Exploratory Research and Hypothesis in Medicine **2021** vol. 6(3) | 99–109
DOI: 10.14218/ERHM.2021.00009

Original Article

RUNX2 as a Potential Prognosis Biomarker and New Target for Human Lung Cancer

Dan Xiao[1], Ke Liu[2], Jun Chen[2], Yiyi Gong[3], Xiaobo Zhou[4*] and Jia Huang[3*]

[1]Department of Respiratory Medicine, Xishui People's Hospital, Hubei Province, P.R. China; [2]Department of Dermatology, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University, School of Medicine, Shanghai, P.R. China; [3]Department of Dermatology, Huashan Hospital, Fudan University, Shanghai, P.R. China; [4]Department of Dermatology, Xinhua Hospital, Shanghai Jiaotong University, School of Medicine, Shanghai, P.R. China

Received: February 19, 2021 | **Revised:** March 16, 2021 | **Accepted:** March 22, 2021 | **Published:** April 1, 2021

Abstract

Background and objectives: The role of runt-related transcription factor 2 (RUNX2) in tumorigenesis and tumor progression in many cancers has been identified except for lung cancer. This study aimed to explore the expression level, functions and prognostic values of RUNX2 in lung cancer.

Methods: ONCOMINE was utilized to compare RUNX2 expression difference between cancers and normal tissue. GEPIA was used to further detect the expression pattern of RUNX2 between lung cancer and corresponding normal lung tissue. Then, the prognosis value of RUNX2 in lung cancer was evaluated through Kaplan-Meier Plots. The genomic alterations of RUNX2 neighbor genes were analyzed by cBioPortal, and GO enrichment was conducted to identify functional categories of RUNX2 and genes associated with RUNX2 alterations.

Results: ONCOMINE and GEPIA revealed the upregulated expression level of RUNX2 in lung cancer compared to normal lung tissue. Higher RUNX2 level was associated with lower progression-free survival and overall survival in lung cancer patients. GO enrichment implicated that cell proliferation, invasion and metastasis-related genes as well as the functions of cell adhesion, cell migration, and cell proliferation were significantly associated with RUNX2 alterations.

Conclusions: Our findings indicated the value of RUNX2 as a novel prognostic biomarker and a potential therapeutic target for lung cancer.

Keywords: RUNX2; Lung cancer; Bioinformatics analysis; Prognosis; Therapeutic target.

Abbreviations: RUNX2, Runt-related transcription factor 2; EMT, epithelial mesenchymal transition; TGF-β, transforming growth factor-β; LC, lung cancer; LUAD, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; NSCLC, non-small cell lung cancer; GEPIA, gene expression profiling interactive analysis; FP, progression-free survival; OS, overall survival; PPS, post progression survival; HPA, human protein atlas; PPI, protein–protein interaction; GO, gene ontology; BP, biological process; CC, cellular component; MF, molecular function.

[*]**Correspondence to**: Xiaobo Zhou, Department of Dermatology, Xinhua Hospital, Shanghai Jiaotong University, School of Medicine, 1665 Kongjiang Road, Shanghai 200092, China. ORCID: http://orcid.org/0000-0001-8827-2921. E-mail: dr.zhouxb@foxmail.com; Jia Huang, Department of Dermatology, Huashan Hospital, Fudan University, No.12 Urumuqi Zhong Road, Shanghai 200040, China. ORCID: http://orcid.org/0000-0001-6249-7516. Tel: +86-21-2327-1699-5606, Fax: +86-21-5307-8128, E-mail: huang_jia_hj@foxmail.com

How to cite this article: Xiao D, Liu K, Chen J, Gong Y, Zhou X, Huang J. RUNX2 as a Potential Prognosis Biomarker and New Target for Human Lung Cancer. *Exploratory Research and Hypothesis in Medicine* 2021;6(3):99–109. doi: 10.14218/ERHM.2021.00009.

Introduction

The runt-related transcription factor (RUNX) family includes RUNX1, RUNX2 and RUNX3, which are indispensable for normal lineage-specific development.[1] Among which, RUNX2 is necessary during embryogenesis for skeletal development and is a key regulator for morphogenesis of organs like thyroid and breast.[2–4] Moreover, RUNX2 exerts its biological functions via the activation of downstream signaling pathways such as epithelial mesenchymal transition (EMT), cell cycle, cell apoptosis, NOTCH, WNT, RAS and transforming growth factor-β (TGF-β)/Smad, *etc.*[5,6]

RUNX2 is increasingly implicated in tumor biology for potential tumorigenicity. It was reported that enhanced RUNX2 could transcriptionally induce gene expression involved in tumor initiation, development and metastasis.[7] For example, uncontrolled RUNX2 expression was observed in the carcinogenic process.[5] In

© 2021 The Author(s). This article has been published under the terms of Creative Commons Attribution-Noncommercial 4.0 International License (CC BY-NC 4.0), which permits noncommercial unrestricted use, distribution, and reproduction in any medium, provided that the following statement is provided. "This article has been published in *Exploratory Research and Hypothesis in Medicine* at https://doi.org/10.14218/ERHM.2021.00009 and can also be viewed on the Journal's website at https://www.xiahepublishing.com/journal/erhm".

melanoma, RUNX2 participated in the regulation of the EMT process.[8] Upregulation of RUNX2 expression level could recover the cancer cell invasion in cervical cancer.[9] Additionally, in the late-stage thyroid, breast and prostate cancer, upregulated expression of RUNX2 could accelerate bone metastasis.[10,11] These revealed that RUNX2 could function as an oncogene and emerged as a promising therapeutic target to treat cancer. Therefore, extensive work has been done. It was confirmed that in cervical cancer, downregulated RUNX2 could slow the tumor growth and metastasis through inhibiting the cancer cell proliferation, invasion, migration, and reversing the EMT process.[12] Suppressing the expression of RUNX2 in metastatic prostate and breast cancer cells could extremely decrease tumor progression and metastasis *in vivo*.[11,13] Knockdown of RUNX2 in esophageal carcinoma cells could obviously inhibit cell growth, migration and invasion, and increased cell apoptosis and suppressed tumor growth.[14]

Nearly 25% of all cancer-related morbidity was contributed to lung cancer (LC) in 2020.[15] Even more dire, greater than half of all LC patients have metastasized when diagnosed (57%), which may explain the unsatisfactory 5-year survival rate (5%).[15] Therefore, effective and strong prognostic biomarkers and potential drug targets are urgently needed to improve diagnosis and prognosis, and personalized drug treatments of LC.

The dysregulated expression level of RUNX2 and its relationship with LC have been partly reported. It was demonstrated that RUNX2 expression level was upregulated in lung squamous cell carcinoma (LUSC) and lung adenocarcinoma (LUAD) tissues.[16,17] Moreover, RUNX2 overexpression could promote EMT and increase the expression of EMT related genes including TWIST1, SNAIL1 and VIMENTIN, which directly reflect an increased migratory and invasion capacity of LUAD cells.[18] These results suggested that RUNX2 could serve as a candidate target for LC therapy. Actually, previous research has reported that targeting RUNX2 could inhibit LC cell growth.[19] Downregulation RUNX2 expression could decrease non-small cell lung cancer (NSCLC) tumor volume and weight.[20] It was also reported that the proliferation, migration, and invasion of human LC cells could be repressed by downregulating the expression level of RUNX2.[21,22]

Numerous RNA and DNA research indicated their critical role in determining the biological and biomedical functions with the development of second-generation sequencing technology.[23] Nowadays bioinformatic analyses has become one of the hottest research priorities in medicine. Specifically, bioinformatic analyses were carried out to predict gene targets and analyze their potential roles in various cancers.[23] However, biological information analysis has not been be applied comprehensively to explore the potential role of RUNX2 in LC. Based on the publicly available gene information, containing gene expression, gene variation and epigenetic mutations, *etc.*, we aimed to identify the expression pattern and variation of RUNX2 in LC and explore its prognostic value and potential functions in LC. Unlike previous studies that explored the function of RUNX2 through experimental methods, the bioinformatics analyses performed in our study will provide a comprehensive evaluation of RUNX2 in LC.

Materials and methods

ONCOMINE database analysis

ONCOMINE is a database of high-throughput sequencing data of cancers (www.oncomine.org), with which we could assess and visualize the differential expression of specific genes from all available public datasets and conduct differential expression analyses. ONCOMINE was utilized to compare the RUNX2 mRNA expression difference between clinical cancer samples and corresponding normal tissue samples. A student's t-test was used to calculate the significant expression differences between tumor and normal tissues. The cut-off values were set at a P-value of 0.05 and fold change of 2.

Gene Expression Profiling Interactive Analysis (GEPIA)

The online database Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/) is a newly developed interactive website to analyze the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) projects.[24] It was used to find the expression differences of RUNX2 between LUAD, LUSC and corresponding normal lung samples. The threshold was set as following: P-value of 0.01, fold change of 2, matched TCGA normal and GTEx data.

Survival prediction analysis of lung cancer patients

The Kaplan-Meier Plotter database (www.kmplot.com) is one of the most effective analytical tools to evaluate survival and prognosis information.[25] The prognosis value of RUNX2 was predicted in patients bearing LC by Kaplan-Meier Plots, which provides an assessment of gene expression data and survival information of 2,437 LC patients. The survival curve of progression-free survival (FP), overall survival (OS) and post progression survival (PPS) in patients with LUAD and LUSC was generated using the JetSet best probe set with the median as the cutoff. Log-rank test results with P-value < 0.05 were considered statistically significant.

Immunohistochemistry validation by Human Protein Atlas

The Human Protein Atlas (HPA) (https://www.proteinatlas.org/) is an extensive database for exploration of the expression pattern of mRNA and protein in cancer and normal tissue from a large volume of proteomics and transcriptomics data.[26] Therefore, the cancer and normal tissue atlas of the HPA were used to generate a list of cancer types detailing the gene and protein expression of RUNX2.

RNA extraction and real-time quantitative polymerase chain reaction (RT-qPCR)

The human LUSC cell line SK-MES-1, LUAD cell line A549 and normal lung bronchus epithelial cell line HBE were obtained from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). HBE (control group) was cultured with dulbecco's modified eagle's medium (DMEM) (Hyclone, Logan City, UT) and SK-MES-1, A549 (experimental group) were cultured with RPMI1640 containing 10% fetal bovine serum (FBS, Gibco, New Zealand), 1% antibiotic-antimycotic (Gibco, Grand Island, NY) at a density of $3\times10^4/cm^2$ onto the 10-cm culture dish for 24 hours. Then total RNA was extracted from the collected cells using Trizol Reagent (Invitrogen, Carlsbad, CA), and cDNA was converted from 2 μg total RNA using 200 U of reverse transcriptase (MMLV RT) and 20 pM oligo dT (Promega Corporation, Madison, WI, USA) at 30 °C for 10 min, 42 °C for 60 min, 95 °C for 5 min, and 5 °C for 5 min. The forward primer (5-TCTG-

GCCTTCCACTCTCAGT-3) and reverse primer (5-TATGGAGT-GCTGCTGGTCTG-3) were synthesized based on the Runx2 mRNA sequence. Quantitative PCR was performed with a protocol as follows: initial denaturation at 95 °C for 10 min, followed by 40 cycles at 95 °C for 30 s, 58 °C and 72 °C for 45 s, using the Strata Gene Mx3000p (Agilent Technologies, Inc., Santa Clara, CA, USA). Each gene of interest was normalized to GAPDH and the fold change was compared relative to the control sample. Each assay was performed in triplicate. The statistical analyses were processed using the statistical software Statistical Package for the Social Sciences (version 19.0, SPSS, Chicago). The data are represented as the mean ± standard derivation. A student's t-test was employed to analyze the differences between the control and experimental group, with $p < 0.05$ considered statistically significant.

cBioPortal analysis

The LUAD (TCGA, Firehose Legacy) and LUSC (TCGA, Firehose Legacy) datasets containing 517 and 501 pathologically reported cases, respectively, were selected for further analyses of RUNX2 using the cBioPortal (https://www.cbioportal.org/). The spectrum of genomic alterations included mutations, putative copy-number alterations (CNA) from GISTIC, mRNA expression z-scores (RNA Seq V2 RSEM) and protein expression Z-scores (RPPA).

Construction of a protein–protein interactions (PPI) network

The STRING database (https://string-db. org/cgi/input.pl) aims to collect, score, and integrate all publicly available sources of PPI information and to complement these with computational predictions. RUNX2 and the targets closely related to RUNX2 alterations (top 100) were put into STRING to build the PPI network to achieve a comprehensive and objective global network, including direct (physical) as well as indirect (functional) interactions. The cutoff of the PPI confidence score was 0.7 and the disconnected nodes were hidden.

Gene ontology analysis

DAVID (http://david.abcc.ncifcrf.gov/) is a database for annotation, visualization and integrated discovery.[27] Gene Ontology (GO) is a widely confirmed gene functional enrichment database which is generally used to explore the enriched GO terms.[28] GO functional annotation enrichment analysis could predict the target gene function through three aspects including biological process (BP), cellular component (CC) and molecular function (MF). Therefore, GO enrichment analysis was conducted to identify functional categories of RUNX2 and the genes closely associated with RUNX2 alterations. Enriched GO terms were identified according to the cut-off criterion of P-value < 0.05 and gene counts more than 2. The top 20 GO terms of BP, CC and MF were selected to display.

Results

The transcriptional level of RUNX2 in multiple types of tumors

The mRNA expression level of RUNX2 in multiple types of cancers was compared with that in the normal samples using the ONCOMINE database (Fig. 1). As shown in Figure 1a, the expression level of RUNX2 was obviously increased in LC patients of three datasets. In Garber's dataset,[29] RUNX2 was upregulated in all LC types compared to normal samples, including LUAD with a fold change of 2.829 (Fig. 1b), LUSC with a fold change of 3.703 (Fig. 1c), large-cell lung carcinoma with a fold change of 3.051 (Fig. 1d) and small cell lung carcinoma with a fold change of 1.768 (Fig. 1e).

Association between RUNX2 expression and the clinicopathological parameters of lung cancer patients

The mRNA expression level of RUNX2 between LC and normal lung tissues was evaluated by GEPIA. The results indicated that RUNX2 expression level was significantly increased in LUAD and LUSC tissues than that in the lung tissues (Fig. 2a–b). The relationship of RUNX2 expression with LUAD and LUSC tumor stage was also analyzed, however, no significant difference was mined (Fig. 2c–d).

Increased RUNX2 expression indicated a poor prognosis of lung cancer patients

The prognostic role of RUNX2 in patients with LC was investigated using Kaplan–Meier plots. The results indicated that the upregulated RUNX2 mRNA expression level was negatively correlated with the FP and OS in all LC patients (Fig. 2e–f, $p < 0.001$). Despite a decreasing trend of PPS in patients with increased expression of RUNX2, there was no statistically significant difference (Fig. 2g, $p > 0.05$).

Increased RUNX2 mRNA and protein levels validated by HPA and RT-qPCR

The HPA database was utilized to identify the expression level of RUNX2 in different normal and cancer tissues. The results demonstrated that NK-cells, dendritic cells, T-cells, cervix uterine, tonsil, appendix, bone marrow, granulocytes, lymph node, spleen contained the top ten normal tissue expression of RUNX2 mRNA (Fig. 3a). In addition, the seven tissues achieved by comparison of RUNX2 protein expression level, were salivary gland, spleen, lymph node, tonsil, bone marrow, nasopharynx, and bronchus (Fig. 3b). Among all types of cancer, thyroid cancer, pancreatic cancer, breast cancer and LC showed the highest expression level of RUNX2 mRNA and urothelial cancer, LC, skin cancer and lymphoma presented the highest expression value of RUNX2 protein (Fig. 3c–d). Our finding demonstrated that RUNX2 mRNA and protein expression levels were both obviously increased in LC. Moreover, the increased protein expression level of RUNX2 was also detected in LUAD and LUSC tissues compared to normal lung tissues (Fig. 3e–g). As shown in Figure 3h, the LUAD (A549) and LUSC (SK-MES-1) cells revealed significantly higher expression of RUNX2 with the level of 1.850±0.5075-fold and 1.773 ± 0.3810-fold more than that of the normal lung bronchus epithelial cells (HBE). The analysis above suggests that RUNX2 was a specificity-increased biomarker in LC.

Analysis of RUNX2 and the altered neighbor genes in lung cancer patients

The RUNX2 alterations and neighbor genes altered by RUNX2 alterations were analyzed in LUAD and LUSC using the cBioPortal database. As shown in Figure 4, RUNX2 was altered in 44 out

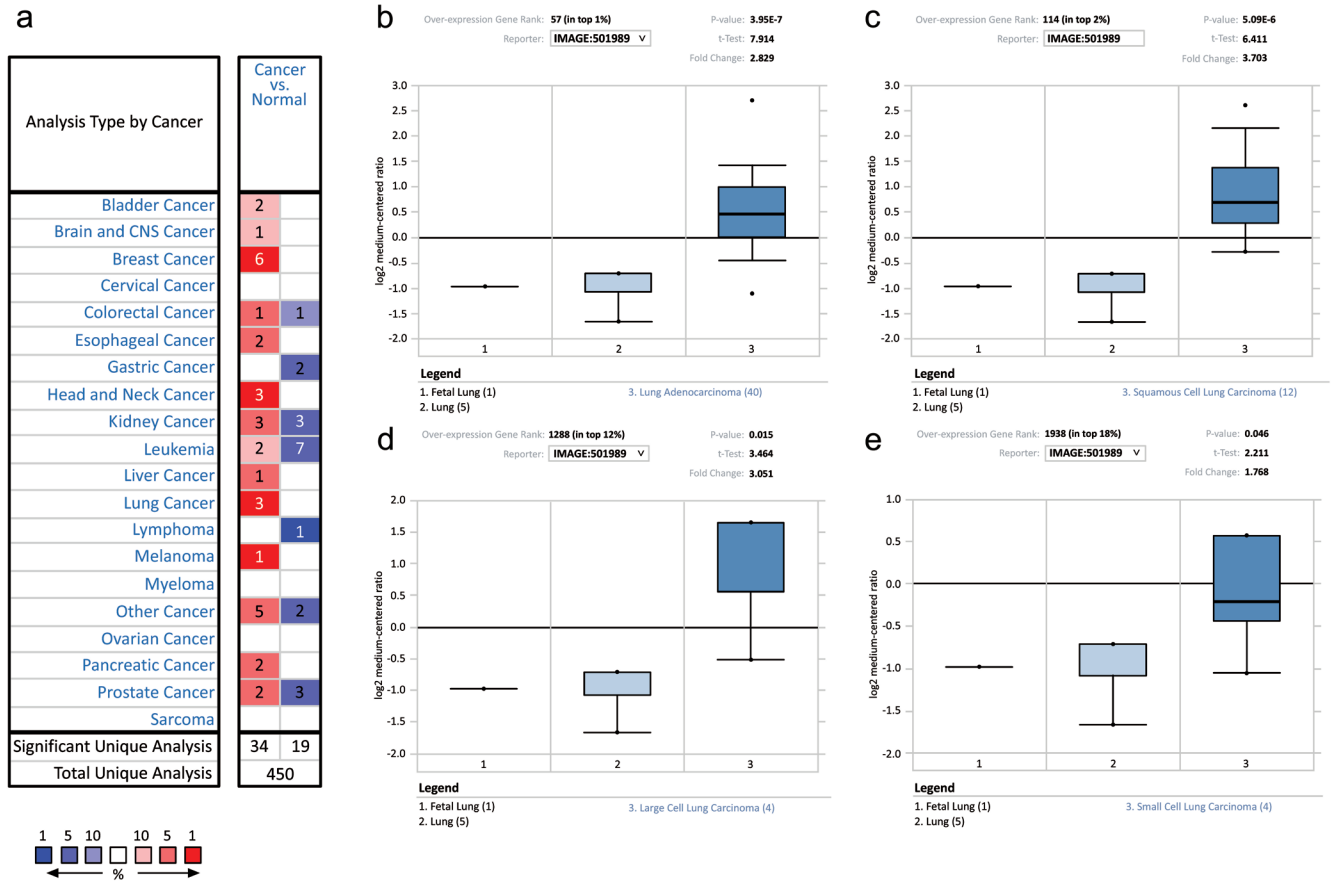

Fig. 1. The mRNA expression level of RUNX2 in various types of cancers (ONCOMINE). (a) Upregulated mRNA expression level of RUNX2 in lung cancer patients from three datasets. RUNX2 was increased in lung adenocarcinoma (b), squamous cell lung carcinoma (c), large-cell lung carcinoma (d) and small cell lung carcinoma (e) tissues compared with normal tissues.

of 517 patients with LUAD (8.51%) (Fig. 4a, c) and 42 out of 501 patients with LUSC (8.38%) (Fig. 4b, d). The top 10 neighbor genes which were most frequently altered by RUNX2 in LUAD and LUSC are listed in Tables 1 and 2, respectively, and all altered neighbor genes information is shown in Table S1–2. The results indicated that cell proliferation, invasion and metastasis-related genes DPYSL3, VCAN, ANTXR1, SULF1, COL1A2, COL3A1, COL6A3 were closely associated with RUNX2 alterations in LUAD (Table 1), and LOXL2, LTBP2, TPM1, TIMP3, COL5A1, COL6A2 were closely associated with RUNX2 alterations in LUSC (Table 2). Furthermore, the proteins in the PPI network are considered core targets in the pathogenesis of RUNX2 for LUAD (Fig. 5a) and LUSC (Fig. 5b).

GO enrichment analysis

The biological function of RUNX2 and the genes closed related to RUNX2 alterations were evaluated by performing GO enrichment analysis. As shown in Figure 6a, clinically important module genes of LUAD in the BP group were strongly enriched in cell adhesion, oxidation reduction, cell motion, extracellular matrix organization, blood vessel development, cell migration, regulation of cell proliferation and collagen fibril organization, *etc.* The genes in the CC terms were strongly enriched in membrane, extracellular matrix, membrane fraction and cell fraction, etc. (Fig. 6b). The genes in the MF group were significantly enriched in polysaccharide binding, oxidoreductase activity, acting on peroxide as acceptor and growth factor binding, etc. (Fig. 6c). For LUSC, the clinically significant module genes in the BP group were significantly enriched in cell adhesion, extracellular matrix organization, response to wounding, vasculature development, blood vessel development, collagen metabolic process, collagen fibril organization, cell-substrate adhesion, oxidation reduction, regulation of cell motion, blood vessel morphogenesis, regulation of cell migration, etc. (Fig. 6d). The genes in the CC group were mainly enriched in collagen, extracellular matrix, cell surface, etc. (Fig. 6e). And the genes in the MF group were significantly enriched in extracellular matrix structural constituent, calcium ion binding, growth factor binding, metalloendopeptidase activity, cytokine binding, integrin binding, etc. (Fig. 6f).

Discussion

Functional genome analysis revealed that gene expression is far more complicated than expected and required an ongoing and widespread regulatory landscape.[30] Tumor initiation, progression and metastasis are an outcome of deregulation of multiple signaling pathways via the abnormal function of transcription factors.

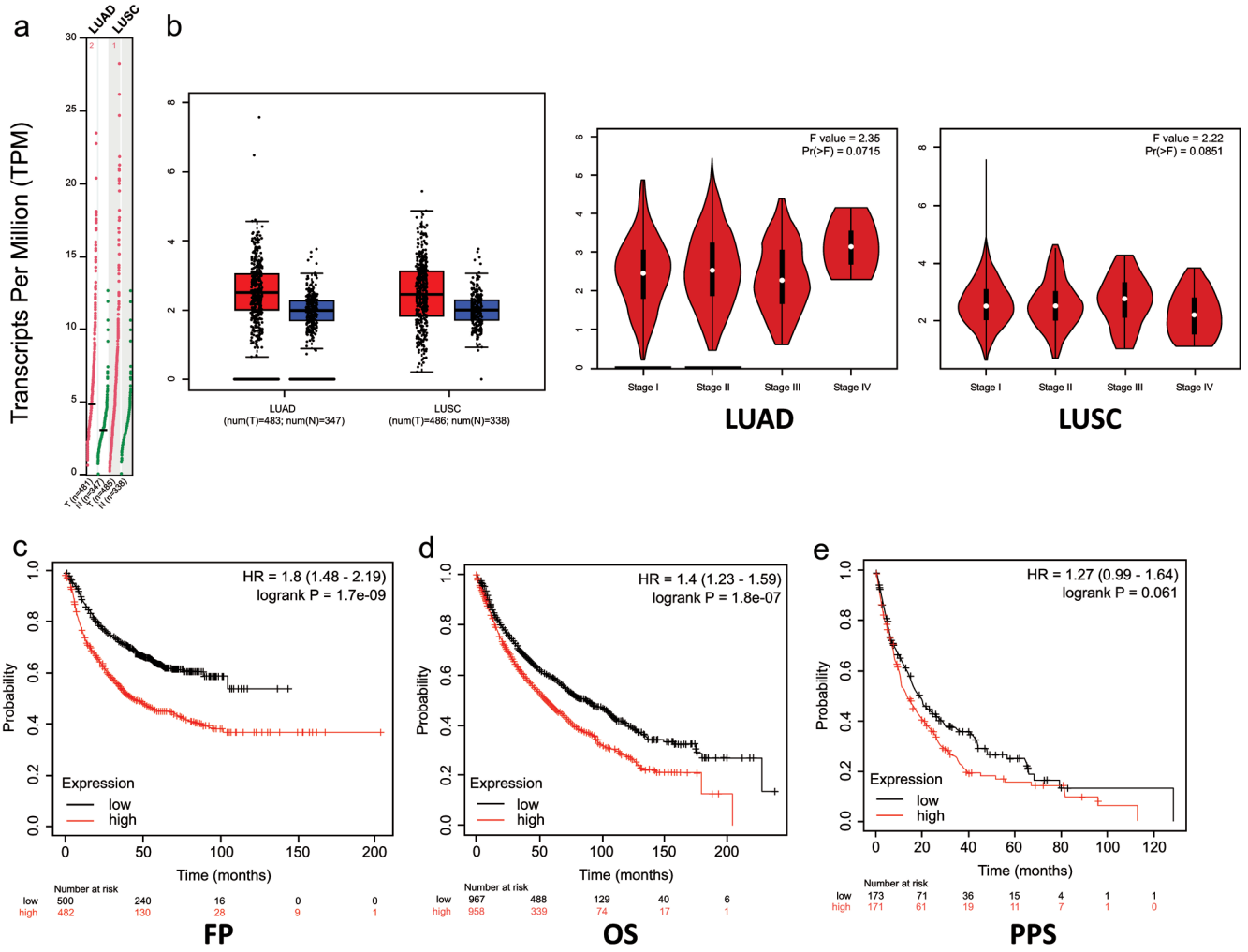

Fig. 2. RUNX2 predicted worse survival in lung cancer patients. The expression level of RUNX2 was increased in lung adenocarcinoma and squamous cell lung carcinoma (a–b) compared with normal lung samples. No significant relationship was found in lung adenocarcinoma (c) and squamous cell lung carcinoma (d) tumor stage with RUNX2 expression level using GEPIA. Increased RUNX2 mRNA level was closely related to a worse FP and OS according to Kaplan-Meier plots (e–g) of patients with lung cancer.

RUNX2 is a lineage-specific transcription factor which is essential in bone development.[31] However, emerging evidence has indicated that RUNX2 may be a pivotal oncogene in tumorigenesis in recent years. The oncogenic potential of RUNX2 was first demonstrated in T-cell lymphoma.[32] After that, a large number of experiments have since proven the involvement of RUNX2 in tumorigenesis.[11,33,34] In the initial step of tumor formation, RUNX2 was overexpressed and caused abnormal activation of pathways like PI3K/Akt, Wnt, TGF-β, BMP, MAPK/ERK and Notch, MET, which were a hallmark of various types of cancers.[35] RUNX2 was documented to be increased in tumor cells and had a negative prognostic value in cancers of the thyroid, breast, colon and prostate.[13,36,37] For example, it was reported that upregulated levels of RUNX2 accelerated prostate cancer aggressiveness by potentiating tumor cell progression and metastasis through promoting filopodia and adhesion in cancer cells.[13,33] In T cell lymphomas, highly expressed RUNX2 contributed to the increased transcriptional activity of genes that accelerate survival, growth, and metastasis in the cancer cells.[38] In pituitary tumors, RUNX2 upregulated the tumor-promoter and inhibited tumor-suppressors, which facilitate bone metastasis.[39] In advanced thyroid, breast and prostate cancer, increased RUNX2 could dramatically accelerate tumor metastasis.[10,11] These above studies revealed that RUNX2 can act as a potential oncogene and therapeutic target in various types of cancers.

Actually, it was demonstrated that downregulated RUNX2 could inhibit tumor development via suppressing the EMT process of tumor cells and blocking cell proliferation and invasion in cervical cancer.[12] Downregulated RUNX2 in metastatic prostate and breast cancer cells could significantly reduce tumor metastasis.[11,13] RUNX2 knockdown could effectively increase esophageal carcinoma cell apoptosis, inhibit cell invasion and eventually suppress tumor enlargement and metastasis.[14]

LC is one of the most common malignancies leading to substantial mortality worldwide, because of a high proportion of LC patients diagnosed in an advanced stage.[40] Therefore, new diagnostic markers and therapeutic targets for LC are urgently required. The relationship between the dysregulated expression of RUNX2 and LC has been partly reported. It was yielded that RUNX2 overexpression could promote EMT and increase the migratory and invasion capacity of LUAD cells.[18] Targeting RUNX2 could inhibit LC

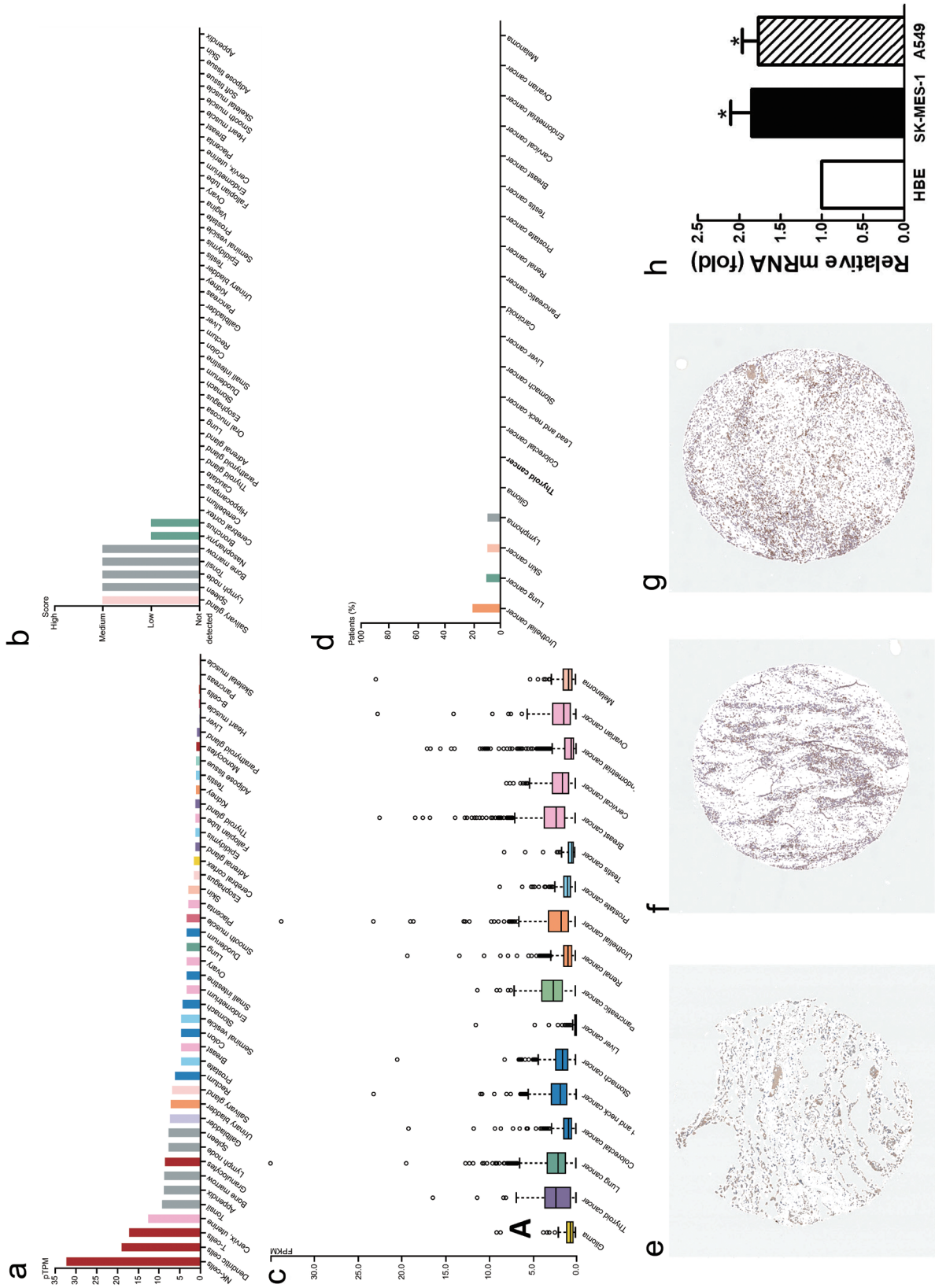

Fig. 3. Immunohistochemistry and gene expression of RUNX2 based on the Human Protein Atlas and PT-qPCR experiment. (a) RUNX2 mRNA expression level in different normal tissue; (b) RUNX2 protein expression level in different normal tissue; (c) RUNX2 mRNA expression in different tumor tissue; (d) RUNX2 protein expression in different tumor tissue. The expression of RUNX2 protein in lung (e), lung adenocarcinoma (f) and squamous cell lung carcinoma (g). The expression of RUNX2 mRNA in SK-MES-1, A549 and HBE (h).

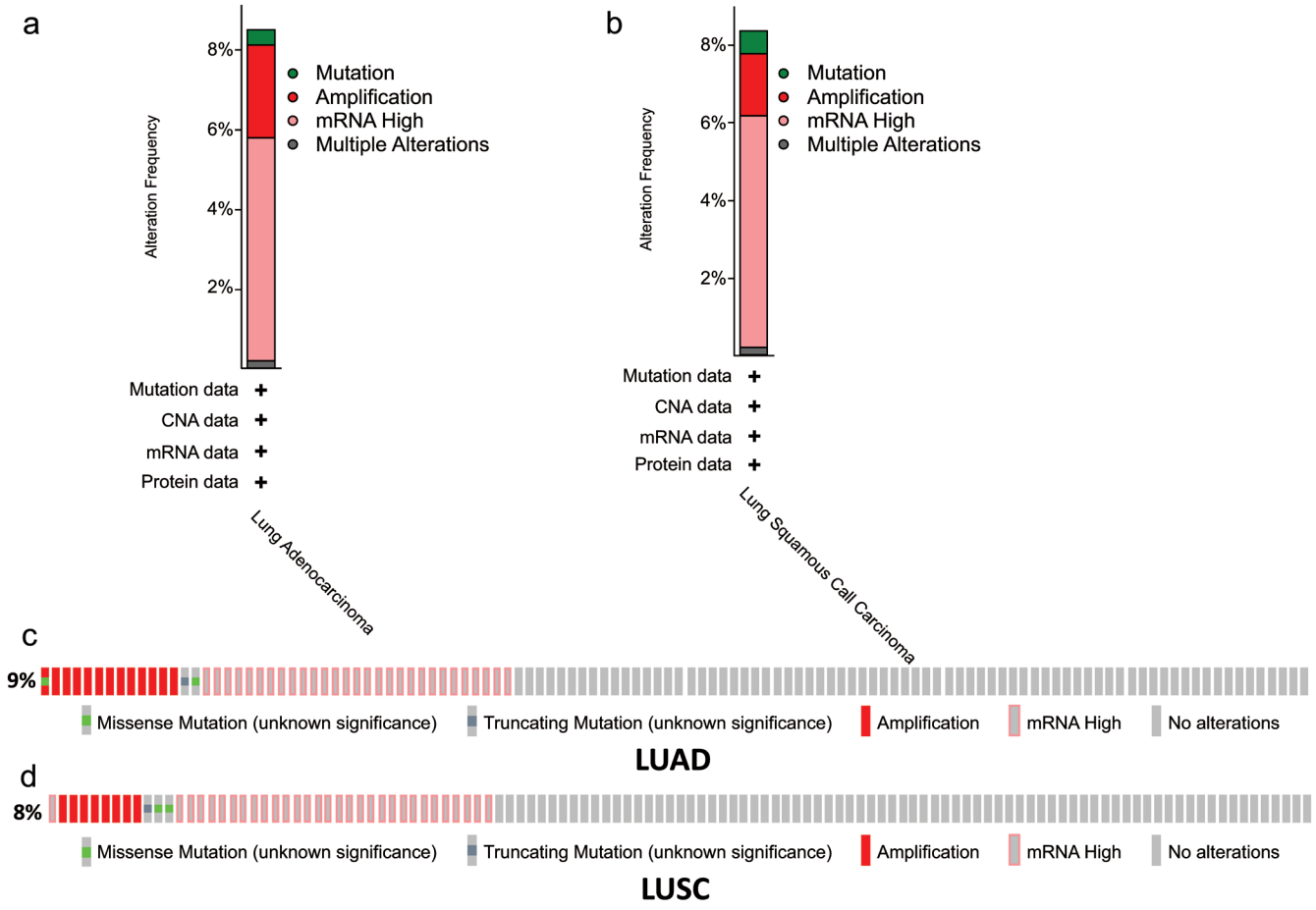

Fig. 4. Analysis of RUNX2 and the altered neighbor genes in lung cancer patients using the cBioPortal database. The RUNX2 alterations and neighbor genes altered by RUNX2 alterations in LUAD (a, c) and LUSC (b, d).

cell growth and greatly reduce tumor volume and weight.[19,20]

In recent decades, bioinformatic analyses are increasingly applied to explore the relationships between genes and cancers. Although the function of RUNX2 in the tumorigenesis and the prognostic values in many cancers have been partially validated, further bioinformatics analysis of LC and RUNX2 has not been performed. The current study is a first attempt to explore the mRNA and protein expression, potential functions and distinct prognostic values of RUNX2 in LC.

RUNX2 was reported to overexpress in primary or metastatic LC samples compared to that of normal tissue samples.[18] In the present study, ONCOMINE and GEPIA databases manifested the

Table 1. The top 10 significant genes correlated with RUNX2 in lung adenocarcinoma (cBioPortal database)

Correlated Gene	Cytoband	Spearman's Correlation	p-Value	q-Value
DPYSL3	5q32	0.572521	2.35E−46	4.72E−42
COL1A2	7q21.3	0.558711	8.92E−44	8.97E−40
ALPK2	18q21.31–q21.32	0.54582	1.78E−41	9.93E−38
VCAN	5q14.2–q14.3	0.545571	1.97E−41	9.93E−38
SERPINF1	17p13.3	0.544201	3.42E−41	1.37E−37
COL3A1	2q32.2	0.542773	6.04E−41	1.88E−37
ALDH1L2	12q23.3	0.542577	6.53E−41	1.88E−37
ANTXR1	2p13.3	0.537946	4.06E−40	9.32E−37
SULF1	8q13.2–q13.3	0.537882	4.16E−40	9.32E−37
COL6A3	2q37.3	0.535679	9.84E−40	1.98E−36

Table 2. The top 10 significant genes correlated with RUNX2 in squamous cell lung carcinoma (cBioPortal database)

Correlated Gene	Cytoband	Spearman's Correlation	p-Value	q-Value
PMEPA1	20q13.31	0.666687883	1.08E−65	2.17E−61
APCDD1L	20q13.32	0.647424619	7.46E−61	7.53E−57
LOXL2	8p21.3	0.6412603	2.23E−59	1.50E−55
COL5A1	9q34.3	0.637548012	1.66E−58	8.40E−55
COL6A2	21q22.3	0.624767517	1.37E−55	5.52E−52
LTBP2	14q24.3	0.622393683	4.60E−55	1.55E−51
TPM1	15q22.2	0.621275378	8.11E−55	2.34E−51
ADAMTS14	10q22.1	0.619570308	1.92E−54	4.84E−51
TIMP3	22q12.3	0.619182766	2.33E−54	5.23E−51
INHBA	7p14.1	0.616864852	7.44E−54	1.50E−50

increased expression of RUNX2 in human LC compared with lung tissues (Fig. 1, Fig. 2a, b). The prognostic value of RUNX2 in LC patients was documented using the Kaplan–Meier Plotter analysis. As shown in Figure 2e–f, the increased RUNX2 expression was obviously related to worse FP and OS in LC patients throughout a 200-month follow-up period. Furthermore, RUNX2 mRNA and protein were found to have a suitable high expression level in LC tissue when analyzing the expression profile using the HPA database (Fig. 3). Nevertheless, RUNX2 alteration information was detected by cBioPortal, and joint analysis was conducted using GO enrichment. It was implicated that the cell proliferation, invasion and metastasis-related genes, as well as the functions of cell proliferation, adhesion, motion, migration which contributed to tumor formation, development and metastasis were significantly associated with RUNX2 alterations (Fig. 6).

Future directions

Large scale experiments and multicenter clinical trials are still needed to validate this supposition. Besides, further analysis of online data concerning RUNX2 in lung cancer, ruling out confounders such as age, tumor stage, and recurrence status should be further refined.

Conclusions

The current study recognized the increased expression level of RUNX2 in LC. Moreover, the upregulated RUNX2 expression level manifested as the poor prognosis of FP and OS. RUNX2 alterations were closely related to cell proliferation, invasion and metastasis-related genes as well as the functions of cell proliferation, adhesion, motion and migration. We provided a novel method that utilizes available public data to mine the relationship between oncogene and cancer. Our findings showed that the elevated RUNX2 expression level in LC patients and its potential biological functions lead to its possible value as a new predictive factor and effective drug target. Certainly, additional large-scale studies and multicenter clinical trials are needed to corroborate this finding.

Supporting information

Supplementary material for this article is available at https://doi.

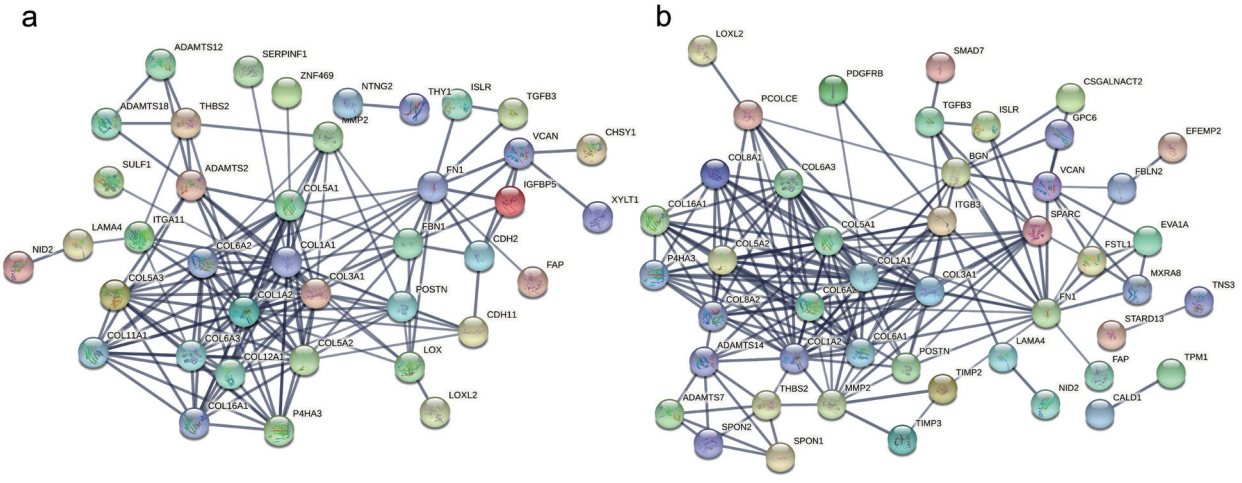

Fig. 5. PPI network of the core targets in the pathogenesis of RUNX2 for LUAD (a) and LUSC (b).

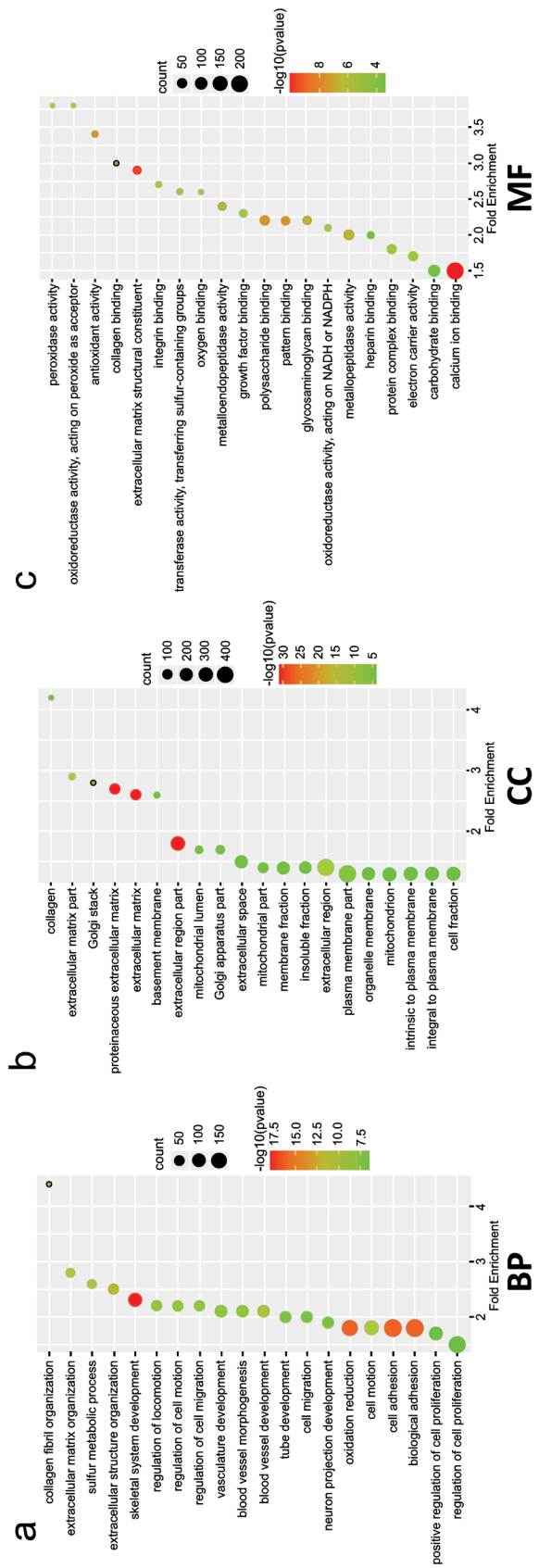

Fig. 6. GO enrichment analysis of RUNX2 and genes closely related to RUNX2 alterations according to the DAVID tool. GO analysis evaluated gene function involved in biological processes (a), cellular components (b) and molecular functions (c) of lung adenocarcinoma and squamous cell lung carcinoma (d–f).

org/10.14218/ERHM.2021.00009.

Table S1. The genes correlated with RUNX2 in lung adenocarcinoma (cBioPortal database).

Table S2. The genes correlated with RUNX2 in squamous cell lung carcinoma (cBioPortal database).

Acknowledgments

None.

Funding

The study is funded by the Interdisciplinary research of 9th People's Hospital affiliated to Shanghai Jiao Tong University School of Medicine (YG2019QNA13).

Conflict of interest

The authors have no conflicts of interest related to this publication.

Author contributions

Study design and administration (JH, XBZ); performance of experiments, analysis and interpretation of data, manuscript writing (DX, KL); critical revision (JH); technical and material support (JC, YYG). All the authors have made a significant contribution to this study and have approved the final manuscript.

Data sharing statement

All data are available from the corresponding authors.

References

[1] Tandon M, Gokul K, Ali SA, Chen Z, Lian J, Stein GS, *et al*. Runx2 mediates epigenetic silencing of the bone morphogenetic protein-3B (BMP-3B/GDF10) in lung cancer cells. Mol Cancer 2012;11:27. doi:10.1186/1476-4598-11-27.

[2] Komori T. Runx2, an inducer of osteoblast and chondrocyte differentiation. Histochem Cell Biol 2018;149(4):313–323. doi:10.1007/s00418-018-1640-6.

[3] Ferrari N, McDonald L, Morris JS, Cameron ER, Blyth K. RUNX2 in mammary gland development and breast cancer. J Cell Physiol 2013;228(6):1137–1142. doi:10.1002/jcp.24285.

[4] Endo T, Kobayashi T. Runx2 deficiency in mice causes decreased thyroglobulin expression and hypothyroidism. Mol Endocrinol 2010;24(6):1267–1273. doi:10.1210/me.2010-0056.

[5] Ito Y, Bae SC, Chuang LS. The RUNX family: developmental regulators in cancer. Nat Rev Cancer 2015;15(2):81–95. doi:10.1038/nrc3877.

[6] Cohen-Solal KA, Boregowda RK, Lasfar A. RUNX2 and the PI3K/AKT axis reciprocal activation as a driving force for tumor progression. Mol Cancer 2015;14:137. doi:10.1186/s12943-015-0404-3.

[7] Villanueva F, Araya H, Briceño P, Varela N, Stevenson A, Jerez S, *et al*. The cancer-related transcription factor RUNX2 modulates expression and secretion of the matricellular protein osteopontin in osteosarcoma cells to promote adhesion to endothelial pulmonary cells and lung metastasis. J Cell Physiol 2019;234(8):13659–13679. doi:10.1002/jcp.28046.

[8] Guo W, Wang H, Yang Y, Guo S, Zhang W, Liu Y, *et al*. Down-regulated miR-23a Contributes to the Metastasis of Cutaneous Melanoma by Promoting Autophagy. Theranostics 2017;7(8):2231–2249. doi:10.7150/thno.18835.

[9] Ouyang P, Wu K, Su L, An W, Bie Y, Zhang H, *et al*. Inhibition of human cervical cancer cell invasion by IL-37 involving runt related transcription factor 2 suppression. Ann Transl Med 2019;7(20):568. doi:10.21037/atm.2019.09.38.

[10] Pratap J, Lian JB, Javed A, Barnes GL, van Wijnen AJ, Stein JL, *et al*. Regulatory roles of Runx2 in metastatic tumor and cancer cell interactions with bone. Cancer Metastasis Rev 2006;25(4):589–600. doi:10.1007/s10555-006-9032-0.

[11] Pratap J, Imbalzano KM, Underwood JM, Cohet N, Gokul K, Akech J, *et al*. Ectopic runx2 expression in mammary epithelial cells disrupts formation of normal acini structure: implications for breast cancer progression. Cancer Res 2009;69(17):6807–6814. doi:10.1158/0008-5472.CAN-09-1471.

[12] Li R, Liu J, Qi J. Knockdown of long non-coding RNA CCAT1 suppresses proliferation and EMT of human cervical cancer cell lines by down-regulating Runx2. Exp Mol Pathol 2020;113:104380. doi:10.1016/j.yexmp.2020.104380.

[13] Akech J, Wixted JJ, Bedard K, van der Deen M, Hussain S, Guise TA, *et al*. Runx2 association with progression of prostate cancer in patients: mechanisms mediating bone osteolysis and osteoblastic metastatic lesions. Oncogene 2010;29(6):811–821. doi:10.1038/onc.2009.389.

[14] Lu H, Jiang T, Ren K, Li ZL, Ren J, Wu G, *et al*. RUNX2 Plays An Oncogenic Role in Esophageal Carcinoma by Activating the PI3K/AKT and ERK Signaling Pathways. Cell Physiol Biochem 2018;49(1):217–225. doi:10.1159/000492872.

[15] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70(1):7–30. doi:10.3322/caac.21590.

[16] Yang DP, Lu HP, Chen G, Yang J, Gao L, Song JH, *et al*. Integrated expression analysis revealed RUNX2 upregulation in lung squamous cell carcinoma tissues. IET Syst Biol 2020;14(5):252–260. doi:10.1049/iet-syb.2020.0063.

[17] Yang DP, Huang WY, Chen G, Chen SW, Yang J, He RQ, *et al*. Clinical significance of transcription factor RUNX2 in lung adenocarcinoma and its latent transcriptional regulating mechanism. Comput Biol Chem 2020;89:107383. doi:10.1016/j.compbiolchem.2020.107383.

[18] Herreno AM, Ramirez AC, Chaparro VP, Fernandez MJ, Cañas A, Morantes CF, *et al*. Role of RUNX2 transcription factor in epithelial mesenchymal transition in non-small cell lung cancer lung cancer: Epigenetic control of the RUNX2 P1 promoter. Tumour Biol 2019;41(5):1010428319851014. doi:10.1177/1010428319851014.

[19] Bai X, Meng L, Sun H, Li Z, Zhang X, Hua S. MicroRNA-196b Inhibits Cell Growth and Metastasis of Lung Cancer Cells by Targeting Runx2. Cell Physiol Biochem 2017;43(2):757–767. doi:10.1159/000481559.

[20] Ma F, Xie Y, Lei Y, Kuang Z, Liu X. The microRNA-130a-5p/RUNX2/STK32A network modulates tumor invasive and metastatic potential in non-small cell lung cancer. BMC Cancer 2020;20(1):580. doi:10.1186/s12885-020-07056-0.

[21] Liang R, Cao X, Li Y, Chen S, Wu Y, Ma Z. MicroRNA-466 regulates the proliferation, migration and invasion of the human lung cancer cells by targeting transcription factor RUNX2. J BUON 2020;25(6):2650–2656.

[22] Li H, Zhou RJ, Zhang GQ, Xu JP. Clinical significance of RUNX2 expression in patients with nonsmall cell lung cancer: a 5-year follow-up study. Tumour Biol 2013;34(3):1807–1812. doi:10.1007/s13277-013-0720-4.

[23] Sealfon SC, Chu TT. RNA and DNA microarrays. Methods Mol Biol 2011;671:3–34. doi:10.1007/978-1-59745-551-0_1.

[24] Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017;45(W1):W98–W102. doi:10.1093/nar/gkx247.

[25] Cui Y, Miao C, Hou C, Wang Z, Liu B. Apolipoprotein C1 (APOC1): A Novel Diagnostic and Prognostic Biomarker for Clear Cell Renal Cell Carcinoma. Front Oncol 2020;10:1436. doi:10.3389/fonc.2020.01436.

[26] Uhlen M, Bjorling E, Agaton C, Szigyarto CA, Amini B, Andersen E, *et al*. A human protein atlas for normal and cancer tissues based on

antibody proteomics. Mol Cell Proteomics 2005;4(12):1920–1932. doi:10.1074/mcp.M500279-MCP200.
[27] Shi L, Yu L, Zou F, Hu H, Liu K, Lin Z. Gene expression profiling and functional analysis reveals that p53 pathway-related gene expression is highly activated in cancer cells treated by cold atmospheric plasma-activated medium. PeerJ 2017;5:e3751. doi:10.7717/peerj.3751.
[28] Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, *et al*. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 2000;25(1):25–29. doi:10.1038/75556.
[29] Garber ME, Troyanskaya OG, Schluens K, Petersen S, Thaesler Z, Pacyna-Gengelbach M, *et al*. Diversity of gene expression in adenocarcinoma of the lung. PNAS 2001;98(24):13784–13789. doi:10.1073/pnas.241500798.
[30] ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. Nature 2012;489(7414):57–74. doi:10.1038/nature11247.
[31] Schroeder TM, Jensen ED, Westendorf JJ. Runx2: a master organizer of gene transcription in developing and maturing osteoblasts. Birth Defects Res C Embryo Today 2005;75(3):213–225. doi:10.1002/bdrc.20043.
[32] Blyth K, Cameron ER, Neil JC. The RUNX genes: gain or loss of function in cancer. Nat Rev Cancer 2005;5(5):376–387. doi:10.1038/nrc1607.
[33] Pratap J, Lian JB, Stein GS. Metastatic bone disease: role of transcription factors and future targets. Bone 2011;48(1):30–36. doi:10.1016/j.bone.2010.05.035.
[34] Morrissey C, Brown LG, Pitts TE, Vessella RL, Corey E. Bone morphogenetic protein 7 is expressed in prostate cancer metastases and its effects on prostate tumor cells depend on cell phenotype and the tumor microenvironment. Neoplasia 2010;12(2):192–205. doi:10.1593/neo.91836.
[35] Bailey JM, Singh PK, Hollingsworth MA. Cancer metastasis facilitated by developmental pathways: Sonic hedgehog, Notch, and bone morphogenic proteins. J Cell Biochem 2007;102(4):829–839. doi:10.1002/jcb.21509.
[36] Sancisi V, Borettini G, Maramotti S, Ragazzi M, Tamagnini I, Nicoli D, *et al*. Runx2 isoform I controls a panel of proinvasive genes driving aggressiveness of papillary thyroid carcinomas. J Clin Endocrinol Metab 2012;97(10):E2006–E2015. doi:10.1210/jc.2012-1903.
[37] Wang X, Li L, Wu Y, Zhang R, Zhang M, Liao D, *et al*. CBX4 Suppresses Metastasis via Recruitment of HDAC3 to the Runx2 Promoter in Colorectal Carcinoma. Cancer Res 2016;76(24):7277–7289. doi:10.1158/0008-5472.CAN-16-2100.
[38] Blyth K, Vaillant F, Hanlon L, Mackay N, Bell M, Jenkins A, *et al*. Runx2 and MYC collaborate in lymphoma development by suppressing apoptotic and growth arrest pathways in vivo. Cancer Res 2006;66(4):2195–2201. doi:10.1158/0008-5472.CAN-05-3558.
[39] Zhang HY, Jin L, Stilling GA, Ruebel KH, Coonse K, Tanizaki Y, *et al*. RUNX1 and RUNX2 upregulate Galectin-3 expression in human pituitary tumors. Endocrine 2009;35(1):101–111. doi:10.1007/s12020-008-9129-z.
[40] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68(6):394–424. doi:10.3322/caac.21492.