Tropical Journal of Pharmaceutical Research March 2023; 22 (3): 543-552
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v22i3.11

Original Research Article

Identification of immune regulatory protein-coding genes in the development of lung adenocarcinoma (LUAD)

Wen Ge[1], Hua Tang[2], Jing Wang[3], Yichun Xu[4,5,*], Haitao Ma[1*]
[1]Department of Thoracic Surgery, The First Affiliated Hospital of Soochow University, 215006, Suzhou, P. R. China, [2]Department of Thoracic and Cardiovascular Surgery, Changzheng Hospital, affiliated to Naval Medical University, 200003, Shanghai, P. R. China, [3]Department of Thoracic and Cardiovascular Surgery, Shuguang Hospital, affiliated to Shanghai University of Traditional Chinese Medicine, 201203, Shanghai, P. R. China, [4]National Engineering Research Center for Biochip, ShanghaiBiochip Limited Corporation, 201203, Shanghai, P. R. China, [5]Department of Pathology, Shanghai Tongji Hospital, Tongji Hospital Affiliated to Tongji University, 200065, Shanghai, P. R. China

For correspondence:* ***Email:* *Haitao_ma61@163.com (Haitao Ma); crystalxyc@hotmail.com (Yichun Xu)*

Sent for review: 9 September 2022 *Revised accepted: 19 February 2023*

Abstract

Purpose: *To verify the differential expressed genes (DEGs) based on The Cancer Genome Atlas (TCGA) using RNA-seq data from eight LUAD patients*
Methods: *TCGA database was used for extraction and analysis of RNA-seq profile in LUAD patients. Then RNA sequencing was performed using the tissue samples from eight LUAD patients for comparison. Weighted correlation network analysis (WGCNA) was applied to establish the co-expression modules and identify hub genes using RNA-seq data from the eight LUAD patients. Protein-protein interaction (PPI) analysis was used to reveal hub genes and GO analysis was performed for functional annotation including immune responses.*
Results: *ALDH3A1, METTL7B, WFDC2 and CDH23 that had close correlation with the immune system were identified by TCGA and validated by RNA-seq from the eight LUAD patients. IL17REL was revealed as a potential oncogene in LUAD.*
Conclusion: *This study highlighted potentially useful biomarkers associated with LUAD development and diagnosis.*

Keywords: *Biomarkers. Immune regulator. Lung adenocarcinoma. Weighted correlation network analysis.*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Lung cancer contains small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), in which the latter takes up around eighty percent among all cases [1, 2]. LUAD, the top prevalent subtype, occupies approximately 60 percent of NSCLC, and LUAD is most frequently found in non-smokers and in East Asian populations [3]. Despite advancements in treatment, the 5-year survival rate in patients with LUAD is merely 20 percent [4]. Hence, the discovery of potential approaches to diagnosing and treating LUAD might help reduce the morbidity and mortality.

High-throughput RNA-sequencing, (RNA-Seq) provides a platform to analyze transcriptome comprehensively and seek new potential therapeutic targets[5]. High throughput data from TCGA is a rich resource for screening differential gene expression. Knowing the molecular characteristics of LUAD is very important for early diagnosis and further better treatment. Recently, some novel biomarkers have been identified for LUAD. For example, low expression of RS1 or inositol polyphosphate-5-phosphatase b (INPP5B) might be a potential independent prognostic indicator for LUAD [6]. Higher CDC25C expression was upregulated in LUAD, which could accelerate malignant progression of LUAD, and restrained efficacy nivolumab in LUAD treatment [7]. As with other types of lung cancer, LUAD treatment has become more biomarker-driven [8]. Hence, the exploration of novel diagnostic or prognostic biomarkers might still facilitate the treatment of LUAD. Thus, the purpose of this study was to verify the differential expressed genes (DEGs) based on The Cancer Genome Atlas (TCGA) using RNA-seq data from eight LUAD patients.

METHODS

TCGA data analysis

The RNA-seq profiles from TCGA database were extracted, which included 522 pairs of tumor tissue samples and adjacent normal ones. 'Maftools' in R package was applied to visualize data. Additionally, the edgeR package was applied for analysis of DEGs. The cut-off criteria were absolute log2 fold change >1 and p-value <0.05. In addition, hierarchical clustering was applied using the Pheatmap package in R. The cluster profile package was applied for pathway analysis and biological function analysis. Potential biological functions were predicted using Gene Set Enrichment Analysis (GSEA).

Sample collection and ethical statement

To verify the results, data from TCGA data was analyzed. Normal and neoplastic tissues were collected from eight patients when they had thoracoscopic surgery for LUAD, and then the tissue samples were analyzed for RNA-seq profile. The study was approved by the Ethics Committee in Shuguang Hospital (2021-931-06-01). Informed written consent was obtained. All subjects were Chinese with random sex and age. The baseline characteristics of the eight LUAD patients were shown in Table 1.

Table 1: Clinical characteristics of study population

LUAD patients (n=8)	N (%)
Gender	
Male	4 (50)
Female	4 (50)
Age (yr) at diagnosis	
<60	4 (50)
≥60	4 (50)
Tobacco	
Yes	5 (62.5)
No	3 (37.5)
Histology type	
Microinvasive adenocarcinoma (MIA)	4 (50)
Invasive adenocarcinoma (IA)	4 (50)
EGFR	
Positive	3 (37.5)
Negative	5 (62.5)
TNM	
I	7 (87.5)
II	1 (12.5)
Differentiation	
Well	6 (75)
Moderate	1 (12.5)
Poor	1 (12.5)

RNA extraction and analysis of the eight pairs of samples

The normal and tumor tissue samples from the eight LUAD patients were preserved in RNAlater solution (Thermo Fisher Scientific Inc, Waltham, MA USA), after resection, and kept at -80°C. Total RNA was extracted using TRIzol™ reagent as described previously (Thermo Fisher Inc, Waltham, MA USA). NanoDrop One spectrophotometer (Thermo Fisher Inc, Waltham, MA USA) was applied for determining RNA concentration and the Agilent 4200 Bioanalyzer was used to evaluate RNA quality. RNA sequencing was carried out on an Illumina platform and paired-end 150 bp raw reads were generated. The preliminary processing of the data was as described before [9]. Bioinformatic analysis was performed on R v.3.6.3. The identification of DEGs and GSEA was performed. WGCNA analysis was aimed to discover co-expressed gene modules, and to find connections within gene networks and phenotypes of hub genes.

The interacting proteins and GO analysis

To further target and screen more meaningful hub genes, the STRING database (https://string-

db.org/) was used to construct a PPI network [10, 11]. The STRING software was applied for analysis of functional protein association network

RESULTS

DEGs and enrichment analysis in LUAD patients from TCGA dataset

DEG analysis revealed 6210 upregulated and 2430 downregulated genes (Figure 1A). The GSEA further revealed DEGs by GO categories. The 10 most obviously enriched GO terms for biological process (BP), molecular function (MF), and cellular component (CC) were shown in Figure 1B, C and D. Furthermore, the GSEA analysis revealed enrichment of hub genes in immune-related GO terms, including ACTIVATION OF IMMUNE RESPONSE (GO:0002253), adaptive immune response (GO:0002250), B CELL MEDIATED IMMUNITY (GO:0019724), IMMUNOGLOBULIN COMPLEX (GO:0019814), and IMMUNOGLOBULIN RECEPTOR BINDING (GO:0034987) (Figure 2A-E).

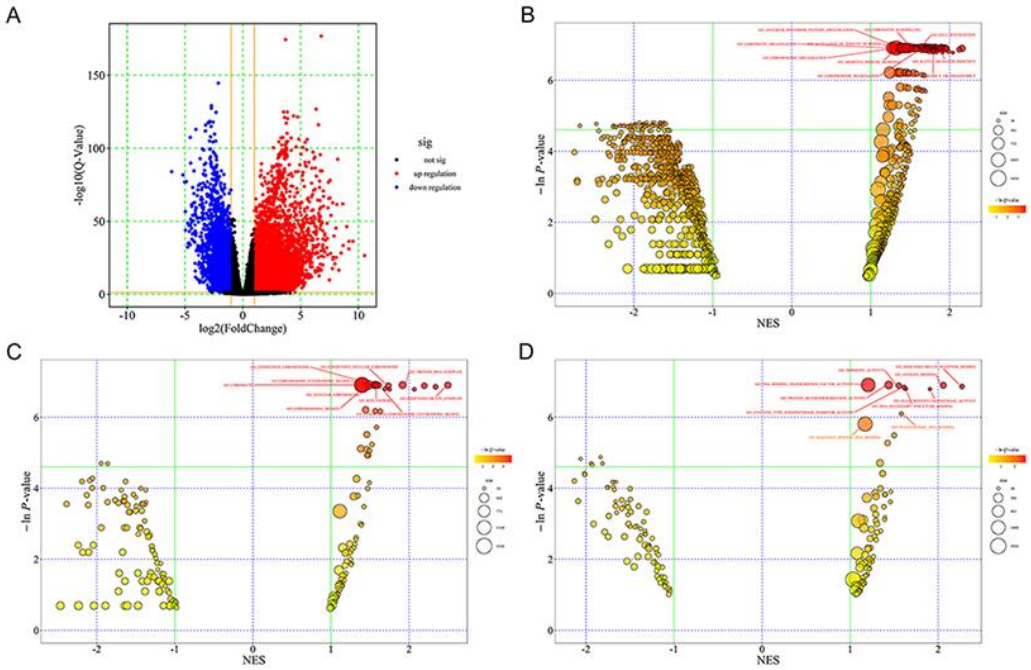

Figure 1: DEGs and enrichment analysis in LUAD patients of TCGA dataset.
(A) Volcano analysis exhibited differentially expression with edgeR. (B) GSEA analysis exhibited differentially enriched GO categories in biological process (BP). (C) GSEA analysis exhibited differentially enriched GO categories in cellular component (CC). (D) GSEA analysis exhibited differentially enriched GO categories in molecular function (MF).

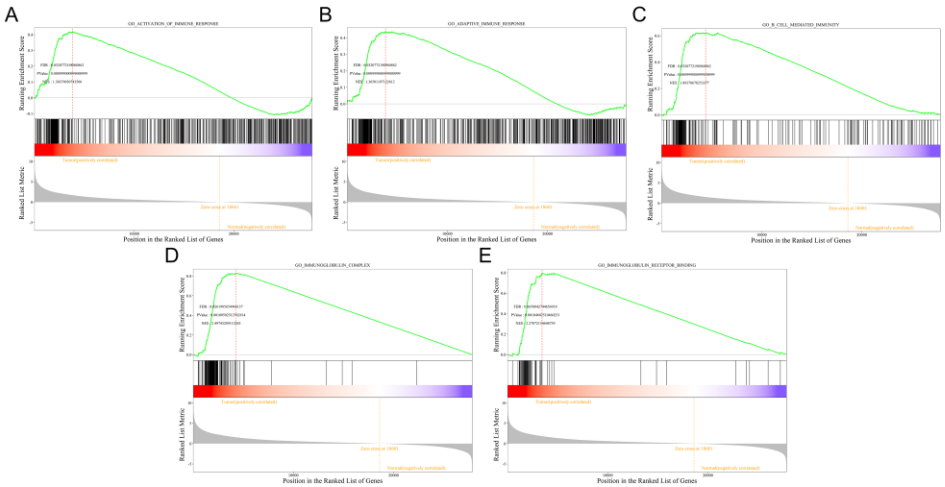

Figure 2: The immune related and enriched GO terms in LUAD patients of TCGA dataset. GSEA revealing enrichment of (A) activation of immune response. (b) adaptive immune response. (C) B cell mediated immunity. (D) immunoglobulin complex. (E) immunoglobulin receptor binding

DEGs and enrichment analysis in tissue samples from 8 LUAD patients

DEGs were listed using a heatmap (Figure 3A). The DEGs analysis revealed 626 upregulated and 470 downregulated genes. The differential enriched GO terms by GSEA analysis for RNA-seq are shown in Figure 3B-D. GSEA analysis of RNA-seq revealed that enrichment of hub genes in immune related GO categories, including ADAPTIVE IMMUNE RESPONSE (GO:0002250), ACTIVATION OF IMMUNE RESPONSE (GO:0002253), IMMUNE RESPONSE REGULATING SIGNALING PATHWAY (GO:0002764), ADAPTIVE IMMUNE RESPONSE BASED ON SOMATIC RECOMBINATION OF IMMUNE RECEPTORS BUILT FROM IMMUNOGLOBULIN SUPERFAMILY DOMAINS (GO:0002460), IMMUNOGLOBULIN COMPLEX (GO:0019814), IMMUNOGLOBULIN COMPLEX CIRCULATING (GO:0042571) AND IMMUNOGLOBULIN RECEPTOR BINDING (GO:0034987) (Figure 4A-G).

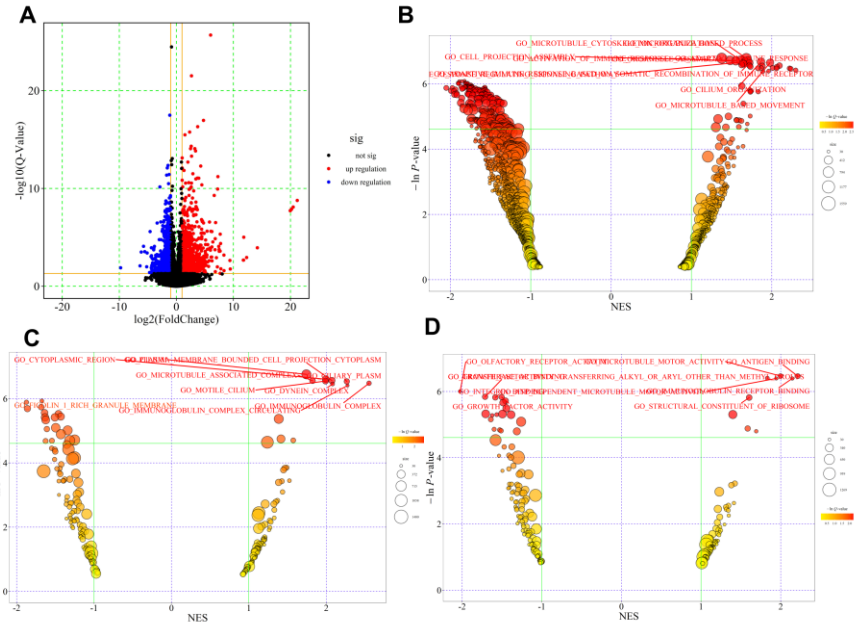

Figure 3: DEGs and enrichment analysis between tumor and adjacent tissues of LUAD patients.
(A) Volcano analysis exhibited differentially expression in LUAD patients. Red dots are for up-regulated genes and green dots for down-regulated genes. (B) GSEA analysis exhibited differentially enriched GO categories in biological process (BP) of LUAD patients. (C) GSEA analysis exhibited differentially enriched GO categories in cellular component (CC) of LUAD patients. (D) GSEA analysis exhibited differentially enriched GO categories in molecular function (MF) of LUAD patients

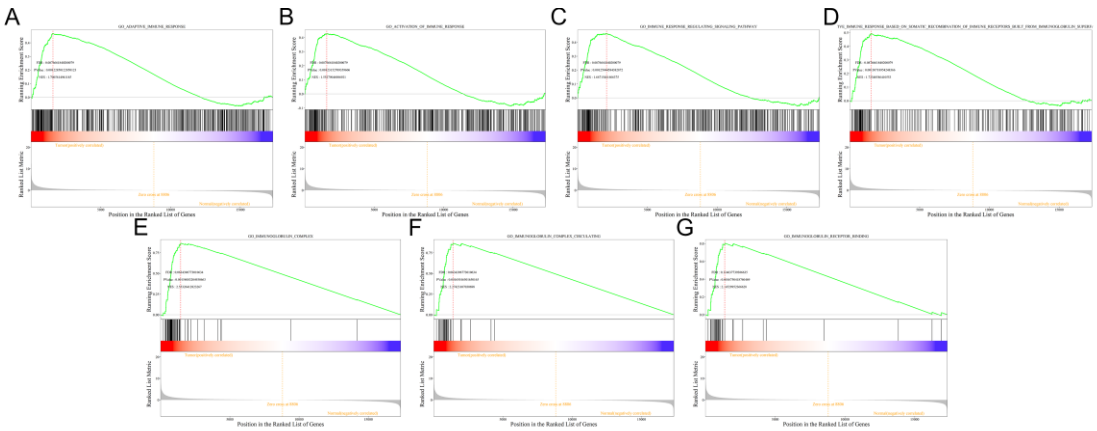

Figure 4: The immune related and enriched GO terms in LUAD patients
GSEA revealing enrichment of (A) adaptive immune response, (b) activation of immune response, (C) immune response regulating signaling pathway, (D) adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains, (E) immunoglobulin complex, (F) immunoglobulin complex circulating, (G) immunoglobulin receptor binding

WGCNA analysis for co-expression network

The co-expression network was analyzed using WGCNA after normalization of expressed genes. After highly similar modules were merged, the co-expression modules were built and delineated in different colors (Figure 5A). Then, 436 genes in the midnightblue module did not belong to others. The counts of genes were 5326 (black), 2641 (brown), 467 (cyan), 1508 (green), 310 (grey60), 395 (lightcyan), 2219 (pink), 747 (purple), 5345 (salmon), and 606 (tan). The module–trait associations were built on WGCNA algorithm and shown in Figure 5B. Among the 11 modules, the midnightblue module was the most relevant for LUAD (Cor: 0.81; $P=1.24\times10^{-4}$), indicating most relevant mRNA expression profiles in LUAD. Furthermore, 436 genes of this module were chosen for the heatmap (Figure 5C). The module membership in midnightblue module showed positive correlation with gene significance (Cor: 0.65; $P=1.1\times10^{-53}$) (Figure 5D).

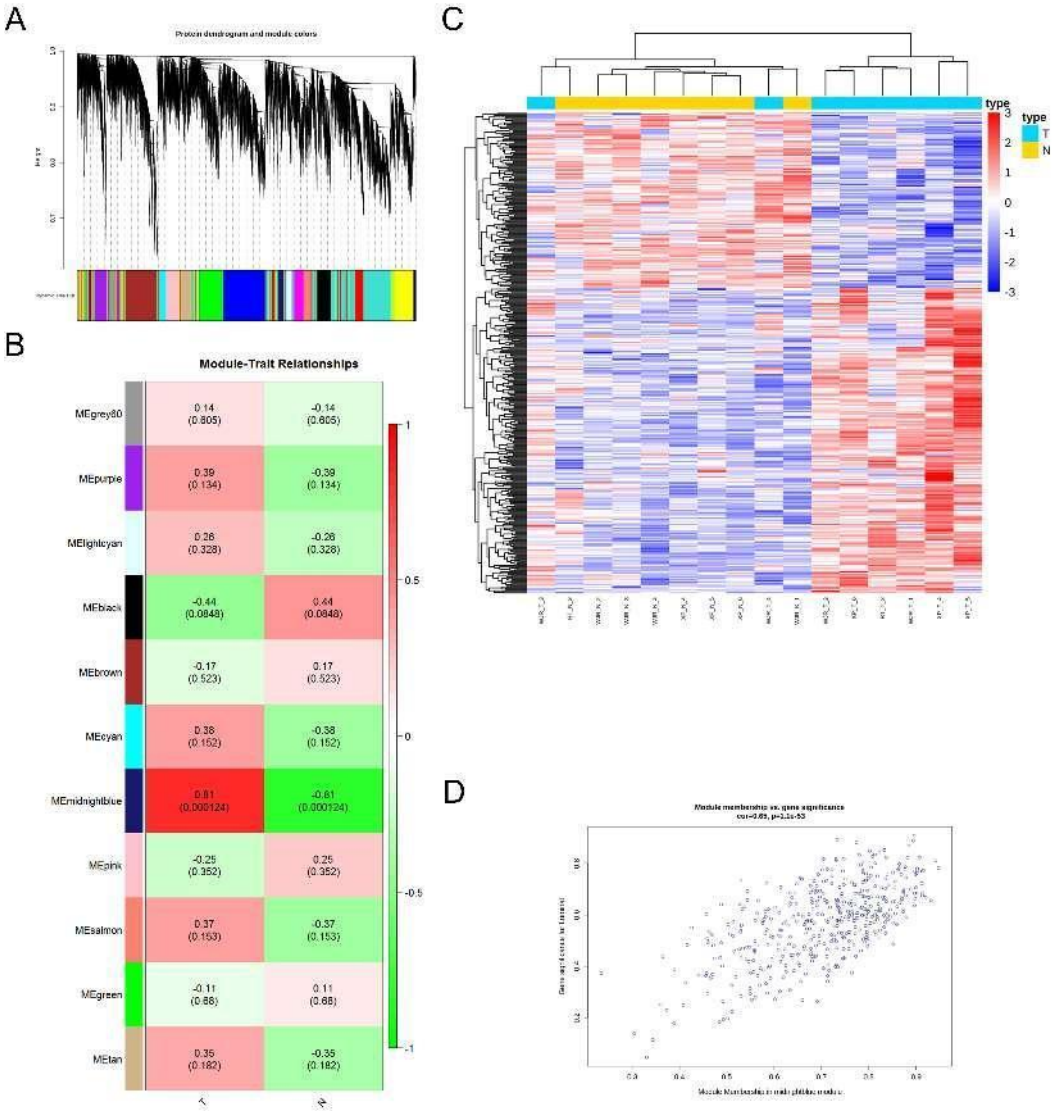

Figure 5: WGCNA analysis for mRNA.
(A) Clustering dendrograms of genes with dissimilarity based on the topological overlap (B) Module–trait associations. Each row shows a module eigengene, each cell displays the corresponding correlation as well as P-value. (C) Hierarchical clustering shows a distinguishable expression profiling between tumor and adjacent tissues of LUAD patients. (D) Scatter plots of module eigengenes in correlation with histologic grade in the midnightblue module.

The interacting proteins and GO analysis

A total of 8640 DEGs were identified in TCGA dataset, while 1096 DEGs were found in RNA-seq and 436 phenotype-specific genes in the midnightblue module. Thereafter, a Venn diagram was created to distinguish overlapped hub genes and 47 specific genes showed their association with LUAD (Figure 6A). Then, a heatmap was used to present hub gene expressions (Figure 6B). both TCGA and RNA-

seq cohorts derived from our source. To better understand key genes for LUAD, a PPI network for these 41 common genes was established on STRING (Figure 6C). Interestingly, aldehyde dehydrogenase 3 family member A1 (ALDH3A1), cytochrome P450 family 26 subfamily B member 1 (CYP226B1), methyltransferase like 7B (METTL7B), breast carcinoma amplified sequence 1 (BCAS1), mucin 6, oligomeric mucus/gel-forming (MUC6), WAP four-disulfide core domain 2 (WFDC2) and cadherin related 23 (CDH23) were in the core of PPI networks. For the membership between midnightblue module and gene significance, the coefficient for module membership in midnightblue module >0.6 and gene significance >0.6 were set as the cut-off criteria and a total of 188 genes were identified. Combined with the 41 hub genes, a Venn diagram was generated to identify the overlapping key genes specifically associated with the occurrence of LUAD, a total of 28 key genes were identified. The 28 key gene expressions in TCGA dataset and RNA-seq were shown in Figure 6D. Among the 28 key genes, ALDH3A1, METTL7B, WFDC2 and CDH23, the core position genes of PPI networks, were included. ALDH3A1 was involved in the processes of oxidation reduction, response to oxygen levels, response to oxygen containing compound, closely related to oxidative stress. METTL7B-regulated metabolites were significantly enriched in the glutathione metabolic pathway, accompanied by a decrease in reactive oxygen species (ROS), an upregulation of the expression and enzymatic activity of the antioxidant genes glutathione peroxidase 4 (GPX4), heme oxygenase 1 (HMOX1) and superoxide dismutase (SOD1) [12]. WFDC2 encodes human epididymis protein 4 (HE4) protein and expresses in pulmonary epithelial cells. CDH23, encoding calcium dependent cell-cell adhesion glycoproteins, is a member of the cadherin superfamily.

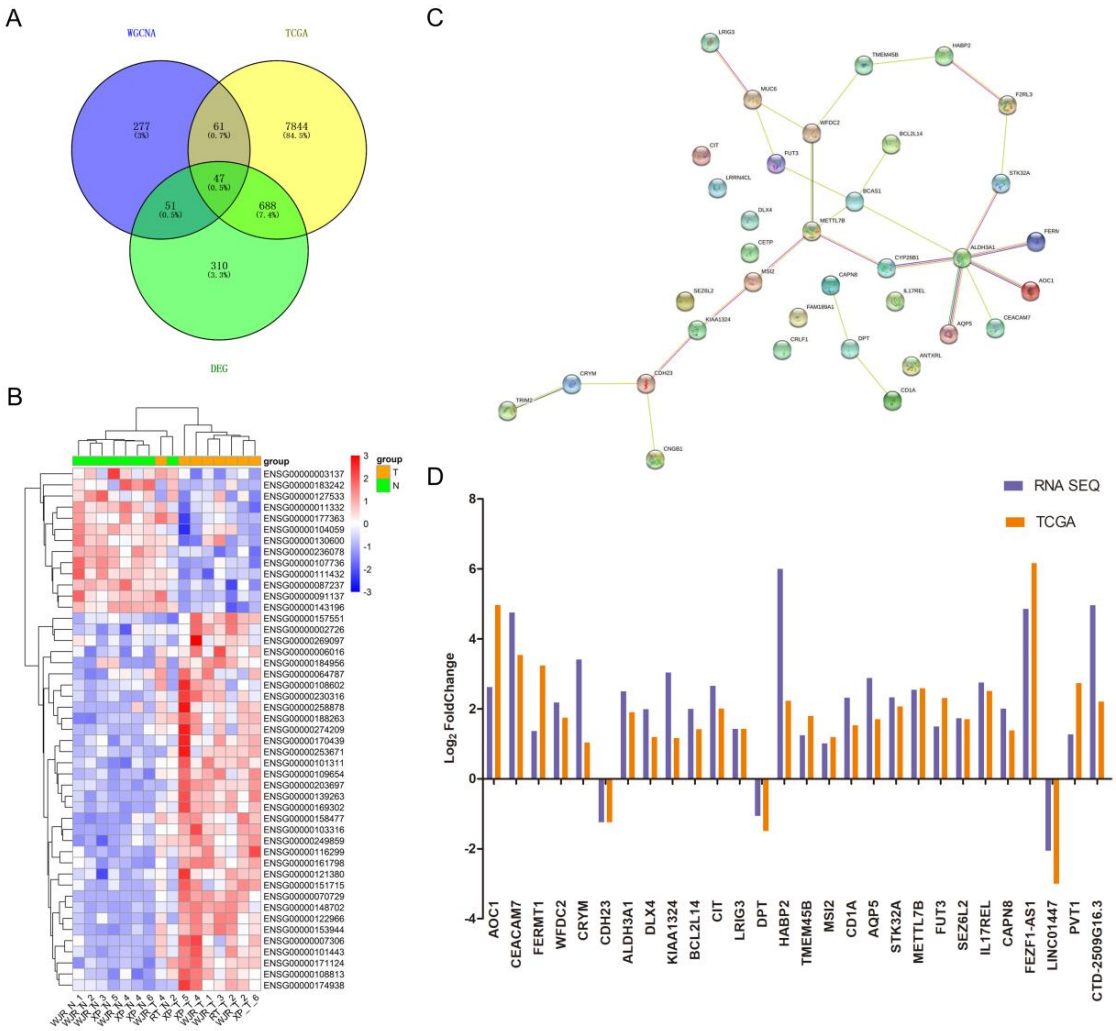

Figure 6: PPI construction, module analysis, and lncRNA-mRNA co-expression network.
(A) A Venn diagram of the genes from the module and edgeR. (B) Top 20 significant KEGG pathways. (C) PPI of DEGs. (D) The global view of the network

IL17REL as a new biomarker of LUAD

Among the 28 key genes (Figure 6D), IL-17REL attracted special attention while its association with cancer has not yet been mentioned. In our LUAD cohort, IL-17REL was involved in RESPONSE TO CYTOKINE (GO: 0034097), CYTOKINE MEDIATED SIGNALING PATHWAY (GO: 0019221), IMMUNE RECEPTOR ACTIVITY (GO: 0140375), INTERLEUKIN 17 RECEPTOR ACTIVITY (GO: 0030368) and MOLECULAR TRANSDUCER ACTIVITY (GO: 0060089), which were mainly associated with immune regulatory. In the GEPIA database (http://gepia.cancer-pku.cn/), IL17REL expression was elevated in LUAD tumor tissue samples (483 *vs*. 347; $P < 0.01$) (Figure 7A). TCGA database also revealed that IL17REL expressions were upregulated in LUAD tumor tissue samples (515 *vs*. 59; $P = 9.887\times10^{-9}$) (Figure 7B). The UALCAN database (http://ualcan.path.uab.edu) was applied for identifying genes interplayed with IL17REL, indicating that 204 genes were interacted with the IL17REL gene. The most expression-related genes of IL17REL included Wnt family member 11 (WNT11), RAS protein activator like 1 (RASAL1), mirror-image polydactyly 1 (MIPOL1), forkhead box N3 (FOXN3) and BCL2 apoptosis regulator (BCL2), with all the correlation coefficients were above 0.6.

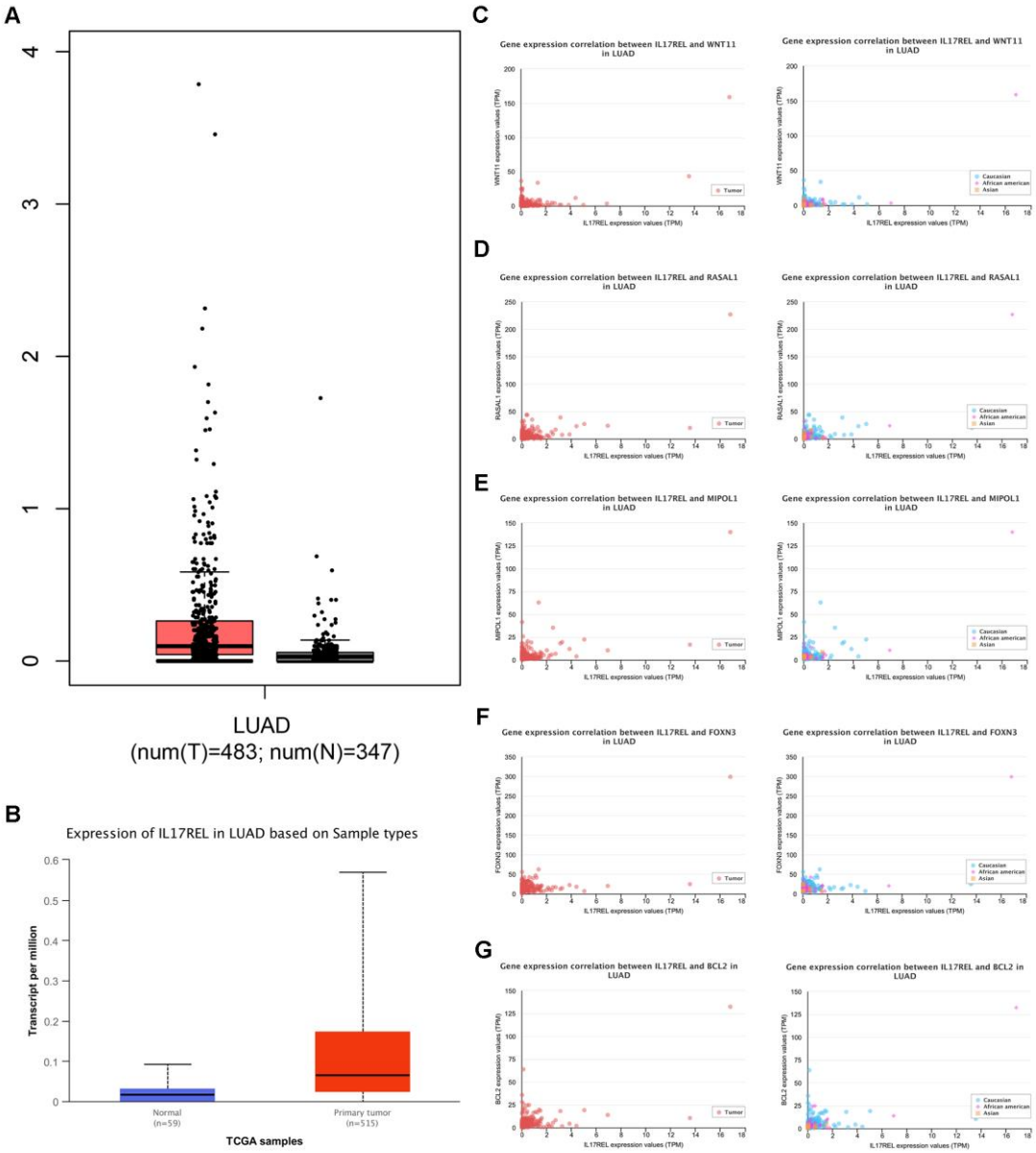

Figure 7: Expression of IL17REL and its expression correlation in LUAD
(A) Expression of IL17REL in LUAD tissues in GEPIA database. (B) Expression of IL17REL in LUAD and normal tissues of TCGA database. (C-G) Expression correlation between IL17REL and WNT11/RASAL1/MIPOL1/FOXN3/BCL2 in LUAD

DISCUSSION

In this current study, researchers first observed that a few DEGs were mainly enriched in immune system gene sets in LUAD patients from TCGA database. The similar results were then confirmed in the RNA-seq data from the eight patients. Both in the enriched gene sets of TCGA dataset and RNA-seq analyzed by GESA, the occurrence of LUAD was linked to the processes of adaptive immune response and immune response activation. In the cohort of TCGA database, B cell mediated immunity was also found to be a vital category. Anti-tumor B cells mainly rely on the secretion of antibodies against tumor-associated antigens (TAA) and provide costimulatory signals to TAA-specific CD4+ T cells to activate T cells. Antibodies produced by B cells can directly kill tumor cells by means of antibody-dependent cytotoxicity (ADCC) and phagocytosis and can also activate T cell immune responses by presenting TAA to T cells through DC cells. B cells can also act as a professional antigen presenting cell (APC) to activate T cells [13]. In the cohort of RNA-seq, immune response regulating signaling pathway was an enriched gene set in LUAD patients. Many studies demonstrated that targeting the metabolic pathways in tumor tissues might overcome deleterious impacts of metabolic competition on tumors and the immune system to facilitate tumor immunogenicity [14].

Four hub genes were identified in this research, including ALDH3A1, METTL7B, WFDC2 and CDH23. As an important NAD(P)+dependent enzyme, ALDH3A1 was involved in the processes of oxidation reduction, response to oxygen levels and oxygen containing compound, with effects on energy metabolism. Immune responses might target metabolic pathways in anticancer immunotherapy by metabolic reprogramming of immune or cancer cells [15]. ALDH3A1 had potential contribution to proliferation, cellular metabolism, and apoptosis suppression [16]. Clinical survival analysis also demonstrated that high ALDH3A1 expressions were associated with bad prognosis of lung cancer patients [17]. ALDH3A1 overexpression and activation facilitated tumor stemness, epithelial–mesenchymal transition (EMT) and elevated PD-L1 expressions, suggesting that aldehyde detoxification might tune the immunological output of malignancies [18]. Exosomes carrying ALDH3A1 from A549 cells enhance migratory and invasive capacities of recipients via facilitating glycolysis [17]. METTL7B, a member of the methyltransferase-like family, participated in tumorigenesis, invasiveness, and migration in various malignancies [19]. METTL7B expression has been verified elevated in LUAD tumor tissue samples and serum in clinical stage [20]. Furthermore, METTL7B is a promising biomarker that can reverse EGFR-TKIs resistance in LUAD by upregulated antioxidant enzymes expressions via mRNA m6A modification [12]. METTL7B is implicated in modulating inflammatory cytokines production and macrophage polarization [21]. HE4, as a product of the WFDC2 gene, may be promising in diagnosing, prognosis, and treatment of LUAD. Serum HE4 could be used for monitoring recurrence of LUAD in patients after treatment [22]. HE4 can activate STAT3 signaling to elevate IL8 and HIF1A in immune cells, resulting in promoted angiogenesis and immunosuppression [23]. HE4 is also closely related to immune regulation. One study found that HE4 suppressed tumor immune microenvironment to upregulate PD-L1 expressions in cancer cells and macrophages [24]. CDH23 acted vitally in intercellular adhesion of cancers, thereby improving prognosis of patients [25]. Elevated CDH23 expression facilitated infiltration of monocytes, which could respond to initiate immunotherapy [26].

\his study is the first to report impacts of IL-17REL on LUAD. IL-17REL, a homolog of IL17RE, is a member of the IL17R family. Bioinformatics analysis speculated that IL-17REL might played vital roles in initiating the Th2-mediated immune responses according to the protein sequences [27]. Researchers also found the IL-17REL was elevated in LUAD tissue samples and co-expressed with several oncogenic genes including WNT11, RASAL1, MIPOL1, FOXN3, and BCL2, which were closely related to tumorigenesis [28].

CONCLUSION

DEGs including ALDH3A1, METTL7B, WFDC2 and CDH23 that had close correlation with the immune system, were identified by TCGA and RNA-seq. Furthermore, the present study revealed that IL-17REL may serve as an oncogene in LUAD. These data altogether demonstrated that these results highlight potentially useful candidate biomarkers associated with LUAD development in diagnosis.

DECLARATIONS

Acknowledgements

None.

Funding

This work was supported by Jiangsu Natural Science Fund (Grant No. BK20191174) and Shanghai Rising-Star Program (Grant No.19QB1404700).

Ethical approval

The study was approved by the Ethics Committee in Shuguang Hospital (2021-931-06-01).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Haitao Ma and Yichun Xu conceived and designed the work, Wen Ge, Hua Tang, and Jing Wang collected the data, Wen Ge and Yichun Xu analysed and interpreted the data, Wen Ge wrote a draft of the manuscript which was read, modified and approved by all authors for publication while Haitao Ma and Yichun Xu did the critical review for important intellectual content.

REFERENCES

1. *Siegel RL, Miller KD, Fuchs HE,Jemal A. Cancer Statistics, 2021. CA: a cancer journal for clinicians 2021; 71(1): 7-33.*
2. *Peng L, Deng HY, Liu ZK, Shang QW, Huang KL, Zheng QQ, Li W,Wang Y. Wedge Resection vs. Stereotactic Body Radiation Therapy for Clinical Stage I Non-small Cell Lung Cancer: A Systematic Review and Meta-Analysis. Front Surg 2022; 9: 850276.*
3. *Sharma SV, Bell DW, Settleman J,Haber DA. Epidermal growth factor receptor mutations in lung cancer. Nature reviews. Cancer 2007; 7(3): 169-181.*
4. *Lu T, Yang X, Huang Y, Zhao M, Li M, Ma K, Yin J, Zhan C,Wang Q. Trends in the incidence, treatment, and survival of patients with lung cancer in the last four decades. Cancer management and research 2019; 11: 943-953.*
5. *Stark R, Grzelak M,Hadfield J. RNA sequencing: the teenage years. Nature reviews. Genetics 2019; 20(11): 631-656.*
6. *Deng J, Lin X, Li Q, Cai XY, Wu LW, Wang W, Zhang B, Li YL, Hu J,Lin NM. Decreased INPP5B expression predicts poor prognosis in lung adenocarcinoma. Cancer cell international 2022; 22(1): 189.*
7. *Zhang W, Shang X, Yang F, Han W, Xia H, Liu N, Liu Y,Wang X. CDC25C as a Predictive Biomarker for Immune Checkpoint Inhibitors in Patients With Lung Adenocarcinoma. Frontiers in oncology 2022; 12: 867788.*
8. *Deb D, Moore AC,Roy UB. The 2021 Global Lung Cancer Therapy Landscape. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer 2022; 17(7): 931-936.*
9. *Lei K, Zhang L, He Y, Sun H, Tong W, Xu Y,Jin L. Immune-associated biomarkers for early diagnosis of Parkinson's disease based on hematological lncRNA-mRNA co-expression. Bioscience reports 2020; 40(12).*
10. *von Mering C, Huynen M, Jaeggi D, Schmidt S, Bork P,Snel B. STRING: a database of predicted functional associations between proteins. Nucleic acids research 2003; 31(1): 258-261.*
11. *Yu G, Wang L, Li Y, Ma Z,Li Y. Identification of drug candidate for osteoporosis by computational bioinformatics analysis of gene expression profile. European Journal of Medical Research 2013; 18(1): 5.*
12. *Song H, Liu D, Wang L, Liu K, Chen C, Wang L, Ren Y, Ju B, Zhong F, Jiang Xet al. Methyltransferase like 7B is a potential therapeutic target for reversing EGFR-TKIs resistance in lung adenocarcinoma. Molecular cancer 2022; 21(1): 43.*
13. *Hong S, Zhang Z, Liu H, Tian M, Zhu X, Zhang Z, Wang W, Zhou X, Zhang F, Ge Qet al. B Cells Are the Dominant Antigen-Presenting Cells that Activate Naive CD4(+) T Cells upon Immunization with a Virus-Derived Nanoparticle Antigen. Immunity 2018; 49(4): 695-708.e694.*
14. *Kareva I. Metabolism and Gut Microbiota in Cancer Immunoediting, CD8/Treg Ratios, Immune Cell Homeostasis, and Cancer (Immuno)Therapy: Concise Review. Stem cells (Dayton, Ohio) 2019; 37(10): 1273-1280.*
15. *Xia L, Oyang L, Lin J, Tan S, Han Y, Wu N, Yi P, Tang L, Pan Q, Rao Set al. The cancer metabolic reprogramming and immune response. Molecular cancer 2021; 20(1): 28.*
16. *Fan F, Yin R, Wang L, Zhao S, Lv D, Yang K, Geng S, Yang N, Zhang X,Wang H. ALDH3A1 driving tumor metastasis is mediated by p53/BAG1 in lung adenocarcinoma. Journal of Cancer 2021; 12(16): 4780-4790.*
17. *Wang C, Xu J, Yuan D, Bai Y, Pan Y, Zhang J,Shao C. Exosomes carrying ALDOA and ALDH3A1 from irradiated lung cancer cells enhance migration and invasion of recipients by accelerating glycolysis. Molecular and cellular biochemistry 2020; 469(1-2): 77-87.*
18. *Terzuoli E, Bellan C, Aversa S, Ciccone V, Morbidelli L, Giachetti A, Donnini S,Ziche M. ALDH3A1 Overexpression in Melanoma and Lung Tumors Drives Cancer Stem Cell Expansion, Impairing Immune Surveillance through Enhanced PD-L1 Output. Cancers 2019; 11(12).*

19. Li W, Xu S, Peng N, Zhang Z, He H, Chen R, Chen D, Fan J,Wang X. Downregulation of METTL7B Inhibits Proliferation of Human Clear Cell Renal Cancer Cells In Vivo and In Vitro. Frontiers in oncology 2021; 11: 634542.
20. Ali J, Liu W, Duan W, Liu C, Song J, Ali S, Li E,Wang Q. METTL7B (methyltransferase-like 7B) identification as a novel biomarker for lung adenocarcinoma. Annals of translational medicine 2020; 8(18): 1130.
21. Huang D,Yan H. Methyltransferase like 7B is upregulated in sepsis and modulates lipopolysaccharide-induced inflammatory response and macrophage polarization. Bioengineered 2022; 13(5): 11753-11766.
22. Muley T, He Y, Rolny V, Wehnl B, Escherich A, Warth A, Stolp C, Schneider MA, Meister M, Herth FJet al. Potential for the blood-based biomarkers cytokeratin 19 fragment (CYFRA 21-1) and human epididymal protein 4 (HE4) to detect recurrence during monitoring after surgical resection of adenocarcinoma of the lung. Lung cancer (Amsterdam, Netherlands) 2019; 130: 194-200.
23. James NE, Emerson JB, Borgstadt AD, Beffa L, Oliver MT, Hovanesian V, Urh A, Singh RK, Rowswell-Turner R, DiSilvestro PAet al. The biomarker HE4 (WFDC2) promotes a pro-angiogenic and immunosuppressive tumor microenvironment via regulation of STAT3 target genes. Scientific reports 2020; 10(1): 8558.
24. Rowswell-Turner RB, Singh RK, Urh A, Yano N, Kim KK, Khazan N, Pandita R, Sivagnanalingam U, Hovanesian V, James NEet al. HE4 Overexpression by Ovarian Cancer Promotes a Suppressive Tumor Immune Microenvironment and Enhanced Tumor and Macrophage PD-L1 Expression. Journal of immunology (Baltimore, Md. : 1950) 2021; 206(10): 2478-2488.
25. Singaraju GS, Sagar A, Kumar A, Samuel JS, Hazra JP, Sannigrahi MK, Yennamalli RM, Ashish F,Rakshit S. Structural basis of the strong cell-cell junction formed by cadherin-23. The FEBS journal 2019; 287(11): 2328-2347.
26. Park JA, Wang L,Cheung NV. Modulating tumor infiltrating myeloid cells to enhance bispecific antibody-driven T cell infiltration and anti-tumor response. Journal of hematology & oncology 2021; 14(1): 142.
27. Gaffen SL. Structure and signalling in the IL-17 receptor family. Nature reviews. Immunology 2009; 9(8): 556-567.
28. Liu L, Cheng X, Yang H, Lian S, Jiang Y, Liang J, Chen X, Mo S, Shi Y, Zhao Set al. BCL-2 expression promotes immunosuppression in chronic lymphocytic leukemia by enhancing regulatory T cell differentiation and cytotoxic T cell exhaustion. Molecular cancer 2022; 21(1): 59.