoto Encyclopedia of Genes and Genomes), and immune infiltration analysis were used to analyze the possible regulatory network of miR-195-3p.

Results: Compared with normal lung tissue, miR-195-3p is down expressed in LUAD tissue (P < 0.001). The low miR-195-3p expression in LUAD was significantly associated with N stage (P = 0.046), pathologic stage (P = 0.011), and gender (P = 0.010). Low miR-195-3p expression predicted a poorer overall survival (HR: 0.60; 95% CI: 0.45–0.81; P = 0.001) and disease-specific survival (HR: 0.55; 95% CI: 0.37–0.80; P = 0.002). The expression of miR-195-3p (HR: 0.488; 95% CI: 0.304–0.784; P = 0.003) was independently correlated with OS in LUAD patients. High expression of miR-195-3p genes, including ABC2, AGMAT, ARNTL2, ATP6V0A4, CDC25A, CDK1, FAM111B, GJB2, GRIP1, HMG1A2, HOXA9, KIF14, SYT2, and TFAP2A, were associated with poor OS in LUAD. GO and KEGG analysis suggested that miR-195-3p was related to the phagosome pathway. MiR-195-3p may promote the function of B cells, dendritic cells, eosinophils, immature dendritic cells, macrophages, Mast cells, NK cells, plasmacytoid dendritic cells, and follicular helper T cells.

Conclusion: Low miR-195-3p expression is significantly associated with poor survival in LUAD, which may be a promising prognostic biomarker for LUAD.

Keywords: lung adenocarcinoma, miR-195-3p, gene expression, prognosis, immune infiltration, biomarker

Introduction

Lung cancer is one of the malignant tumors that seriously threaten human health.1 Lung cancer accounts for 11.6% of all cancers and has the highest incidence, with 18.4 deaths from each type of cancer.2 Non-small cell lung cancer (NSCLC) accounts for 80% of all lung cancers and is the most common histological form of lung cancer.3 Lung adenocarcinoma (LUAD) is the most aggressive histological
type of lung cancer, and the incidence of LUAD has been increasing rapidly.\textsuperscript{4} Compared with other types of lung cancer, LUAD has a slower growth rate and has no specific early clinical symptoms.\textsuperscript{5} Due to the similarity of symptoms of common respiratory diseases, it is easy to misdiagnose.\textsuperscript{6} Approximately 75\% of patients are diagnosed with advanced or metastatic cancer and often miss major surgery opportunities. With the improvement of modern clinical diagnosis methods, although the overall survival rate and quality of life have improved, the survival rate is still unsatisfactory, only about 15\%.\textsuperscript{7} Therefore, there is an urgent need to find validated tumor markers for LUAD to guide clinical diagnosis.

MicroRNAs (miRNAs or miRs), typically 18–23 nucleotides in length, have received widespread attention as oncogenes or inhibitors that regulate gene activity at the post-transcriptional and translational levels.\textsuperscript{8} Numerous studies have shown that aberrantly expressed miRNAs in various types of cancer are associated with cell development, cell proliferation, apoptosis, and tumorigenesis.\textsuperscript{9,10} miR-195-3p was a clinical biomarker for renal cell carcinoma.\textsuperscript{11} Cancer is a very heterogeneous disease with complex interactions between malignant cells and the tumor microenvironment (TME).\textsuperscript{12} The TME is composed of various immune cells, mesenchymal-derived cells, and extracellular matrix (ECM), which influence all stages of tumorigenesis by interacting directly with tumor cells.\textsuperscript{13} MiR-195 inhibits NSCLC by targeting CHEK1.\textsuperscript{14} MiR-195 suppresses lung adenocarcinogenesis by regulating CD4+ T cell activation through the CCDC88C/Wnt signaling pathway.\textsuperscript{15} Hsa-miR-195-3p acts as one of the maturators of hsa-miR-195. However, the expression of miR-195-3p in LUAD and its relationship with clinical features and immune infiltration are still unclear.

The Cancer Genome Atlas (TCGA) is a landmark cancer genomics program that has molecularly characterized more than 20,000 primary cancers and matched normal samples across 33 cancer types. TCGA has generated more than 2.5 PB of genomic, epigenomic, transcriptomic, and proteomic data. These data have improved our ability to diagnose, treat, and prevent cancer and continue to be publicly available to anyone in the research community. Based on the Cancer Genome Atlas (TCGA) database, this study analyzed the expression of miR-195-3p in LUAD and normal lung tissues, the relationship between miR-195-3p expression and the clinical characteristics of patients, and the relationship between 195–3p expression and prognosis in LUAD patients. Target genes of miR-195-3p were predicted by several software. Enrichment analysis, and immune infiltration analysis were used to analyze the possible regulatory network of miR-195-3p. The results of this study may provide a promising prognostic biomarker for LUAD.

**Materials and Methods**

**Clinical Information**

Analytical methods referred to the published reference.\textsuperscript{16} Molecule: hsa-miR-195-3p [MIMAT0004615]. The data includes miRNAseq data and clinical data from the BCGSC level 3 miRNA profile analysis of the TCGA (https://portal.gdc.cancer.gov/) and LUAD (lung adenocarcinoma) project.

**Differential Expression of miR-195-3p in LUAD Patients**

**Unpaired Samples**

Analytical methods referred to the published reference.\textsuperscript{17} Molecule: hsa-miR-195-3p. Data transformation: log2 transformation of miRNAseq data in RPM format for inter-sample expression comparison. Data Filtering: Retain paired samples.

**Paired Samples**

Analytical methods referred to the published reference.\textsuperscript{17} Molecule: hsa-miR-195-3p. Data transformation: log2 transformation of miRNAseq data in RPM format for inter-sample expression comparison. Data Filtering: None.

**ROC Curves**

Analytical methods referred to the published reference.\textsuperscript{16} The molecule is hsa-miR-195-3p. Clinical variables: Normal vs Tumor. Disease: LUAD.

**The Correlation Between miR-195-3p Expression and Clinical Information in LUAD Patients**

**Clinical Relevance**

Analytical methods referred to the published reference.\textsuperscript{16} The molecule is hsa-miR-195-3p. The subgroup is median. The clinical variables include N stage, pathological stage, and gender.

**Logistics Analysis**

Analytical methods referred to the published reference.\textsuperscript{16} Dependent variable: hsa-miR-195-3p.
The Relationship Between the Expression of miR-195-3p and the Prognosis of LUAD Patients

Kaplan–Meier Method
Analytical methods referred to the published reference. Molecule: hsa-miR-195-3p. Prognosis type: overall survival (OS) and disease specific survival (DSS).

Cox Regression
Analytical methods referred to the published references. Prognosis type: Overall Survival. Included variables: clinical characteristics and hsa-miR-195-3p.

Target Gene Prediction of miR-195-3p
The target genes of miR-195-3p were predicted by several databases. The LUAD mRNA average expression file was downloaded from the website (http://bioinfo.life.hust.edu.cn/miR_path/download.html). Venn diagram was used to obtain the shared genes of miR-195-3p target genes and LUAD down-regulated genes.

The Correlation Between Expression of miR-195-3p Target Genes and Prognosis
Analytical methods referred to the published reference. Molecules: ABCC2 [ENSG00000023839], AGMAT [ENSG00000116771], ARNTL2 [ENSG00000029153], CDC25A [ENSG00000164045], CDK1 [ENSG000000170312], FAM111B [ENSG00000189057], GJB2 [ENSG00000149948], HOXA9 [ENSG00000078399], KIF14 [ENSG00000118193], TFAP2A [ENSG00000137203]. Grouping: 0–50 vs 50–100. Prognosis type: overall survival (OS). Data: RNAseq data and clinical data from level 3 HTSeq-FPKM format from the TCGA LUAD project.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Analysis
The DAVID database was utilized to perform GO and KEGG analysis for miR-195-3p targets.

Sing Sample Gene Set Enrichment Analysis (SsGSEA) Analysis of Immune Infiltration
Analytical methods referred to the published references. Molecule: hsa-miR-195-3p.

Analysis of Statistics
Analytical methods referred to the published reference.

Results
Clinical Characteristics
There were 521 patients in this study (Table 1). According to the T stage, we had 173 patients with T1 (33.4%), 277 patients with T2 (53.5%), 49 patients with T3 (9.5%), and 19 patients with T4 (3.7%). The N stage included 336 N0 (66.3%), 95 N1 (18.7%), 74 N2 (14.6%), and 2 N3 (0.4%). The M stage included 351 N0 (93.9%), and 23 N1 (6.1%). The pathological stage included 283 Stage I (55.1%), 123 Stage II (23.9%), 84 Stage III (16.3%), and 24 Stage IV (4.7%). According to the primary therapy outcome, we had 68 patients with PD (15.7%), 35 patients with SD (8.1%), 6 patients with PR (1.4%), and 324 patients with CR (74.8%). According to the gender, we had 278 females (53.4%) and 243 males (46.6%). According to race, we had 8 Asian patients (1.8%), 53 Black or African American patients (11.6%), and 394 White patients (86.6%). According to age, there were 240 patients (≤65, 47.8%) and 262 patients (>65, 52.2%). According to the residual tumor, there were 342 R0 patients (95.3%), 13 R1 patients (3.6%), and 4 R2 patients (1.1%). According to the anatomic neoplasm subdivision, there were 202 patients with left (39.9%) and 304 patients with right (60.1%). According to the anatomic neoplasm subdivision 2, there were 62 central lungs (33.3%) and 124 peripheral lungs (66.7%). The number pack years smoked included 176 patients (<40, 49.7%) and 178 patients (≥40, 50.3%). The smokers included 76 no (15%) and 431 yes (85%). The median age was 66.5 years, with a range of 59 to 72 years.

Correlation Between miR-195-3p Expression and Clinical Features in LUAD Patients
MiR-195-3p expression in LUAD tissues was significantly lower than that in normal lung tissues (1.990±0.030, n=46 vs 2.629±0.157, n=521, P<0.001) (Figure 1A). The expression of MiR-195-3p in in LUAD tissues (1.734±0.084, n=46) was significantly lower than that of matched normal tissues (2.629±0.157, n=46) (P<0.001) (Figure 1B). The AUC of miR-195-3p was 0.659 (Figure 1C). MiR-195-3p expression was related to gender (P=0.012) (Table 1). The low expression of miR-195-3p in LUAD patients is significantly associated with N stage.
| Characteristic                  | Overall | Low Expression of hsa-miR-195-3p | High Expression of hsa-miR-195-3p | p         | Method     |
|--------------------------------|---------|----------------------------------|----------------------------------|-----------|------------|
| T stage, n (%)                 |         |                                  |                                  |           |            |
| T1                             | 173 (33.4%) | 74 (41.3%)                      | 99 (19.1%)                       | 0.057     | Chisq. test|
| T2                             | 277 (53.5%) | 148 (28.6%)                     | 129 (24.9%)                      |           |            |
| T3                             | 49 (9.5%)   | 24 (4.6%)                       | 25 (4.8%)                        |           |            |
| T4                             | 19 (3.7%)    | 13 (2.5%)                       | 6 (1.2%)                         |           |            |
| N stage, n (%)                 |         |                                  |                                  |           |            |
| N0                             | 336 (66.3%) | 161 (31.8%)                     | 175 (34.5%)                      |           |            |
| N1                             | 95 (18.7%)   | 50 (9.9%)                       | 45 (8.9%)                        |           |            |
| N2                             | 74 (14.6%)   | 47 (9.3%)                       | 27 (5.3%)                        |           |            |
| N3                             | 2 (0.4%)     | 1 (0.2%)                        | 1 (0.2%)                         |           |            |
| M stage, n (%)                 |         |                                  |                                  |           |            |
| M0                             | 351 (93.9%) | 187 (50%)                       | 164 (43.9%)                      | 0.623     | Chisq. test|
| M1                             | 23 (6.1%)    | 14 (3.7%)                       | 9 (2.4%)                         |           |            |
| Pathologic stage, n (%)        |         |                                  |                                  |           |            |
| Stage I                        | 283 (55.1%) | 134 (26.1%)                     | 149 (29%)                        |           | Chisq. test|
| Stage II                       | 123 (23.9%) | 58 (11.3%)                      | 65 (12.6%)                       |           | Chisq. test|
| Stage III                      | 84 (16.3%)   | 51 (9.9%)                       | 33 (6.4%)                        |           | Chisq. test|
| Stage IV                       | 24 (4.7%)    | 15 (2.9%)                       | 9 (1.8%)                         |           |            |
| Primary therapy outcome, n (%) |         |                                  |                                  |           |            |
| PD                             | 68 (15.7%)   | 40 (9.2%)                       | 28 (6.5%)                        | 0.060     | Fisher test|
| SD                             | 35 (8.1%)    | 12 (2.8%)                       | 23 (5.3%)                        |           |            |
| PR                             | 6 (1.4%)     | 4 (0.9%)                        | 2 (0.5%)                         |           |            |
| CR                             | 324 (74.8%)  | 147 (33.9%)                     | 177 (40.9%)                      |           |            |
| Gender, n (%)                  |         |                                  |                                  | 0.012     | Chisq. test|
| Female                         | 278 (53.4%) | 124 (23.8%)                     | 154 (29.6%)                      |           |            |
| Male                           | 243 (46.6%) | 136 (26.1%)                     | 107 (20.5%)                      |           |            |
| Race, n (%)                    |         |                                  |                                  | 0.538     | Fisher test|
| Asian                          | 8 (1.8%)     | 4 (0.9%)                        | 4 (0.9%)                         |           |            |
| Black or African American      | 53 (11.6%)   | 22 (4.8%)                       | 31 (6.8%)                        |           |            |
| White                          | 394 (86.6%)  | 195 (42.9%)                     | 199 (43.7%)                      |           |            |
| Age, n (%)                     |         |                                  |                                  | 0.861     | Chisq. test|
| ≤65                            | 240 (47.8%)  | 121 (24.1%)                     | 119 (23.7%)                      |           |            |
| >65                            | 262 (52.2%)  | 129 (25.7%)                     | 133 (26.5%)                      |           |            |
| Residual tumor, n (%)          |         |                                  |                                  | 0.917     | Fisher test|
| R0                             | 342 (95.3%)  | 183 (51%)                       | 159 (44.3%)                      |           |            |
| R1                             | 13 (3.6%)    | 8 (2.2%)                        | 5 (1.4%)                         |           |            |
| R2                             | 4 (1.1%)     | 2 (0.6%)                        | 2 (0.6%)                         |           |            |
| Anatomic neoplasm subdivision, n (%) |         |                                  |                                  | 0.703     | Chisq. test|
| Left                           | 202 (39.9%)  | 104 (20.6%)                     | 98 (19.4%)                       |           |            |
| Right                          | 304 (60.1%)  | 150 (29.6%)                     | 154 (30.4%)                      |           |            |
| Anatomic neoplasm subdivision 2, n (%) |         |                                  |                                  | 0.638     | Chisq. test|
| Central Lung                   | 62 (33.3%)   | 37 (19.9%)                      | 25 (13.4%)                       |           |            |
| Peripheral Lung                | 124 (66.7%)  | 68 (36.6%)                      | 56 (30.1%)                       |           |            |
| number_pack_years_smoked, n (%)|         |                                  |                                  |           |            |
| <40                            | 176 (49.7%)  | 87 (24.6%)                      | 89 (25.1%)                       |           |            |
| ≥40                            | 178 (50.3%)  | 95 (26.8%)                      | 83 (23.4%)                       |           |            |
| Smoker, n (%)                  |         |                                  |                                  | 0.070     | Chisq. test|
| No                             | 76 (15%)     | 30 (5.9%)                       | 46 (9.1%)                        |           |            |
| Yes                            | 431 (85%)    | 222 (43.8%)                     | 209 (41.2%)                      |           |            |
| Age, median (IQR)              |         |                                  |                                  | 0.111     | Wilcoxon   |
Correlation Between miR-195-3p Expression and Prognosis in LUAD Patients

Low expression of miR-195-3p in LUAD patients was associated with poor OS (HR: 0.60; 95% CI: 0.45–0.81; P=0.001) and DSS (HR: 0.55; 95% CI: 0.37–0.80; P=0.002) (Figure 1G–H). The univariate analysis of the Cox regression model of the OS prognostic factors in Table 3 showed that low miR-195-3p expression levels (HR: 0.592; 95% CI: 0.440–0.796; P<0.001) were related to T stage (HR: 2.385; 95% CI: 1.634–3.481; P<0.001), N stage (HR: 2.617; 95% CI: 1.946–3.519; P<0.001), M stage (HR: 2.330; 95% CI: 1.360–3.991; P=0.002), pathologic stage (HR: 2.766; 95% CI: 2.030–3.768; P=0.046), pathologic stage (P=0.011), and gender (P=0.010) (Figure 1D–F, and Table 2).
Table 2 Expression of miR-195-3p in Relation to Clinical Characteristics (Logistic Regression)

| Characteristics                                      | Total (N) | Odds Ratio (OR) | P value |
|------------------------------------------------------|-----------|-----------------|---------|
| T stage (T3&T4 vs T1&T2)                            | 518       | 0.816 (0.487–1.359) | 0.436   |
| N stage (N1&N2&N3 vs N0)                            | 507       | 0.685 (0.472–0.992) | 0.046   |
| M stage (M1 vs M0)                                  | 374       | 0.733 (0.298–1.716) | 0.481   |
| Primary therapy outcome (PD&SD&PR vs CR)            | 433       | 0.786 (0.508–1.214) | 0.278   |
| Pathologic stage (Stage III & Stage IV vs Stage I & Stage II) | 514       | 0.571 (0.368–0.877) | 0.011   |
| Gender (Male vs Female)                             | 521       | 0.633 (0.447–0.895) | 0.010   |
| Race (Black or African American & White vs Asian)   | 455       | 1.060 (0.248–4.532) | 0.935   |
| Age (>65 vs ≤65)                                    | 502       | 1.048 (0.738–1.489) | 0.792   |
| Residual tumor (R1&R2 vs R0)                        | 359       | 0.806 (0.287–2.147) | 0.668   |
| Anatomic neoplasm subdivision (Right vs Left)       | 506       | 1.090 (0.763–1.556) | 0.637   |
| Anatomic neoplasm subdivision 2 (Peripheral Lung vs Central Lung) | 186       | 1.219 (0.659–2.278) | 0.531   |
| number_pack_years_smoked (≥40 vs <40)               | 354       | 0.854 (0.562–1.296) | 0.459   |
| Smoker (Yes vs No)                                  | 507       | 0.614 (0.370–1.005) | 0.055   |

Table 3 Association of OS and Clinical Characteristics in LUAD (Cox Regression)

| Characteristics                                      | Total (N) | Univariate Analysis | Multivariate Analysis |
|------------------------------------------------------|-----------|---------------------|-----------------------|
|                                                      |           | HR (95% CI)         | P value               | HR (95% CI)         | P value               |
| T stage (T1&T2 vs T3&T4)                             | 509       | 2.385 (1.634–3.481) | <0.001                | 1.802 (0.855–3.797) | 0.121                 |
| N stage (N0 vs N1&N2&N3)                            | 498       | 2.617 (1.946–3.519) | <0.001                | 1.607 (0.980–2.632) | 0.060                 |
| M stage (M0 vs M1)                                  | 365       | 2.330 (1.360–3.991) | 0.002                 | 1.204 (0.443–3.273) | 0.716                 |
| Pathologic stage (Stage I & Stage II vs Stage III & Stage IV) | 505       | 2.766 (2.030–3.768) | <0.001                | 1.595 (0.783–3.250) | 0.198                 |
| Primary therapy outcome (PD&SD&PR vs CR)            | 426       | 0.368 (0.261–0.519) | <0.001                | 0.270 (0.163–0.446) | <0.001                |
| Gender (Female vs Male)                             | 512       | 1.024 (0.765–1.371) | 0.873                 | 0.201 (0.137–0.294) | 0.001                 |
| Race (Asian vs Black or African American & White)   | 455       | 2.604 (1.364–18.609)| 0.340                 | 3.902 (1.510–10.084)| 0.005                 |
| Age (≤65 vs >65)                                    | 502       | 1.210 (0.900–1.625) | 0.206                 |                      |                      |
| Residual tumor (R0 vs R1&R2)                        | 350       | 4.044 (2.256–7.250) | <0.001                | 3.902 (1.510–10.084)| 0.005                 |
| Anatomic neoplasm subdivision (Left vs Right)       | 498       | 1.015 (0.751–1.372) | 0.923                 |                      |                      |
| Anatomic neoplasm subdivision 2 (Central Lung vs Peripheral Lung) | 179       | 0.922 (0.575–1.479) | 0.737                 |                      |                      |
| number_pack_years_smoked (<40 vs ≥40)               | 348       | 1.049 (0.732–1.504) | 0.793                 |                      |                      |
| Smoker (No vs Yes)                                  | 498       | 0.917 (0.607–1.384) | 0.679                 |                      |                      |
| hsa-miR-195-3p (Low vs High)                        | 512       | 0.592 (0.440–0.796) | <0.001                | 0.488 (0.304–0.784) | 0.003                 |

P<0.001), primary therapy outcome (HR: 0.368; 95% CI: 0.261–0.519; P=0.001), and residual tumor (HR: 4.044; 95% CI: 2.256–7.250; P<0.001). The multivariate analysis showed that miR-195-3p (HR: 0.488; 95% CI: 0.304–0.784; P=0.003), primary therapy outcome (HR: 0.270; 95% CI: 0.163–0.446; P<0.001), and residual tumor (HR:3.902; 95% CI: 1.510–10.084; P=0.005) were independently related to OS (Table 3).

Relationship Between miR-195-3p Target Genes and Survival of LUAD Patients

There were 470 miR-195-3p target genes, 3066 LUAD up-regulated genes, and 51 common genes in Figure 2. High ABC2C expression was related to poor OS (HR: 1.46; 95% CI: 1.09–1.95; P=0.01) (Figure 3A). High AGMAT expression was related to poor OS (HR: 1.52; 95% CI: 1.14–2.03; P=0.005) (Figure 3B). High ARNTL2 expression was related to poor OS (HR: 1.62; 95% CI: 1.21–2.17; P=0.001) (Figure 3C). High CDC25A expression was related to poor OS (HR: 1.44; 95% CI: 1.078–1.92; P=0.014) (Figure 3D). High CDK1 expression was related to poor OS (HR: 1.84; 95% CI: 1.37–2.48; P<0.001) (Figure 3E). High FAM111B expression was related to poor OS (HR: 1.66; 95% CI: 1.24–2.22; P=0.001) (Figure 3F). High GJB2 expression was related to poor OS (HR: 1.75; 95% CI: 1.31–2.35; P<0.001) (Figure 3G).
Figure 2 The common genes between miR-195-3p target genes and LUAD upregulated genes. (Venn diagram).

Figure 3 Correlation of miR-195-3p target gene expression with prognosis of LUAD patients. (A) ABC2, (B) AGMAT, (C) ARNTL2, (D) CDC25A, (E) CDK1, (F) FAM111B, (G) GJB2, (H) GRIP1, (I) HMGA2, (J) HOXA9, (K) KIF14, and (L) TFAP2A. Overall survival, OS.
High GRIP1 expression was related to poor OS (HR: 1.46; 95% CI: 1.09–1.95; P=0.011) (Figure 3G). High HMGA2 expression was related to poor OS (HR: 1.35; 95% CI: 1.01–1.80; P=0.041) (Figure 3I). High NHS expression was related to poor OS (HR: 1.37; 95% CI: 1.03–1.83; P=0.032) (Figure 3J). High NDN expression was related to poor OS (HR: 1.67; 95% CI: 1.25–2.23; P=0.001) (Figure 3K). High NHS expression was related to poor OS (HR: 1.78; 95% CI: 1.33–2.38; P<0.001) (Figure 3L).

**Possible Pathways Involved in MiR-195-3p Target Genes**

Common genes are involved in biological processes, including sensory perception of mechanical stimulus, sensory perception of sound, appendage morphogenesis, limb morphogenesis, and response to dexamethasone, etc.; cellular components including cell projection membrane, apical part of cell, cell-cell junction, apical plasma membrane, and brush border membrane, etc.; molecular function including benzodiazepine receptor activity, GABA-gated chloride ion channel activity, inhibitory extracellular ligand-gated ion channel activity, DNA-binding transcription activator activity, RNA polymerase II-specific, and GABA-A receptor activity, etc.; the phagosome pathway (Figure 4 and Table 4).

**The Correlation Between miR-195-3p Expression and Immune Infiltration**

The expression of miR-195-3p is positively correlated with the level of immune infiltration of B cells (0.311±0.077 vs 0.286 ±0.074, P<0.001; r=0.180; Figures 5A, 6A, and 7), dendritic cells (DC) (0.489±0.099 vs 0.45±0.109, P<0.001; r=0.240; Figures 5B, 6B, and 7), Eosinophils (0.399±0.028 vs 0.391 ±0.031, P<0.001; r=0.210; Figures 5C, 6C, and 7), immature dendritic cells (iDC) (0.534±0.059 vs 0.506±0.06, P<0.001; r=0.280; Figures 5D, 6D, and 7), Macrophages (0.621±0.062 vs 0.597±0.071, P<0.001; r=0.210; Figures 5E, 6E, and 7), Mast cells (0.329±0.073 vs 0.291±0.077, P<0.001; r=0.170; Figures 5F, 6F, and 7), NK cells (0.493±0.024 vs 0.479±0.025, P<0.001; r=0.170; Figures 5G, 6G, and 7), plasmacytoid dendritic cells (pDC) (0.613±0.102 vs 0.570±0.098, P<0.001; r=0.290; Figures 5H, 6H, and 7), and follicular helper T cells (TFH) (0.415±0.038 vs 0.402±0.037, P<0.001; r=0.210; Figures 5I, 6I and 7).

![Figure 4 GO analysis of common genes.](https://doi.org/10.2147/IJGM.S350340)
**Discussion**

MiRNAs are associated with the development of some cancers. miR-375 is critical to the advancement of LUAD.\(^{27}\) MiR-139-5p may act as a tumor suppressor gene in the development and progression of NSCLC.\(^{28}\) Low expression of MiR-99a is a poor prognostic factor for patients with LUAD and acts as a tumor suppressor by targeting mTOR.\(^{29}\) miR-210-3p is a promising LUAD diagnostic biomarker.\(^{30}\) Therefore, it is essential to develop new prognostic biomarkers for LUAD.

**Table 4** KEGG Analysis of Common Genes

| Category     | Term                        | Count | %    | P value | Genes                      |
|--------------|-----------------------------|-------|------|---------|----------------------------|
| KEGG_PATHWAY | hsa04145: Phagosome         | 4     | 7.84 | 0.013   | TUBB2A, ITGA2, ATP6V0A4, THBS2 |

**Figure 5** MiR-195-3p expression was associated with 24 LUAD immune cells (grouped comparison plots). (A) B cells, (B) DC, (C) eosinophils, (D) iDC, (E) macrophages, (F) Mast cells, (G) NK cells, (H) pDC, and (I) TFH. ** P<0.01; *** P<0.001.
In the present study, miR-195-3p was lowly expressed in LUAD patients, and patients with low miR-195-3p expression had poorer OS. Low miR-195-3p expression was significantly related to N stage (P = 0.046), pathological stage (P = 0.011), and gender (P = 0.010). The expression of miR-195-3p in LUAD tissue was significantly lower than that in normal lung tissue. The expression of miR-195-3p in patients with advanced N stage was lower than that in the early stage, and the expression of miR-195-3p in patients with stage III–IV was significantly lower than that of stage I–II. These phenomena suggest that miR-195-3p may be involved in the occurrence of LUAD and inhibit proliferation. Male patients showed low expression of miR-195-3p compared to female patients. The cause of this situation requires further investigation. The low expression of miR-195-3p in LUAD patients is related to poor OS (HR: 0.60; 95% CI: 0.45–0.81; P=0.001) and DSS (HR: 0.55; 95% CI: 0.35–0.84; P=0.011).

Figure 6 MiR-195-3p expression was associated with 24 LUAD immune cells (scatter plots). (A) B cells, (B) DC, (C) eosinophils, (D) iDC, (E) macrophages, (F) Mast cells, (G) NK cells, (H) pDC, and (I) TFH.
And miR-195-3p (HR: 0.488; 95% CI: 0.304–0.784; P=0.003) were independently correlated with OS in multivariate analysis. High expression of miR-195-3p targets, including ABCC2, AGMAT, ARNTL2, ATP6V0A4, CDC25A, CDK1, FAM111B, GJB2, GRIP1, HMGA2, HOXA9, KIF14, SYT2, and TFAP2A, were significantly associated with poor OS in LUAD patients.

MiR-195-3p inhibits cervical cancer cell proliferation by targeting BCDIN3D.\textsuperscript{31} Chemokine CCL4 regulates miR-195-3p to induce VEGF-C expression and lymphangiogenesis in oral squamous cell carcinoma (OSCC).\textsuperscript{32} In this study, GO and KEGG analysis suggested that miR-195-3p was related to the phagosome pathway. The molecular mechanism of miR-195-3p-mediated development of LUAD needs to be further investigated.

The immunological component of TME acts as a double-edged sword that can inhibit or promote tumor development.\textsuperscript{33} Infiltrating immune cells in TME is what influences tumor growth and progression, as well as treatment outcome and patient prognosis.\textsuperscript{34} The results of this study showed that miR-195-3p was associated with infiltration of B cells, DC, Eosinophils, iDC, Macrophages, Mast cells, NK cells, pDC, and TFH in LUAD. MiR-195-3p may promote the function of B cells, DC, Eosinophils, iDC, Macrophages, Mast cells, NK cells, pDC, and TFH.

The present study had the limitation that the current analysis was based on LUAD tissues from the TCGA database. The results of the bioinformatics analysis need to be demonstrated by major bench experiments. In the real world, the clinical and biological significance of miR-195-3p in LUAD patients need to be further investigated.

**Conclusion**

The expression of miR-195-3p in LUAD tissue was significantly lower than that in normal lung tissue. The low expression of miR-195-3p in LUAD patients is associated with poor OS. MiR-195-3p was involved in the development of LUAD through the phagosome pathway. The expression of miR-195-3p was significantly associated with immune infiltration. The findings of this study suggested that miR-195-3p could be a novel prognostic biomarker for LUAD.

**Data Sharing Statement**

The datasets generated and analyzed during the present study are available from the corresponding author on reasonable request.
Ethics Approval
This study was submitted to Medical Ethics Committee of Central Hospital of Guangdong Nongken for review and the ethics requirement was waived (Approval NO: [2021]02).

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The data set generated in this study can be obtained from TCGA, which provides free resources. The authors thank MyGene Diagnostics Co., Ltd. for technology support.

Author Contributions
All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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The authors declare that they have no conflict of interest.

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