Novel Lipid Metabolism-Related Gene Signature in Lung Adenocarcinoma Patients Experiencing Lymph Node Metastasis

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Abstract

Accumulating evidence revealed that lipid metabolic reprogramming facilitates lymph node metastasis and cancer progression. This study aimed to perform comprehensive bioinformatic analyses of the lipid metabolism-related impact in lung adenocarcinoma (LUAD) patients experiencing lymph node metastasis (LNM). Clinicopathological information and RNA-sequence data on LUAD patients were collected from the TCGA and GEO databases. Subsequently, 189 differentially expressed lipid metabolism-related genes (LMRGs) were screened for LUAD patients with and without LNM. Based on the risk scoring system related to prognosis, we further correlated the LMRGs with diverse clinicopathological outcomes, genomic alterations, and immune features, as well as immunotherapeutic responses and drug susceptibility.

A three-gene lipid metabolic signature (GPD1L, SPHK1, and ST3GAL4) associated with tumor progression in LUAD with LNM was identified. Analyses revealed that the high-risk group had worse overall survival; an increased proportion of M0 macrophages and degranulating mast cell infiltration; increased non-responders to immunotherapy; and resistance to several common TKI drugs. Interestingly, we also found that high-risk patients had higher tumor mutation burdens and PDL-1 expression, suggesting that this subset of patients may benefit from immunotherapy plus LMRG-targeted therapy. Additionally, the results showed that the differential expressed genes (DEGs) of the high-risk group were enriched in the REACTIVE OXYGEN SPECIES pathway. Overall, we identify a lipid metabolic signature that has prognostic value and provides new insights into molecular mechanisms and precision therapies of lipid metabolism-related LUAD regional metastasis.

Introduction

Lung cancer is a highly malignant tumor worldwide, ranking first in morbidity and mortality[1]. And more than 40% of NSCLCs are lung adenocarcinomas (LUADs)[2, 3]. Despite diagnostic and therapeutic improvements, the 5-year survival rate of metastatic LUAD is approximately 15%[4, 5]. The lymphatic system is the main route for lung cancer metastasis, and lymphatic metastasis is a vital factor affecting the stage and prognosis of lung cancer[6]. Experimental studies have shown that cancer cells spreading to lymph nodes can invade blood vessels in lymph nodes and thus trigger tumor growth in distant organs[7]. Therefore, LNM is closely related to distant metastasis of tumors, systemic spread, postoperative recurrence, and survival of LUAD patients[8, 9]. Thus, further investigations are required to identify LNM-associated pathological genes and the underlying molecular mechanisms.

In recent years, accumulating evidence has indicated that lipid metabolic reprogramming contributes to the metastasis cascade of the lymphatic system, subsequent distant organs, and further malignant progression[10, 11]. Choong-kun Lee et al., through comparative transcriptomic and metabolomic analysis of primary and LNM tumor cells in melanoma mouse models, found that LNM was associated with lipid metabolism disorders involving a metabolic shift toward fatty acid oxidation[12]. Moreover, a study on cervical cancer also showed that the lipid metabolism-related gene FABP5 could promote the tumor epithelial-mesenchymal transition (EMT), lymphangiogenesis, and LNM by reprogramming the

fatty acid metabolism[13]. Collectively, the above-mentioned studies suggest a close relationship between LNM and lipid metabolism disorders. Currently, few studies exist on the relationship between LNM and lipid metabolism levels in LUAD. Given this deficiency, we screened lipid metabolism-related genes (LMRGs) associated with LNM in LUAD by data-mining-based bioinformatic analysis to investigate LNM-specific lipid metabolic gene influences on LUAD prognosis and the potential mechanism.

Materials & Methods

LUAD data acquisition and processing

Clinicopathological information and RNA-sequence data regarding LUAD patients were collected from The Cancer Genome Atlas database (TCGA, <https://www.tcgadatacenter.gov/>). The patients were then divided into LNM (LN+) and non-LNM (LN-) groups, with 482 samples (164 LN+ vs. 318 LN-) included and set as the training cohort. The GSE50081 dataset was downloaded from the Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>) database, with 127 LUAD samples included and set as the verification cohort.

Identification of LMRGs in nodal staging-specific LUAD

Seven lipid metabolism-related pathways, including 189 LMRGs, were extracted from the Kyoto Encyclopedia of Genes and Genomes (KEGG, <http://www.kegg.jp/blastkoala/>). The “limma” package (<https://www.bioconductor.org/packages/limma.html>) [14] was performed to identify differentially expressed genes (DEGs) for the subgroups of LNM (w/wo lymph node metastasis of LUAD). A log₂ (fold change [FC]) > 1, a p-value < 0.05, and a false discovery rate (FDR) < 0.05 were considered as cut-off values.

Cox regression and survival analyses of LMRGs in LUAD

Univariate Cox regression was conducted using the “survival” package in R for LMRGs, and genes with a p-value < 0.01 were considered prognostic-associated genes. Each LMRG from the Cox model was further plotted using the Kaplan-Meier survival curve[15].

Lasso regression construction and verification in LUAD

Subsequently, a Lasso-Cox regression model was constructed using the R package “glmnet” [16]. A LMRG signature was then developed by multivariate Cox regression analysis of genes filtered by Lasso analysis, and a signature risk score was calculated as follows: $GPD1L \times (-0.259) + SPHK1 \times 0.087 + ST3GAL4 \times 0.168$. LUAD patients were then divided into high-risk and low-risk subgroups of the LMRG signature according to the median risk score (median value = 0.98). The time-dependent receiver operating characteristic (ROC) curves were then conducted to evaluate the accuracy of the risk score for both pathological N-stage prediction (pN1-, pN2-, pN3-stage) and survival prediction (12-, 24-, and 36-month survival rates). The above model was validated internally with TCGA and externally with GSE50081 from

the GEO dataset. Additionally, the clinical features and LMRG-based nomogram (<https://cran.r-project.org/web/packages/rms/index.html>) were plotted.

Immunohistochemical (IHC) examination of the LMRGs

To examine three LMRGs expressions at the protein level, we used the results from the Human Protein Atlas (HPA) database and a commercial tissue microarray (TMA). The protein expressions of SPHK1 and ST3GAL4 were validated via the HPA[17, 18]. As no staining data for GPD1L were found in the HPA database, we performed the IHC analyses of GPD1L on a commercial TMA of LUAD (Cat#LUC1601, Shanghai Zhuohao Medical Science and Technology Co. Ltd) [19] containing 80 LUAD tumors and matched adjacent non-tumor tissue with clinical and prognostic information. The TMA slide was incubated with a rabbit polyclonal antibody against the GPD1L protein (1:100 dilution; Abcepta, AP10723a-400). Quantification was based on the percentage of positive cells and the staining intensity. Briefly, the GPD1L positive staining was scored as follows: 0, < 5% of the tumor cells in the lesions; 1, 5–30% of the tumor cells; 2, 31–60% of the tumor cells; and 3, $\geq 61\%$ of the tumor cells. The intensity was graded as follows: 0, negative; 1+, weak; 2+, moderate; and 3+, strong. A final score between 0 and 12 was calculated by multiplying the positive rate and intensity scores. The X-Tile package (Yale University, USA) was performed to calculate the best cut-off points (cut-off = 6) for OS. A final staining index was recorded, in which a score of 0 was considered the negative expression, 1–6 was considered the low expression, and ≥ 6 was considered high expression.

Genomic alteration analysis of LMRGs and corresponding survival analysis

The cBioPortal (<https://www.cbioportal.org/>) is an online database for exploring cancer genomics, including somatic mutations, DNA copy number, and mRNA expression. Here, we used it to analyze the correlation between mutations (OncoPrint display of gene mutation) and LMRG subgroups of LUAD (TCGA, Firehose Legacy) patients. Additionally, the survival analysis for LMRG subgroups was investigated using a KM plot for several genes (TP53, KRAS, and EGFR) that are frequently mutated in LUAD.

Estimation of immune infiltration and correlation analysis between LMRG signature and tumor microenvironment scores

CIBERSORT (R Bioconductor package, <http://cibersort.stanford.edu/>) is a versatile computational method for quantifying cell compositions from gene expression profiles[20]. In this study, we used it to estimate the relationship between the LMRG signature and immune cell infiltrates in the TCGA-LUAD dataset. Subsequently, the ESTIMATE algorithm (R Bioconductor package, <https://bioinformatics.mdanderson.org/estimate/>) was applied to calculate the microenvironment scores based on gene set enrichment analysis[21]. The immune, stromal, and estimate scores were

automatically output to estimate the infiltrating stromal and immune cells for two LMRG signature subgroups.

Prediction of immunotherapeutic response and evaluation of drug sensitivity

The Tumor Immune Dysfunction and Exclusion (TIDE, <http://tide.dfci.harvard.edu/>) algorithm[22] and subclass mapping[23] were used to predict the response to immunotherapy of LMRG subgroups. Moreover, the tumor mutation burden (TMB), microsatellite instability (MSI), and tumor neoantigen scores were also assessed in the TCGA-LUAD cohort[24]. Additionally, we used the Genomics of Drug Sensitivity in Cancer (GDSC, <https://www.cancerrxgene.org/>) database[25] to explore the association between the LMRG subgroup and clinical anti-LUAD drugs, including TKI drugs (e.g., erlotinib, afatinib, selumetinib), chemotherapy drugs (e.g., cisplatin, docetaxel, paclitaxel), and VEGF receptor inhibitors. The half-maximal inhibitory concentration (IC50) of related anticancer drugs in lung cancer cell lines was obtained using the GDSC database.

Functional and pathway enrichment analyses of LMRGs

To further explore the potential biological processes and molecular characteristics for LMRG subsets, we conducted gene set enrichment analysis (GSEA, <http://www.broadinstitute.org/gsea/>)[26] to identify the related biological function enrichment in the LMRG high-risk group. Meanwhile, gene set variation analysis (GSVA) was conducted using the “GSVA” R package[27] to reveal the specific signaling pathways involved in DEGs for the LMRG high-risk and low-risk groups.

Results

Identification of LMRGs associated with LNM in LUAD and evaluation

A total of 482 LUAD patient records were collected from the TCGA database, including 164 cases with LNM and 318 without LNM (Supplemental Table S1). With RNA-seq data for analyzing differentially expressed LMRGs for the LNM and non-LNM groups, 21 DEGs were obtained, including 12 up-regulated and 9 down-regulated genes (Supplemental Table S2). A gene set of 189 LMRGs was extracted from the KEGG database. Through univariate Cox analysis (HR-unicox), six lipid metabolism genes related to prognosis were selected for further Lasso regression analysis. Finally, three prognostic LMRGs (GPD1L, SPHK1, and ST3GAL4) were identified as associated with LNM in LUAD patients. Among them, GPD1L was down-regulated, and SPHK1 and ST3GAL4 were up-regulated in the LUAD-LNM group (Fig. 1a). Subsequently, the patients were further divided into low-risk and high-risk LMRG groups using risk score (risk score = $GPD1L \times [-0.259] + SPHK1 \times 0.087 + ST3GAL4 \times 0.168$) as the cut-off value, and their pathological N-stage and survival difference were evaluated in the TCGA-LUAD cohort (Fig. 1f). As shown in Fig. 1b, the ROC curve based on the high-risk LMRGs exhibited a certain degree of prediction concordance of the pathological N-stage (pN1 AUC = 0.621, pN2 AUC = 0.639, pN3 AUC = 0.716).

Construction and validation of a prognostic model based on LMRG signature

We then tested the prognostic model comprising the LMRG signature in the TCGA-LUAD cohort. As shown in Fig. 1c, patients in the LMRG high-risk group had a significantly worse prognosis ($p = 0.00012$).

Moreover, the LMRG signature exhibited good prognostic efficiency in predicting the overall survival of LUAD patients (12-month AUC = 0.762, 24-month AUC = 0.755, 36-month AUC = 0.716, $n = 482$). We then used the GSE50081 dataset derived from the GEO database to validate the TCGA-based results. As the GEO-based results were consistent with those of the TCGA training set, we found that the high-risk group had a significantly worse prognosis (Fig. 1d), and the LMRG signature model had a good predictive value (12-month AUC = 0.712, 24-month AUC = 0.706, 36-month AUC = 0.69, $n = 127$).

Protein levels confirmed the identified LMRG signature

We then evaluated the protein expression of LMRGs by checking the HPA database. The results indicated that the SPHK1 and ST3GAL4 (data existing in the public domain) were overexpressed in LUAD tumors (Fig. 1e, Supplementary Figure S4a,b). Due to the lack of public data for GPD1L protein expression, we conducted IHC staining using a commercial tissue assay (TMA) of LUAD patients. The result was consistent with the TCGA sequencing data, in which the GPD1L showed low expression in LUAD tumors compared to the adjacent normal tissue (Supplementary Figure S4c). Furthermore, low expression was closely associated with N-staging and poor survival in this LUAD cohort of 75 cases (Supplemental Table S3). The survival curves for the LMRG signature are provided in Fig. 1g.

LMRG signature is a prognostic factor and is associated with unique patterns of genomic alterations in LUAD

We used Cox proportional hazards regression to assess the association between the risk score derived from the three-gene signature (GPD1L, SPHK1, ST3GAL4) and clinical characteristics in LUAD patients (Fig. 2a). Furthermore, a nomogram plot was constructed, including age, sex, pathological T-, N-, M-stage, and LMRGs to predict the patient survival rate. Moreover, the results showed that the LMRG risk score contributed greatly to predicting independent prognostic factors in LUAD (Fig. 2b).

Additionally, we explored the association between LMRGs and the LUAD genomic profile. The somatic mutation analysis presented a high frequency of mutations in TP53 (61%), TTN (52%), and RYR2 (45%) in the high-risk LMRG subgroup (Fig. 2c). Moreover, we were interested in investigating the survival difference between LMRG subgroups of LUAD patients with several common driver genes, including TP53, KRAS, and EGFR. Therefore, we stratified the survival analyses, and the results showed that patients in high-risk LMRG group had a significantly worse prognosis among the patients with TP53, EGFR, and KRAS mutations (Fig. 2d).

Analysis of immune infiltration and relationship between LMRG signature and TME

The immunosuppressive tumor microenvironment (TME) is a major barrier to cancer treatment[28]. In this context, we calculated the immune, stromal, and estimate scores to understand the relationship between metabolic status and TME in LUAD. As shown in Fig. 3b, the LUAD tissue for the high-risk LMRG signature showed significantly lower immune and estimate scores, while no significant differences were found for stromal scores. Here, the LMRG signature was significantly negatively correlated with the immune scores, indicating that fewer immune cell infiltrates existed in the TME with the high-risk LMRG signature.

Additionally, we further analyzed the relationship between LMRGs and immune infiltrates (types of immune cells) in the TME using the CIBERSORT method. A strong correlation was found between the high and low risk of LNM and immune cell content, especially the M0 resting macrophages contributing most to the high-risk group (Fig.3a). Interestingly, we also noted marked enrichment of mast cells activated in the high-risk signature, while the relatively high abundance of resting mast cells was shown in the low-risk signature. The role of mast cells in contributing to tumor progression or lymph node metastasis is discussed further below. Collectively, the results suggested that the LMRGs may impact immunotherapy in LUAD patients.**Fig.3** The correlation between LMRG signature and immune cells in the TME. **a** association analysis between immune cell content and patients with low- and high-risk LMRG signature scores. **b** The relationship between LMRG signature and immune, stromal, and estimate scores; *, **, and *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$.

LMRG signature is associated with the TMB and immunotherapy response in LUAD

Since the TMB, MSI, tumor neoantigen score and PDL-1 expression are currently the most important biomarkers for immunotherapy in clinical practice[29], we first evaluated the relationship between the patients with low- and high-risk signature scores. As shown in Fig. 4a, we noted that the TMB significantly increased in high-risk patients with no significant difference in the MSI and tumor neoantigen peptide levels between the two groups. Additionally, predicting the response to immunotherapy based on the TIDE database further showed that the proportion of patients with no response to immunotherapy in high-risk group was significantly higher than that in low-risk group ($p < 0.001$; Fig. 4b). Meanwhile, a significant increase was observed in the expression of CD274 (PDL-1; $p = 4e - 03$) and LAG3 ($p = 1.426e - 05$) in the high-risk signature subgroup (Fig. 4c). The marked difference in immune response between the two groups suggested that the gene sets in the low- and high-risk groups could be used as therapeutic targets for LUAD.

Relationship between LMRG signature and anticancer drug susceptibility

Since drug resistance is a major issue in cancer therapy, we subsequently evaluated the drug sensitivity to commonly used chemotherapeutic or immunotherapeutic drugs for LUAD. The relationship between risk score and drug sensitivity was explored through the GDSC database. The results demonstrated that compared with low-risk patients, high-risk patients were associated with greater IC50s for classical TKI drugs (erlotinib, afatinib, and selumetinib), suggesting that high-risk patients were resistant to some classical TKIs (Fig. 5a). Conversely, the IC50 of the following drugs in the high-risk patients was markedly lower than in the low-risk group, including TKI drugs pazopanib and sunitinib, VEGF receptor inhibitor motesanib, chemotherapy drugs cisplatin/docetaxel/paclitaxel, cell cycle inhibitor, and PPAR (an important pathway of lipid metabolism) inhibitor olaparib, indicating that high-risk patients were sensitive to such drugs (Fig. 5b). Together, these results showed that LMRGs were related to drug susceptibility and may be helpful for drug resistance surveillance.

LMRGs enriched in cancer-associated pathways and pathological processes in LUAD

To explore potential biological behavior and molecular pathways, we conducted the relevant enrichment analysis involved in LNM for low- and high-risk patients. According to the GSVA analysis, patients in the high-risk LMRG group were significantly associated with several important cancer-associated pathways (e.g., REACTIVE OXYGEN SPECIES PATHWAY, HEDGEHOG SIGNALING, and TNFA SIGNALING VIA NFKB pathways), while patients in the low-risk group were strongly related to the SPERMATOGENESIS pathways (Fig. 6a). For pathway enrichment analysis, the GSEA results showed that the high-risk signature group was highly related to the classic cancer-associated or metastatic cancer-related pathways, such as DNA replication, mismatch repair, and cell cycle, which further led to a worse prognosis among LUAD patients (Fig. 6b, c).

Discussion

In LUAD, patients presenting with lymph node metastasis (LNM) consistently indicate poor prognoses[30]. In recent years, growing evidence has suggested that lipid-metabolic reprogramming can play an essential role in regulating the survival of metastatic cancer cells as well as other resident immune or stromal cells in the TME of LNM. Therefore, our study aimed to identify lipid metabolic-related signatures related to clinicopathological features and could be used as a predictive tool in LUAD patients with lymph node metastasis.

In the present study, we identified a three-gene signature of LMRGs associated with distinct clinical characteristics, immune features, drug sensitivities, signaling pathways, and different prognoses, namely, GPD1L, SPHK1, and ST3GAL4.

Here, GPD1L, whose full name is glycerol-3-phosphate dehydrogenase-1-like, has glycerol-3-phosphate dehydrogenase activity. Liu et al.[31] reported that GPD1L expression was low in oral and HPV-opharyngeal cancer, and patients with early head and neck squamous cell carcinoma with reduced

GPD1L levels had a higher risk of LNM. SPHK1, full name sphingosine kinase 1, has been confirmed by Nagahashi et al., [32] who demonstrated that SPHK1 was up-regulated in patients with obesity-related breast cancer. In breast cancer-bearing mice, targeted inhibition of SPHK1 signaling could reduce obesity-related inflammation, S1P signal transduction, and lung metastasis and prolong survival. Additionally, several studies have shown that SPHK1 catalyzes the formation of S1P and promotes tumor cell proliferation, migration, invasion, and EMT[33]. ST3GAL4 encodes for β -galactosidase- α 2,3-sialyltransferase 4, showing involvement in the biosynthesis of tumor antigens sulfo-sLe (x) and sLe (x). Moreover, these elevated ST3GAL4 expression levels enhance the invasive ability of gastric adenocarcinoma cells[34].

Accordingly, the above studies resemble our results in terms of gene expression and physiopathological functions. It is suggested that GPD1L, SPHK1, and ST3GAL4 may be used as prognostic biomarkers for LUAD with LNM. Additionally, this signature may participate in the immune response in the TME by affecting various immune cells. Interestingly, we noted many types of antitumor immune cells (e.g., M1 macrophages, activated NK cells, and CD8 + T-cells) in the high-risk LMRG group, as well as a higher TMB score and higher expression of PDL-1 and LAG3 (immune checkpoint genes), which were expected to benefit from the immunotherapy and correlated with better clinical outcomes. We assumed that the final poor prognosis might be due to the infiltration of M0 macrophages and activated mast cells, which can create an immunosuppressive microenvironment in the LUAD-cohort. Based on previous literature, Frossi[35] demonstrated that the mast cells modulate immune responses and the pathogenesis of inflammatory disorders and cancers by turning the “resting state” into the “degranulating state” like a switch. Mast cell infiltration has been positively associated with tumor progression and poor prognoses, including gastrointestinal cancer[36], colorectal cancer[37], pancreatic cancer[38], and LUAD[39]. Specifically, activated mast cells can release excessive histamine, IL-10, and IL-12, which directly and indirectly influence T-cell function, polarizing engaged naive CD4 + T-cells toward a Th2 phenotype[40] as well as favoring the recruitment of Treg cells[41]. Moreover, compelling evidence obtained from MC-deficient mice showed that mast cells could also support MDSC activity in a B16 metastatic melanoma model[42]. Additionally, Rabenhorst[43] revealed that mast cells could enhance neoangiogenesis in T-cell neoplasms by producing VEGF and TNF- α . Furthermore, in a recent study on lung carcinoma, Qu[44] showed that mast cells could be recruited into the LUAD TME and promoted EMT of A549 cancer cells through the IL-8/Wnt/ β -catenin pathway. However, although the lipid metabolism-related disorder may condition the poor tumor outcome, we suggest the above situation could be greatly improved by targeting the LMRGs. Since many types of anticancer immune cells were also recruited in the site with increased TMB and expression of immune checkpoints, we hypothesized that targeted LMRG therapy might release the host potential antitumor effects.

Therefore, we further focus on the drug susceptibility and pathological mechanism in the high-risk LMRG subgroup. Conversely, patients in the high-risk group had increased tolerance to some classical TKIs and were sensitive to some chemotherapy drugs (e.g., cisplatin, docetaxel, paclitaxel), VEGF receptor inhibitor motesanib, and PPAR inhibitor olaparib. Furthermore, the mechanism may be related to the REACTIVE OXYGEN SPECIES PATHWAY and TNFA SIGNALING VIA NFKB signaling, as well as classic cancer-related

or metastatic cancer-associated pathways (e.g., DNA replication, mismatch repair, and cell cycle). As also found in a previous study, Canli et al.[45] provided convincing evidence that increased ROS can transform non-invasive tumors into invasive tumors by crosstalk between the ROS molecules, oxidative DNA damage, and TNF- α regulated signaling to orchestrate a microenvironment for promoting tumor progression. The results suggest LUAD patients with LNM who fail to respond to immunotherapy could be classified as having the high-risk LMRG signature and further considered for combined treatment with relevant chemotherapeutic drugs, VEGF inhibitors, or PPAR inhibitors.

Inevitably, the present study and multi-omics analyses generally have several limitations. First, this is a retrospective study based on public datasets. Thus, the relatively small sample size and inherent selection bias may have influenced the results. Second, we only validated the protein expression of LMRGs between the LUAD tumor and normal tissue due to a lack of knowledge regarding the lymph node metastatic site staining in the HPA database and the commercial TMA. Therefore, more experiments incorporating multiplatform analyses should be performed to confirm the association between the lipid metabolism-related signature and LUAD. Third, the LMRG-specific immune infiltration, immunotherapy response, and drug sensitivity were realized through bioinformatic algorithms with RNA-seq data. Accordingly, the available evidence is limited. Overall, our study only reflects certain aspects of the clinical and immune features in LNM-specific LUAD from the perspective of lipid metabolism reprogramming.

Conclusions

In summary, we identified an LMRG signature based on LUAD data stratified by LNM. Moreover, this finding of the three-gene signature not only reveals distinct metabolic features and the immune status of the TME for LUAD patients with or without LNM; it provides new insights into the exploration of molecular mechanisms and precision therapies of LUAD regional metastasis.

Declarations

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Author Contributions

SL conceived and directed this study, performed the bioinformatic analyses, and wrote the manuscript. ZSC analyzed the data and revised the manuscript. YMX and MH provided critical suggestions and material. YMZ, XYL, and JNZ performed a careful pathologic evaluation and the staining experiment. WHL and JXH supervised the study and contributed to the manuscript editing.

Availability of supporting data

The datasets used and analyzed in this study are available from the corresponding author on reasonable request.

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Conflict of interests

The authors declare no potential conflicts of interest.

Ethical Approval

Given that the datasets and commercial products used in this study are available in the public domain for research use, ethical approval was obtained from the Ethics committee of the First Affiliated Hospital of Guangzhou Medical University, and informed consent was waived.

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Figures

Figure 1

Identification of LMRGs in nodal staging-specific LUAD and the prognostic LMRG signature construction. **a** volcano plot of the up-and down-regulated lipid metabolism genes related to lymph node metastasis of lung adenocarcinoma in TCGA database. **b** ROC curve analysis for predicting the pathological N-stage for high-risk and low-risk LMRG subgroups. **c** ROC curve analysis of survival difference between LMRG subgroups in TCGA cohort. **d** ROC curve analysis of survival difference between LMRG subgroups in GSE50081 dataset. **e** Verification of identified LMRG signature in protein expression level using the HPA database (SPHK1/ST3GAL4) and IHC staining on a commercial tissue microarray (GPD1L). **f** Using the

median of risk score as a cut-off value for the prediction (risk score = 0.98) to divide LUAD patients into high- and low-risk LMRG subgroups. **g** Kaplan-Meier survival curve of LMRG signature in LUAD.

Figure 2

The LMRG signature can be used as a prognostic factor for LUAD and its differential genomic profiles. **a** Forest plot showing multivariate Cox regression analysis of the LMRG signature risk score associated with age, gender, and pathological staging of TCGA-LUAD cohort. **b** Nomogram developed using multivariate Cox regression analysis to evaluate LUAD prognosis (12-, 24-, 36-month survival rates). **c** Differential somatic mutations in LUAD with low and risk LMRG signature. **d** KM survival curve of OS rates associated with LMRGs in LUAD patients with several frequently mutated genes (e.g., TP53 mutation, KRAS mutation, EGFR mutation, $p < 0.05$).

Figure 3

Figure 3

The correlation between LMRG signature and immune cells in the TME. **a** association analysis between immune cell content and patients with low- and high-risk LMRG signature scores. **b** The relationship between LMRG signature and immune, stromal, and estimate scores; *, **, and *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$.

Figure 4

Figure 4

The LMRG signature has potential immunotherapeutic implications. **a** The distribution of TMB, MSI, and neoantigens in the low- and high-LMRG signature. **b** TIDE databases predict the responses of patients in different signature subgroups to immunotherapy. **c** Evaluation of the correlation between immune checkpoint gene expression (PDL-1 and LAG3) and LMRG signature; *, **, and *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$.

Figure 5

Figure 5

The relationship between LMRG signature and drug sensitivity. **a** The upper panels show representative IC50-resistant drugs between LMRG signatures. **b** The lower panels show representative IC50-sensitive

drugs between LMRG signatures; the IC50 value of each drug was displayed in each graph for drug sensitivity measurement.

Figure 6
a

Figure 6

Functional and pathway enrichment analyses of LMRG signature. **a** Gene set variation analysis (GSVA) showed the correlations between high-risk LMRGs and cancer-related functions. **b** Gene set enrichment analysis (GSEA) showed the associations between high-risk LMRGs and classic cancer-related or metastatic cancer-related pathways. **c** Downstream genes involved in the related pathway, which are potential target molecules for intervention.

Supplementary Files

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