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The *PITX* gene family as potential biomarkers and therapeutic targets in lung adenocarcinoma

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Abstract

The *PITX* gene family of transcription factors have been reported to regulate the development of multiple organs. This study was designed to investigate the role of *PITXs* in lung adenocarcinoma (LUAD).

In this study, the transcriptional levels of the 3 identified *PITXs* in patients with LUAD were examined using the gene expression profiling interactive analysis interactive web server. Meanwhile, the immunohistochemical data of the 3 *PITXs* were obtained in the Human Protein Atlas website, and western blotting was additionally conducted for further verification. Moreover, the association between the levels of *PITXs* and the stage plot as well as overall survival of patients with LUAD was analyzed.

We found that the mRNA and protein levels of *PITX1* and *PITX2* were higher in LUAD tissues than those in normal lung tissues, while those of *PITX3* displayed no significant differences. Additionally, *PITX1* and *PITX3* were found to be significantly associated with the stage of LUAD. The Kaplan-Meier Plot showed that the high level of *PITX1* conferred a better overall survival of patients with LUAD while the high level of *PITX3* was associated with poor prognosis.

Our study implied that *PITX1* and *PITX3* are potential targets of precision therapy for patients with LUAD while *PITX1* and *PITX2* are regarded as novel biomarkers for the diagnosis of LUAD.

Abbreviations: GEPIA = gene expression profiling interactive analysis, HPA = the Human Protein Atlas, LUAD = lung adenocarcinoma, OS = overall survival, TPM = transcripts per million.

Keywords: biomarkers, lung adenocarcinoma, *PITX* gene family, prognosis

1. Introduction

Lung adenocarcinoma (LUAD), one of the most frequent histological types of lung cancer, is generally induced by an aberration in a driver oncogene.[1] The pathogenesis of LUAD is a multistep process implicated with the alterations of various vital events, involving mitochondrial dysfunction, altered lipid metabolism, endoplasmic reticulum stress, oxidative stress, inflammation, as well as epigenetic changes.[2–5] Currently, despite considerable advancements in diagnostic and therapeutic methods, the 5-year overall survival (OS) rate of LUAD remains still very low.[6,7] Therefore, a set of sensitive prognostic markers and potential drug targets should be verified to improve the accuracy of prognosis prediction and individualized treatments.

The *PITX* homeobox gene family, including 3 identified members in vertebrates, possesses significant roles in eye, vertebrate pituitary, branchial arch, brain, and hindlimb development, in addition to a key function in modulating left-right asymmetry. The expression of *PITX* activators is deregulated in some human malignancies, including lung cancer,[8] colorectal carcinoma,[9] breast cancer,[10] melanoma[11] and so on. Up to date, a total of 3 *PITX* transcription factors have been verified in mammalian cells, which were named according to their discovery time (*PITX1*, *PITX2*, and *PITX3*).[12] Among these 3 genes, *PITX1* and *PITX2* have been reported to be implicated with lung cancer. For instance, *PITX1* was decreased in lung cancer cell lines and tissues. Sixty-two percent patients with lung cancer displayed no expression of *PITX1* and the lower expression of *PITX1* was significantly associated with higher tumor stages.[8] In a recent bioinformatics analysis, the role of *PITX1* has been uncovered. However, this study did not focus on the other 2 members of *PITX* family, meanwhile, the study did not verify the protein expression of *PITX1* using molecular biology experiments.[13] In non-small cell lung cancer patients, the DNA methylation level of *PITX2* has been proven to be significantly related to the risk of occurrence and progression of lung cancer.[14]

The dysregulated expression of *PITX* transcription factors and their relationship with clinicopathological features and prognosis have been partly reported in human LUAD. To the best of our knowledge, bioinformatics analysis has yet been applied to investigate the roles of *PITX* genes in LUAD. On the basis of the

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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analyses on the expression or variation in copy numbers for thousands of genes published online, we analyzed and validated the expression of different *PITX* transcription factors in patients with LUAD in detail to determine their expression patterns, potential functions, and distinct prognostic values in LUAD.

2. Methods

2.1. Ethics statement

This study was approved by the Academic Committee of Shaoxing People's Hospital, Shaoxing Hospital of Zhejiang University, and conducted based on the principles expressed in the Declaration of Helsinki. All the datasets mentioned in our study were retrieved from the published literature, which confirmed that all written informed consent was acquired.

2.2. GEPIA (gene expression profiling interactive analysis) dataset

GEPIA (http://gepia.cancer-pku.cn/index.html) serves as a newly generated interactive web server designed by Zefang Tang, Chenwei Li, and Boxi Kang of Zhang Lab, Peking University, aiming to analyze the RNA sequencing expression data of 9736 tumors and 8587 normal samples from the GTEx project and the Cancer Genome Atlas database in a standard processing manner.[15] GEPIA provides customizable functions including tumor/normal differential expression analysis, profiling according to cancer types or pathological stages, patient survival analysis, similar gene detection, correlation analysis, and dimensionality reduction analysis. In the present study, we mainly employed GEPIA to detect the expression of *PITX* genes in LUAD and normal lung samples. Additionally, GEPIA was also employed to obtain the transcripts per million (TPM) of *PITX* genes to display their relative expression level.

2.3. The OS and stage plot of PITX genes

Similarly, we used the GEPIA database to get the OS and stage plot information of *PITX* genes. The method for differential gene expression analysis is 1-way ANOVA, using pathological stage as variable for calculating differential expression. The log rank *P* value and hazard ratio with 95% confidence intervals were showed on the plot. $P < .05$ was statistically significant.

2.4. Immunohistochemical data

The Human Protein Atlas (HPA, https://www.proteinatlas.org/) is a Swedish-based program initiated in 2003 with the aim to map all human proteins in cells, tissues, and organs using the integration of various omics technologies, including antibody-based imaging, mass spectrometry-based proteomics, transcriptomics and systems biology. Based on the immunohistochemical data of patients with or without LUAD in HPA, we further verified the expression of *PITX* genes.

2.5. Western blotting

Paired adjacent tissues and carcinoma tissues were obtained from 12 patients with LUAD in Shaoxing People's Hospital, Shaoxing Hospital of Zhejiang University. Protein extraction, SDS-PAGE, as well as immunodetection were all performed according to previous research.[16] In detail, total proteins from whole lung tissues were extracted using a Pro-Prep Protein Extraction Solution according to the manufacturer's instructions. The antibodies for *PITX1*, *PITX2,* and *PITX3* were obtained from Santa Cruz Biotechnology Inc. (Dallas, TX). Protein expression levels were normalized to the matched proteins or GAPDH. The immunoblotting densitometry was quantified by the Gel Logic 6000 PRO Imaging System (Carestream Health, Inc. Rochester, NY).

2.6. Statistical analysis

The obtained data were presented as mean ± standard deviation and assessed by the 2-tailed Student *t* test. A difference of $P < .05$ was considered statistically significant.

3. Results

3.1. TPM of PITX genes in patients with LUAD

After analyzing 347 normal lung tissues and 483 LUAD tissues based on GEPIA online website, we found that the mRNA levels of *PITX1* and *PITX2* were significantly higher in patients with LUAD than normal control ($P < .05$), while *PITX3* mRNA exhibited no significant difference between 2 groups ($P > .05$) (Fig. 1A-C). TPM is a measurement of the proportion of transcripts in the pool of RNA which perfectly explains the transcript abundance. In this study, *PITX1* and *PITX2* in LUAD group were observed to have higher levels ($P < .05$), indicating that these 2 genes possessed more transcripts in LUAD tissues. Similarly, as for *PITX3*, no significant difference was found between normal group and LUAD group ($P > .05$) (Fig. 1D-E).

3.2. Validation of the expression of PITX genes

To enhance the reliability of the GEPIA database, we searched the immunohistochemical data of the 3 genes in the HPA website. In consistent with the above results, the immunohistochemical data from HPA demonstrated that the protein levels of *PITX1* and *PITX2* were higher in LUAD tissues (Fig. 2A). Meanwhile, we detected the protein expression of *PITX* genes in clinical carcinoma tissues and adjacent tissues from patients with LUAD using western blotting, the result (Fig. 2B) of which further validated our previous hypothesis.

3.3. Correlation between PITX genes expression and tumor stage in patients with LUAD

Meanwhile, we analyzed the correlation between *PITX* genes expression and tumor stage in patients with LUAD. The results demonstrated that the expression levels of *PITX1* and *PITX3* displayed a strong correlation with the tumor stage in patients with LUAD, while the *PITX2* expression in different stages did not significantly differ (Fig. 3A-C).

3.4. Correlation between PITX genes expression and OS in patients with LUAD

Subsequently, we further analyzed the potential association between the expression levels of *PITX* genes and the OS of patients with LUAD (Fig. 4A-C). The Kaplan-Meier analysis showed that *PITX1* and *PITX3* displayed a significant correlation with the OS of patients with LUAD. In detail, the high level of

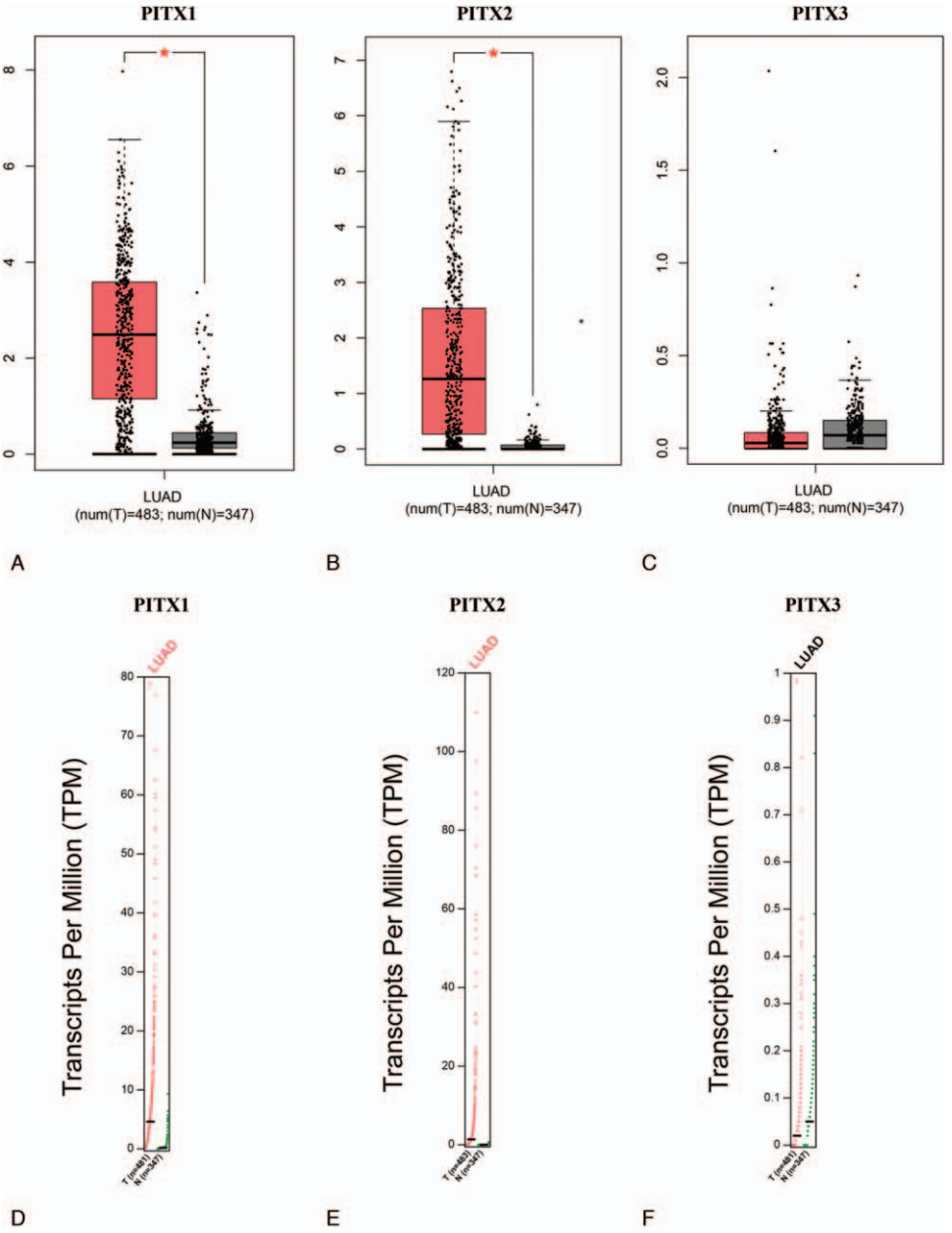

Figure 1. The mRNA expression level and transcripts per million (TPM) of PITX1, PITX2 and PITX3. (A) PITX1; (B) PITX2; (C) PITX3.

PITX1 may contribute to a better prognosis of LUAD while the high level of *PITX3* may lead to a worse prognosis ($P < .05$).

4. Discussion

The dysregulation of *PITX* transcription factors has been reported in various types of cancer. Although the role of *PITX* transcription factors in the carcinogenesis and prognosis of some cancers has been partially verified, further bioinformatics analysis on *PITXs* in LUAD has yet to be carried out. Our study is the first attempt to investigate the mRNA and protein expression as well as prognostic value of different members of *PITX* family in LUAD. We sincerely hope that our study would provide available knowledge, help to improve treatment designs and enhance the accuracy of prognosis prediction for patients with LUAD.

PITX1 is a paired-type homeodomain transcription factor which is expressed in a hindlimb-restricted fashion and mainly functions in the development of mammals.[17] April et al reported that *PITX1* was essential in the morphology of tendon, muscle, and bones of the hindlimb.[18] Additionally, in human primary cells, oncogenic signals could be activated upon the inhibition of *PITX1* expression. Meanwhile, inhibiting *PITX1* could also give rise to the activation of RAS pathway, which is vital for tumorigenesis.[19] The expression of *PITX1* can influence the expression of growth hormone, which possesses antiapoptotic and mutagenic properties and directly affects colorectal cancer risk by modulating the circulation of insulin-like growth factor I in the serum.[20,21] Additionally, *PITX1* rs647161 genetic polymorphism is also significantly related with increased risk of colorectal cancer.[22] A recent study has showed that forced

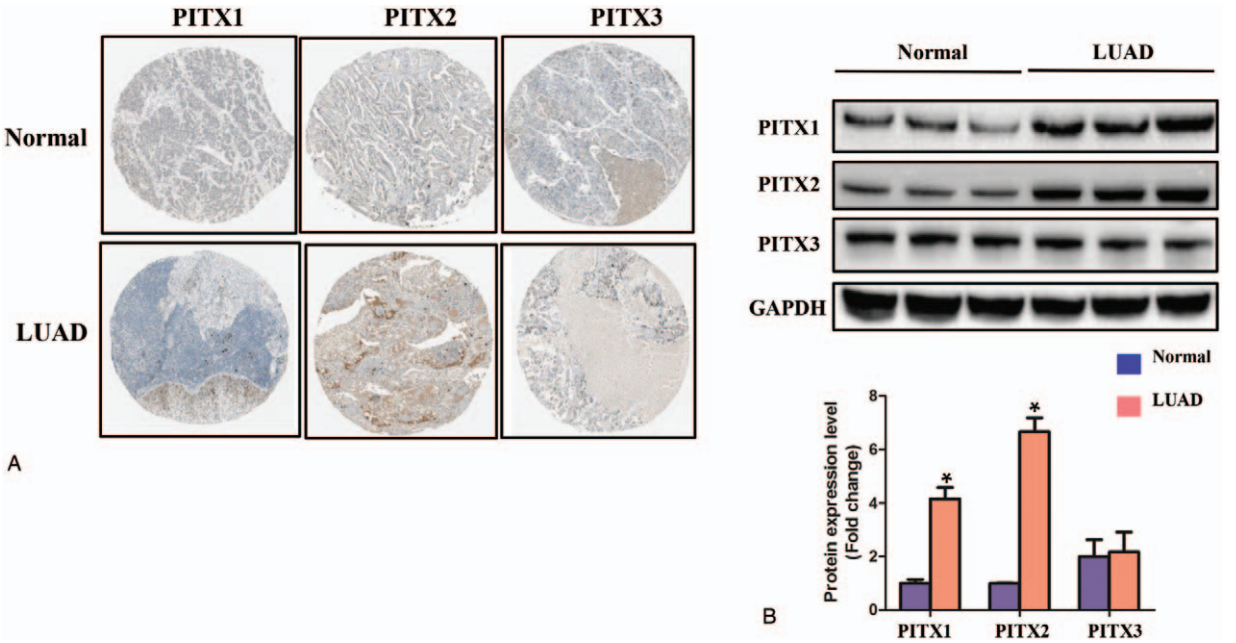

Figure 2. The immunohistochemical staining and western blotting for protein expression of PITX1, PITX2 and PITX3. (A) The immunohistochemical data obtained from HPA database. Each image of immunohistochemistry stands for 1 representative patient. (B) Representative western blots and statistical results (n = 12). $^{*}P < .05$ vs normal group.

expression of *PITX1* is able to give rise to the apoptosis of human osteosarcoma MG-63 cells in a p53-independent manner and the apoptosis of MCF-7 cells in a p53-dependent manner. Mechanically, *PITX1* can directly activate p53 gene transcription by binding to the *PITX1* consensus elements of the p53 promoter in MCF-7 cells.[20] As for lung cancer, *PITX1* has been found to be downregulated in cancer cell lines and tissues, and the lower expression of *PITX1* is also associated with higher tumor stages.[1] However, in our study, we found that the mRNA and protein expression of *PITX1* were significantly higher in patients with LUAD, which may be associated with better prognosis. A recent study has also disclosed that the high DNA methylation of *PITX1* indicates the poor survival condition of patients with LUAD.[13] For our perspective, the difference may be associated with tumor types.

PITX2 initially identified as the gene responsible for the human Rieger syndrome is an autosomal dominant condition resulting in developmental abnormalities.[23]*PITX2* is asymmetrically expressed in the left lateral-plate mesoderm, and mutant mice with laterality defects show altered patterns of *PITX2* expression that correlate with changes in the visceral symmetry (situs).[24] Recent studies have demonstrated that *PITX2* is overexpressed in node-positive colorectal cancer, nonfunctional pituitary adenomas, ovarian cancer, and thyroid cancer.[25,26] In thyroid cancer cells, inhibiting the expression of *PITX2* by shRNA can significantly suppress the capacity of cell growth in soft-agar assay, indicating that *PITX2* may possess oncogenic property and may be implicated with tumor progression.[27] In ovarian cancer, the mRNA as well as protein expression level of *PITX2* is seen to be frequently upregulated particularly in high-grade and

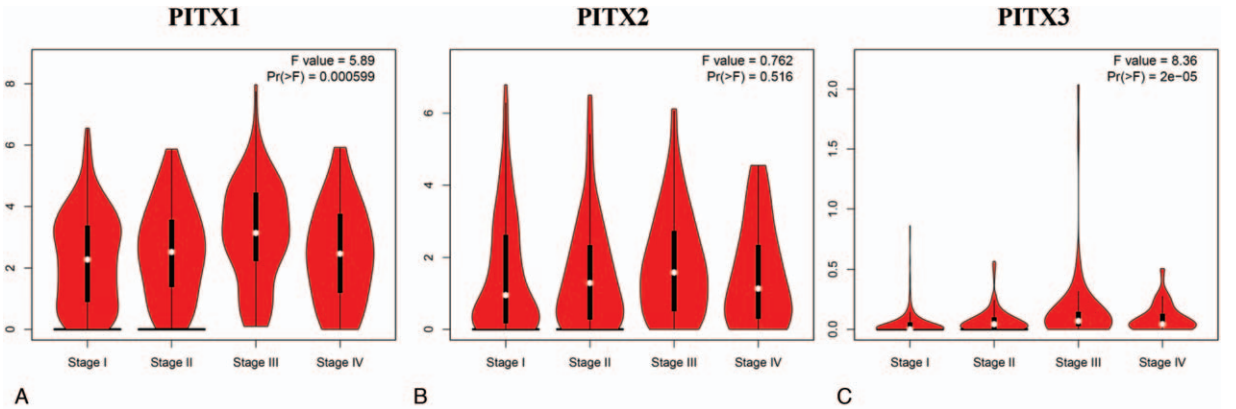

Figure 3. The relationship between the level of PITX genes and tumor stage in patients with LUAD. (A) PITX1; (B) PITX2; (C) PITX3; $^{*}Pr(>F) < 0.05$ represents significant differences among stages.

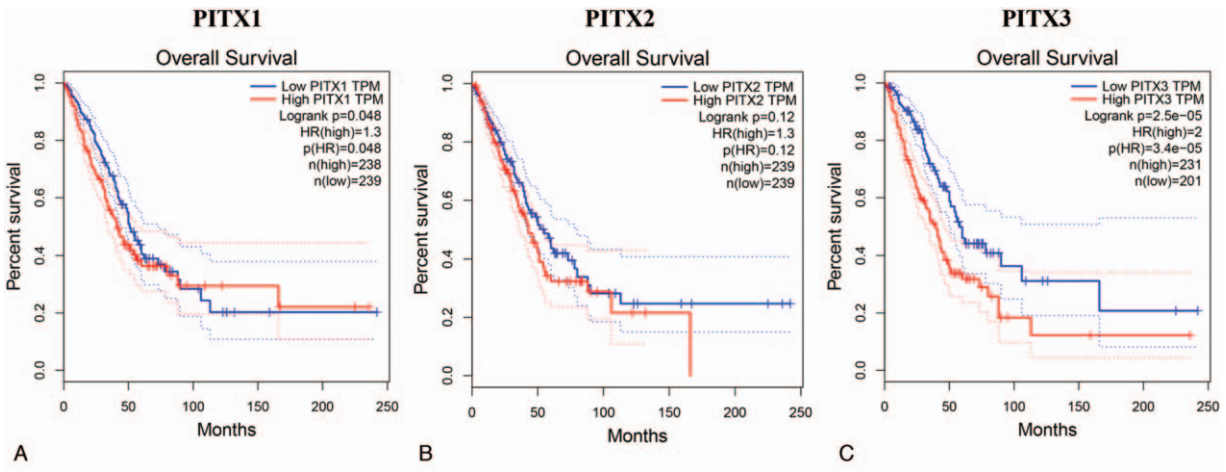

Figure 4. The relationship between the level of PITX genes and overall survival in patients with LUAD. (A) PITX1; (B) PITX2; (C) PITX3.

clear cell subtype of ovarian cancer. In the meantime, *PITX2* also displays the oncogenic property in cell migration/invasion, proliferation, anchorage-independent growth ability, and tumor growth in the tumor xenograft mice model.[28] However, up to date, the prognostic role of *PITX2* in LUAD has yet to be investigated. In this report, we documented that the expression of *PITX2* in patients with LUAD was higher than that in normal tissues, but its expression was not correlated with tumor stage and OS in patients with LUAD. A previous study has demonstrated that miR-17-92 cluster co-localizes with *PITX2* expression, and loss of *PITX2* gives rise to loss of some miRNAs encoded by miR-17-92 as well as its closely related homologue miR-106b-25.[29] Intriguingly, miR-17-92 is upregulated in human lung cancer, which can promote the proliferation of cancer cells.[30] Here, we speculated that the increased expression of *PITX2* in patients with LUAD may enhance lung cancer cell proliferation via increasing the level of miR-17-92.

PITX3, located on chromosome 10q24, is restricted to the developing eye and midbrain dopaminergic progenitor cells from embryonic day 11 throughout adult life in mice.[31] In the brain, the *PITX3* gene specifically localizes to the substantia nigra and retrorubral field.[32] Till now, a number of studies have uncovered that the methylation level of *PITX3* may affect the prognosis of patients with tumor. For instance, it has been found that *PITX3* is hypermethylated in patients with breast cancer.[33] Additionally, *PITX3* DNA promoter methylation has been proved to be strongly associated with biochemical recurrence-free survival in prostate cancer patients after radical prostatectomy.[34] As for patients with head and neck squamous cell carcinoma, *PITX3* DNA methylation also serves as an independent prognostic biomarker.[35] In our study, we found that although the mRNA and protein expression of *PITX3* did not differ between normal group and LUAD group, the level of *PITX3* was significantly related to tumor stage and OS. Prior studies have showed that during development, the expression of Nurr1 can activate *PITX3*, meanwhile, *PITX3* also potentiates SMRT-mediated repression of Nurr1 release, indicating the co-adjustment between *PITX3* and Nurr1.[36,37] On the other hand, activation of thromboxane A2 receptor can activate Nurr1 expression and stimulate proliferation of human lung cancer cells.[38] Whether the regulatory effect of Nurr1 on lung cancer cells is affected by *PITX3* expression warrants further exploration.

In this study, we systemically analyzed the expression and prognostic value of *PITX* gene family in LUAD and provided a thorough understanding on the heterogeneity and complexity of the molecular biological properties of the 3 *PITX* genes. Our studies indicated that the increased expression of *PITX1* and *PITX2* in LUAD tissues might play a vital role in LUAD oncogenesis, therefore *PITX1* and *PITX2* could be promising diagnostic biomarkers for LUAD. Low *PITX1* expression and high *PITX3* expression were significantly associated with poor survival and tumor stage of the patients with LUAD, suggesting that *PITX1* and *PITX3* could be potential therapeutic targets for LUAD.

Author contributions

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Supervision: Chunyi Zhang.
Validation: Chunyi Zhang.
Visualization: Chunyi Zhang.
Writing – original draft: Chunyi Zhang.
Writing – review & editing: Chunyi Zhang.

References

[1] West S, Kumar S, Batra SK, et al. Uncovering and characterizing splice variants associated with survival in lung cancer patients. PLOS Comput Biol 2019;15:e1007469.
[2] Tan Q, Cui J, Huang J, et al. Genomic alteration during metastasis of lung adenocarcinoma. Cell Physiol Biochem 2016;38:469–86.
[3] Lindskog C, Fagerberg L, Hallström B, et al. The lung-specific proteome defined by integration of transcriptomics and antibody-based profiling. FASEB J 2014;28:5184–96.
[4] Ma J, Mannoor K, Gao L, et al. Characterization of microRNA transcriptome in lung cancer by next-generation deep sequencing. Mol Oncol 2014;8:1208–19.
[5] Yuan X, Yu G, Hou X, et al. Genome-wide identification of significant aberrations in cancer genome. BMC Genom 2012;13:342.

[6] Molinier O, Goupil F, Debieuvre D, et al. Five-year survival and prognostic factors according to histology in 6101 non-small-cell lung cancer patients. Resp Med Res 2019;77:46–54.
[7] Wu HY, Pan YY, Kopylov AT, et al. Assessment of serological early biomarker candidates for lung adenocarcinoma by using multiple reaction monitoring-mass spectrometry. proteomics. Clin Appl 2020; e1900095.
[8] Chen Y, Knösel T, Ye F, et al. Decreased PITX1 homeobox gene expression in human lung cancer. Lung Cancer (Amsterdam, Netherlands) 2007;55:287–94.
[9] Knösel T, Chen Y, Hotovy S, et al. Loss of desmocollin 1-3 and homeobox genes PITX1 and CDX2 are associated with tumor progression and survival in colorectal carcinoma. Int J Colorectal Dis 2012;27:1391–9.
[10] Stender JD, Stossi F, Funk CC, et al. The estrogen-regulated transcription factor PITX1 coordinates gene-specific regulation by estrogen receptor-alpha in breast cancer cells. Mol Endocrinol 2011;25:1699–709.
[11] Ohira T, Naohiro S, Nakayama Y, et al. miR-19b regulates hTERT mRNA expression through targeting PITX1 mRNA in melanoma cells. Sci Rep 2015;5:8201.
[12] Charles MA, Suh H, Hjalt TA, et al. PITX genes are required for cell survival and Lhx3 activation. Mol Endocrinol 2005;19:1893–903.
[13] Song X, Zhao C, Jiang L, et al. High PITX1 expression in lung adenocarcinoma patients is associated with DNA methylation and poor prognosis. Pathol Res Pract 2018;214:2046–53.
[14] Dietrich D, Hasinger O, Liebenberg V, et al. DNA methylation of the homeobox genes PITX2 and SHOX2 predicts outcome in non-small-cell lung cancer patients. Diagn Mol Pathol 2012;21:93–104.
[15] Li N, Li L, Chen Y. The identification of core gene expression signature in hepatocellular carcinoma. Oxid Med Cell Longev 2018;2018: 3478305.
[16] Yang HW, Menon LG, Black PM, et al. SNAI2/Slug promotes growth and invasion in human gliomas. BMC Cancer 2010;10:301.
[17] Logan M, Tabin CJ. Role of Pitx1 upstream of Tbx4 in specification of hindlimb identity. Science (New York, NY) 1999;283:1736–9.
[18] DeLaurier A, Schweitzer R, Logan M. Pitx1 determines the morphology of muscle, tendon, and bones of the hindlimb. Dev Biol 2006;299: 22–34.
[19] Kolfschoten IG, van Leeuwen B, Berns K, et al. A genetic screen identifies PITX1 as a suppressor of RAS activity and tumorigenicity. Cell 2005;121:849–58.
[20] Liu DX, Lobie PE. Transcriptional activation of p53 by Pitx1. Cell Death Differ 2007;14:1893–907.
[21] Szeto DP, Rodriguez-Esteban C, Ryan AK, et al. Role of the Bicoid-related homeodomain factor Pitx1 in specifying hindlimb morphogenesis and pituitary development. Genes Dev 1999;13:484–94.
[22] Gunathilake MN, Lee JH, Cho YA, et al. Interaction between physical activity, PITX1 rs647161 genetic polymorphism and colorectal cancer risk in a Korean population: a case-control study. Oncotarget 2018; 9:7590–603.
[23] Gage PJ, Suh H, Camper SA. Dosage requirement of Pitx2 for development of multiple organs. Development (Cambridge, England) 1999;126:4643–51.
[24] Zhou T, Song WF, Shang Y, et al. Halogen inhalation-induced lung injury and acute respiratory distress syndrome. Chin Med J 2018; 131:1214–9.
[25] Moreno CS, Evans CO, Zhan X, et al. Novel molecular signaling and classification of human clinically nonfunctional pituitary adenomas identified by gene expression profiling and proteomic analyses. Cancer Res 2005;65:10214–22.
[26] Meeh PF, Farrell CL, Croshaw R, et al. A gene expression classifier of node-positive colorectal cancer. Neoplasia 2009;11:1074–83.
[27] Huang Y, Guigon CJ, Fan J, et al. Pituitary homeobox 2 (PITX2) promotes thyroid carcinogenesis by activation of cyclin D2. Cell Cycle 2010;9:1333–41.
[28] Fung FK, Chan DW, Liu VW, et al. Increased expression of PITX2 transcription factor contributes to ovarian cancer progression. PloS One 2012;7:e37076.
[29] Wang J, Bai Y, Li N, et al. Pitx2-microRNA pathway that delimits sinoatrial node development and inhibits predisposition to atrial fibrillation. P Natl Acad Sci USA 2014;111:9181–6.
[30] Hayashita Y, Osada H, Tatematsu Y, et al. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res 2005;65:9628–32.
[31] Nunes I, Tovmasian LT, Silva RM, et al. Pitx3 is required for development of substantia nigra dopaminergic neurons. Proc Natl Acad Sci USA 2003;100:4245–50.
[32] van den Munckhof P, Luk KC, Ste-Marie L, et al. Pitx3 is required for motor activity and for survival of a subset of midbrain dopaminergic neurons. Development 2003;130:2535–42.
[33] Dietrich D, Lesche R, Tetzner R, et al. Analysis of DNA methylation of multiple genes in microdissected cells from formalin-fixed and paraffin-embedded tissues. J Histochem Cytochem 2009;57:477–89.
[34] Holmes EE, Goltz D, Sailer V, et al. PITX3 promoter methylation is a prognostic biomarker for biochemical recurrence-free survival in prostate cancer patients after radical prostatectomy. Clin Epigenetics 2016;8:104.
[35] Sailer V, Holmes EE, Gevensleben H, et al. PITX3 DNA methylation is an independent predictor of overall survival in patients with head and neck squamous cell carcinoma. Clin Epigenetics 2017;9:12.
[36] Jacobs FM, van Erp S, van der Linden AJ, et al. Pitx3 potentiates Nurr1 in dopamine neuron terminal differentiation through release of SMRT-mediated repression. Development (Cambridge, England) 2009;136: 531–40.
[37] Volpicelli F, De Gregorio R, Pulcrano S, et al. Direct regulation of Pitx3 expression by Nurr1 in culture and in developing mouse midbrain. PloS One 2012;7:e30661.
[38] Li X, Tai HH. Activation of thromboxane A(2) receptors induces orphan nuclear receptor Nurr1 expression and stimulates cell proliferation in human lung cancer cells. Carcinogenesis 2009;30:1606–13.