Investigation on Potential Correlation Between the RNA-Binding Protein of Evolutionarily Conserved MEX3 Family and Non–Small-Cell Lung Cancer

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Abstract

Background

As mRNA binding proteins, MEX3 (muscle excess 3) family highlights its unique characteristics and plays an emerging role in post-transcriptionally regulating programmed of biological processes, including tumor cell death and immunological relevance. These have been shown to be involved in various diseases, however, the role of MEX3 in non-small-cell lung cancer (NSCLC) has not been fully elucidated.

Results

In this study, we found that the sequence or copy number of *MEX3* gene did not change significantly, which can explain the stability of malignant tumor development through the COSMIC database. Further, gene expression in NSCLC was examined using the Oncomine™ database, and the prognostic value of each gene was analyzed by Kaplan-Meier analysis. The results showed that overexpressed of MEX3A, MEX3B, MEX3C and MEX3D were associated with significantly lower OS in patients with NSCLC and LUAD, while overexpressed of MEX3D was associated with significantly poorer OS in patients with LUSC. We also applied the Tumor Immune Estimation Resource (TIMER) tool to assess the correlations between distinct MEX3 and the infiltrating immune cell landscape.

Conclusion

On this subject, we have learned about the complexity and heterogeneity of NSCLC through MEX3. We found that most of MEX3 is highly expressed in NSCLC. High expression indicates a poor prognosis and has a certain immune correlation. Therefore, these conclusions can lay a framework for the prognosis of NSCLC patients and the development of treatment strategies in the future.

Introduction

RNA-binding proteins (RBPs) are highly species conserved, maintaining the homeostasis of gene expression and regulating almost all RNA post-transcriptional process [1–3]. MEX3 (muscle excess 3) is a member of the evolutionarily conserved RBP family, comprised of four human members (MEX3A-D), which encodes different phosphorylated proteins, exhibits different expression patterns in human genome response [4,5]. Each human MEX3 protein has two K homology (KH) domains bound to RNA, distinguished from other RBPs by a C-terminal RING called the ubiquitin E3 ligase RING (Really Interesting New Gene) domain[4,6]. This particular indicates that the MEX3 proteins play vital role in the balance between self-renewal and differentiation by mediating self-ubiquitination or ubiquitination of target protein and promoting RING-dependent degradation of HLA-A2 (human leukocyte antigen serotype A2) mRNA[6,7]. The ability of MEX3 proteins can not only interact with different RNA sequences, but also diversify the mechanisms enhanced by the RING domain, despite the increasing complexity of regulation,

so far, little evidence support these redundant[8]. Consistent with the multi-pathway disease presented by cancer and the multiple roles of MEX3 in regulating gene expression, MEX3 is involved in multiple biological processes in the occurrence and development of cancer[9]. MEX3 mediates cancer cell proliferation, migration, tumor immune escape mechanism, and the transcription level changes in different cancer types[10–12]; according to the tumor type and family members, the expression of MEX3 is related to the increase or decrease of patient survival rate.

However, there are few studies on the correlation between MEX3 family and lung cancer, and the conclusions are limited. In agreement, here, we draw attention to investigating on large sample of databases to explore MEX3 family expression, prognostic value, and immune-related effects in non–small-cell lung cancer(NSCLC),thereby providing further insights into tumor heterogeneity and targeted therapy.

Materials And Methods

Somatic Mutations of MEX3 in lung cancer.

We applied COSMIC database[13] to establish *MEX3* somatic mutation entries, a free online authoritative resource that provides information on gene mutations, fusion, genome rearrangement, and human cancers copy number variations. Data for this study are from COSMIC v92 version, with a deadline of February 20, 2021.

Expression of MEX3 in NSCLC.

Oncomine™ 4.5 Research Edition[14] (http://www.oncomine.org/) used for the exploration of *MEX3* expression,a web-based database that contains data mining related to cancer microarray, to conduct genome-wide analyses of major cancer types and normal tissues, and to compare transcriptome expression of the data analyzed. It currently contains 715 data sets (86,733 samples). In this study, we used the database to determine the mRNA expression of MEX3 in NSCLC and compared the mRNA levels of lung cancer patients and healthy controls, P = 0.05, fold change value > 1.5, the top 10% genes, as the threshold. Data entries from October 2020 to February 2021, and results were drawn with GraphPad Prism 7 software (GraphPad Software, Inc.).

MEX3 prognostic analysis

Kaplan-Meier plotter[15,16] were used to assess the prognostic relevance of MEX3 A-D specifically expressed in NSCLC samples. In our study, Affymetrix Identity (Jetset best Probe[17], as shown in Table I) identified available genes, used median gene expression values to divide patient samples into high and low expression group, 95% confidence interval (CI) calculated log-rank P value and hazard ratio (HR), "Array quality control" and "Exclude biased array " were selected to obtain numerical results through

univariate Cox regression analysis. Finally, the valuable Kaplan-Meier survival curve (OS, Overall survival) was generated according to these parameters.

Immunological correlation of MEX3

The immune correlation of MEX3 was completed through the Tumor Immune Estimation Resource (TIMER 2.0, timer.comp-genomics.org/), a web server for comprehensive analysis of tumor infiltrating immune cells[18], including The Cancer Genome Atlas (TCGA) of cancer genome maps from 32 cancer types, a total of 10,897 samples, six subsets of TIIC including B cells, CD4 + T cells, CD8 + T cells, macrophages, neutrophils and dendritic cells were involved[19]. Here, we investigate the significance of MEX3A-D mRNA expression data and invasion of six immune cell types in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC).

Results

Four MEX3 genes were assessed for mutations by COSMIC database. Prior to February 20, 2021, the characteristics of *MEX3* in Table II, and the genetic alteration affecting *MEX3* in lung cancer samples in Table III. We found that the regulatory mechanism types in tumors, including lung cancer, are mainly missense mutations. Further, in the regulatory mechanism types study of lung cancer, three regulatory mechanism types were found in Fig. 1, Point Mutations, Copy Number Variation (CNV), Gene Expression. While in these regulatory mechanism types, point mutations mainly occurred in *MEX3B*, with the highest mutation frequency being 1.06%; CNV was confirmed on *MEX3A*, with the highest mutation frequency 1.99%; Gene Expression (Overexpressed) was determined on *MEX3A*, with the highest frequency 14.03%. No translocations, insertions, deletions, or loss of heterozygosity were identified. In summary, the results indicate that the sequence or copy number of the *MEX3* gene has not altered significantly, except for *MEX3A* overexpression, which revealed that it is stable and not easy to mutate, leading to malignant proliferation of tumor cells. It may explain the development of malignant tumors.

Oncomine™ database analysis revealed that MEX3 expression in tissues of NSCLC patients compared to normal. The column bar graph in Fig. 2 was derived from the expression of each gene in tumors of different pathological types. The analysis demonstrated that, compared with normal, expression of MEX3 mRNA were significantly overexpressed in different pathological types. It concluded that: MEX3C does not meet the conditions, and other subtypes are overexpressed. MEX3A[20] can be found in LUAD, LUSC and large cell lung cancer (LCLC), MEX3B[20] in LCLC and MEX3D[21] in LUAD. Furthermore, under the same pathological type and different databases, the distinctions in MEX3 expression were summarized in Table IV.

The prognostic value of MEX3 mRNA expression was examined by Kaplan Meier-plotter [Lung Cancer] 2015 version. Firstly, Fig. 3a demonstrated the prognostic effect of MEX3A mRNA expression. Over-expressed of MEX3A had significantly poor prognosis of NSCLC patients (HR = 1.48; CI, 1.26–1.75; P = 2.9E-06), LUAD (HR = 1.74; CI, 1.36–2.22; P = 9.9E-06), but not in LUSC (HR = 1.00; CI, 0.73–1.36; P = 0.99). Secondly, the effect of MEX3B mRNA expression on prognosis was examined, once again, the survival

curves of patients with NSCLC, LUAD, and LUSC were described (Fig. 3b). Over- expressed of MEX3B was associated with decreased OS in all NSCLC (HR = 1.37; CI, 1.17–1.62; P = 1.4E-04) and LUAD cases (HR = 1.32; CI, 1.04–1.68; P = 0.023), but not in patients with LUSC (HR = 1.05; CI, 0.77–1.42; P = 0.78). As indicated in Fig. 3c, the prognostic value of MEX3C mRNA expression was analyzed. In all patients with NSCLC (HR = 0.81; CI, 0.71–0.91; P = 7.8E-04) and LUAD (HR = 0.50; CI, 0.39–0.63; P = 5.6E-09), over-expressed was related to lower OS, but not to LUSC (HR = 0.87; CI, 0.69–1.11; P = 0.26). Finally, Fig. 3d demonstrates the prognostic effect of MEX3D mRNA expression. Over-expressed of MEX3D had statistical difference in patients with NSCLC (HR = 1.30; CI, 1.14–1.47; P = 5.2E-05), LUAD (HR = 2.13; CI, 1.67–2.72; P = 4.5E-10) and LUSC (HR = 0.76; CI, 0.60–0.96; P = 0.02).

The associations between MEX3 and clinic pathological characteristics in NSCLC patients were explored, also, including Pathological histology, Stage, American Joint Committee on Cancer (AJCC) classification T, Lymph node status (AJCC stage N), Gender, Smoking status and Chemotherapy. Grade, AJCC stage M and Radiotherapy cannot examined because of invalid samples. As demonstrated in Table V, Overexpressed of MEX3A, MEX3B, MEX3C and MEX3D were associated with significantly lower OS in NSCLC and LUAD, while Overexpressed of MEX3D was associated with significantly poorer OS in patients with LUSC. Next, MEX3A, MEX3C and MEX3D overexpressed were identified to significant poorer OS in stage I, while MEX3C also has a consistent prognosis in stage II in Table S1. MEX3A and MEX3B were significantly associated with classification 2,while MEX3A was associated with classification 1 and MEX3B was associated with classification 3□shown in Table S2.

MEX3A was correlated with 0 lymph node status (Table S3), meanwhile, four MEX3s were correlated with gender (Table S4) in NSCLC patients. For MEX3A, it was significantly associated to smoking, but MEX3C and MEX3D no significant (Table S5). In contrast, MEX3 was not associated to prognosis with or without chemotherapy treatment in NSCLC patients (Table S6).

The relationship between MEX3 and the immune microenvironment of LUAD and LUSC, as well as information of tumor purity were obtained by TIMER 2.0 database. In LUAD, MEX3A was positively correlated with tumor purity and negatively correlated with the level of dendritic cell infiltration. MEX3B was positively correlated with tumor purity, CD4 + T cell and macrophage infiltration level. MEX3C, not only correlated with tumor purity, but also with CD8 + T cells, CD4 + T cells, macrophages, and neutrophils infiltration levels were positively correlated. Finally, we found that MEX3D has statistically related with tumor purity, but positively in CD4 + T cells, macrophages and neutrophils (Fig. 4a). In LUSC, similarly, MEX3A was shown to be positively correlated with tumor purity, B cells, negatively with CD4 + T cells. MEX3B and MEX3C were positively correlated in tumor purity, CD8 + T cells and CD4 + T cells. In addition, MEX3D has no signification on tumor purity, but positively correlates with CD4 + T cells and macrophages (Fig. 4b). Here, further, we assessed that overexpressed MEX3 had no statistically significant difference in the survival rate of NSCLC patients, regardless of whether it was 1 year, 3 years or 5 years (P > 0.05).

Discussion

The human MEX3 family is differentially expressed in healthy tissues of different origins[9], so in the same way, we are interested in understanding how it is expressed in abnormal tissues, particularly in cancer. In the human protein atlas, members of the MEX3 family are expressed in heterogeneously types of tumors[22,23]. Many evidences indicated that MEX3A promotes cell proliferation and inhibits cell apoptosis bladder cancer[24,25], gastric cancer[26], colorectal cancer [27]. Furthermore, increased MEX3A levels are also reported in liver cancer, which were significantly associated with a poor patients' survival[28]. This study revealed that over-expression of MEX3A had significantly poor prognosis of NSCLC and LUAD, but not in LUSC. In addition, increased expression of MEX3A in NSCLC patients is also associated with stage I, classification tumor, lymph node status, and male, whether chemotherapy has little effect on the prognosis. For MEX3B,there are few studies on tumors. It may be a ubiquitination of Runx3(runt-related transcription factor 3) and can increase invasion of gastric cancer cells[29]. Our study revealed that high expression of MEX3B mRNA had decreased OS in all NSCLC and LUAD cases. Over-expressed MEX3B was also associated with classification T2 and T3, and gender of NSCLC patients.

Recently, MEX3A and MEX3C proteins have been reported to be negatively correlated posttranslational regulators of several target genes[30]. In colorectal cancer, MEX3C has been identified as an unstable gene that is frequently lost in CIN+ (cervical intraepithelial neoplasia+) tumors[31,32],regulating lipid metabolism through JNK(c-Jun N-terminal kinases) pathway in bladder cancer[33]and breast cancer[34]. Based on these observations, we hypothesized that MEX3C is an important part of influencing metastasis and prognosis in NSCLC. Consistent with our findings, high expression of MEX3C mRNA was associated with poor prognostic OS in NSCLC and LUAD patients. Several study[35,36] demonstrated that MEX3D reverses apoptosis by interacting with au-rich elements (AREs) and enhances the degradation of BCL2 (B-cell lymphoma2) mRNA. Moreover, MEX3D is frequently deleted in various human cancers[37], can also participate in the modulation by chemotherapy in AML (acute myeloid leukemia)[38] and overexpression in androgen-independent prostate cancer[39]. In this study, according to Kaplan-Meier analysis, high expression of MEX3D was observed, it was a good prognostic indicator, not only in LUAD and LUSC, but also in all NSCLC.

In immune response, MEX3B can be used as a co-receptor in the innate antiviral response of toll-like receptor[40]. In melanoma[41], under-expression of MEX3B is associated with antibodies against the programmed cell death 1 (PD-1) receptor, while over-expression can inhibit T cell-mediated tumor elimination. MEX3C is involved in the regulation of proteins, degradation and ubiquitination, and it has been identified as a new type of RNA-binding E3 ubiquitin ligase, which is responsible for post-transcriptional regulation[42]. In present study, our results showed that MEX3B and MEX3C expression was positively related to tumor purity, and CD8 + T cell and CD4 + T cell infiltrating levels. While the function of MEX3A and MEX3D in immune responses has not yet been sufficient research evidence. Abundant evidence[8,9] that, MEX3 proteins have the ability to regulate gene expression in tumor suppression, negative correlation. In the follow-up, we can explore the research of the targeted inhibitor of MEX3 and it could be identified as a marker of immunotherapy detail.

In summary, from the research results, in the COSMIC database, the sequence or copy number of MEX has no major alterations, which can explain its more copy number in malignant tumors, that is, malignant proliferation. After that, we used the Oncomine™ database to detect the expression of MEX3 in NSCLC, and Kaplan-Meier analyzed the prognostic value of genes. The results indicated that in NSCLC and LUAD, the lower prognosis of overexpressed MEX3A, MEX3B, MEX3C, and MEX3D was statistically significant, besides, in LUSC, MEX3D is also highly expressed with poor OS. In addition, MEX3B and MEX3C were positively related to tumor purity, of CD8 + T cells and CD4 + T cells infiltrating levels in TIMER.

These data reflect the potential association of MEX3 in non-small cell lung cancer. We found that most of MEX3 is highly expressed in NSCLC. High expression indicates a poor prognosis and has a certain immune correlation. Therefore, these conclusions can lay a framework for the prognosis of NSCLC patients and the development of treatment strategies in the future. But of course, our research needs to be improved and deepened. In the future, we need to further analyze its post-transcriptional regulatory mechanism and immunomodulatory effects, which is currently the focus of tumor immunotherapy direction.

Declarations

Acknowledgements

Not applicable.

Availability of data and materials

The datasets analyzed in the present study are available in the COSMIC database (https://cancer.sanger.ac.uk/cosmic) and Oncomine^{TM}database (https://www.oncomine.org/resource) and the Kaplan Meier-plotter [Lung Cancer] (http://kmplot.com/analysis/index.php?p=service&cancer=lung) and the TMIER

(http://timer.comp-genomics.org/)

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MZ, HL Z, JJD were responsible for the design of the study and interpretation of the data. LF C, HX H, ZX F and XD L examined the archives and identified the databases included in the study, and they also have revised critically the manuscript for important intellectual content. All authors contributed to the writing of the manuscript. All authors read and approved the final manuscript.

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Tables

Table I. Affymetrix ID of MEX3 family in the Kaplan-Meier plotter.

MEX3	Affymetrix ID (Jetset best probe set)
MEX3A	226346_at
MEX3B	223627_at
MEX3C	218247_s_at
MEX3D	91816_f_at

MEX3, muscle excess 3.

Table II. The characteristics of *MEX3* family in COSMIC.

	Chromosomal location	the distribution of mutations across the primary tissue types			Drug resistance
		Point Mutations	Copy Number Variation	Gene expression	
MEX3A	1q22	Endometrium (3.09%)	Biliary tract (8.33%)	Adrenal gland (30.38%)	None
MEX3B	15q25.2	Large intestine (4.08%)	Skin (1.02%)	Urinary tract (9.06%)	
MEX3C	18q21.1	Central nervous system (6.72%)	Stomach (3.39%)	None	
MEX3D	19p13.3	Cervix (2.45%)	Ovary (1.32%)	Adrenal gland (Over-expressed 49.37%; Under-expressed 37.97)	

MEX3, muscle excess 3.

Table III. Genetic alteration affecting *MEX3* family in lung cancer samples (COSMIC database)

	Percent of mutated samples(number)	Genetic alteration	Number	Percentage (%)
MEX3A	272/38263	Ponit Mutations	16/2608	0.61
		Copy number Variation	20/1006	1.99
		Gene Expression	143/1019	14.03
MEX3B	379/38350	Ponit Mutations	46/2609	1.76
		Copy number Variation	1/1006	0.10
		Gene Expression	55/1019	5.40
MEX3C	311/38693	Ponit Mutations	29/2742	1.06
		Copy number Variation	2/1006	0.99
		Gene Expression(over)	53/1019	5.20
		Gene Expression(under)	21/1019	2.06
MEX3D	262/38693	Ponit Mutations	13/2742	0.47
		Copy number Variation	10/1006	0.99
		Gene Expression	58/1019	5.69

MEX3, muscle excess 3.

Table IV. Expression of MEX3 family in different NSCLC pathological types

MEX3	Fold change	Dataset	Sample number		Total	P-value
			Normal	Cancer		
A, Lung adenocarcinoma vs. normal						
MEX3A	1.937	Hou Lung	65	45	110	$9.11x10^{-14}$
MEX3D	1.649	Landi Lung	49	58	107	$7.58x10^{-9}$
B, Squamous cell lung carcinoma vs. normal						
MEX3A	1.759	Hou Lung	65	27	92	$3.64x10^{-10}$
C, Large cell lung carcinoma vs. normal						
MEX3A	3.534	Hou Lung	65	19	84	$4.17x10^{-7}$
MEX3B	2.475	Hou Lung	65	19	84	$1.94x10^{-5}$

MEX3, muscle excess3; NSCLC, not-small-cell lung cancer.

Table V. Correlation of MEX3 with Pathological histology in NSCLC patients.

	Pathology subtype	Case-low	Case-high	HR(95%CI)	P value
MEX3A	All	576	568	1.48(1.26-1.75)	2.9E-06
	LUAD	339	333	1.74(1.36-2.22)	9.9E-06
	LUSC	136	135	1.00 (0.73-1.36)	0.99
MEX3B	All	589	555	1.37(1.17-1.62)	1.4E-04
	LUAD	340	332	1.32 (1.04-1.68)	0.023
	LUSC	137	134	1.05(0.77-1.42)	0.78
MEX3C	All	962	963	0.81(0.71-0.91)	7.8E-04
	LUAD	360	359	0.50 (0.39-0.63)	5.6E-09
	LUSC	262	262	0.87 (0.69-1.11)	0.26
MEX3D	All	962	963	1.30 (1.14-1.47)	5.2E-05
	LUAD	361	358	2.13 (1.67-2.72)	4.5E-10
	LUSC	262	262	0.76 (0.60-0.96)	0.02

Abbreviation: NSCLC, non-small-cell lung cancer; All stands for NSCLC; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinomas; HR: hazard ratio; CI: confidence interval; Cases-low/high: patient number of low/high expression of the corresponding gene.

Figures

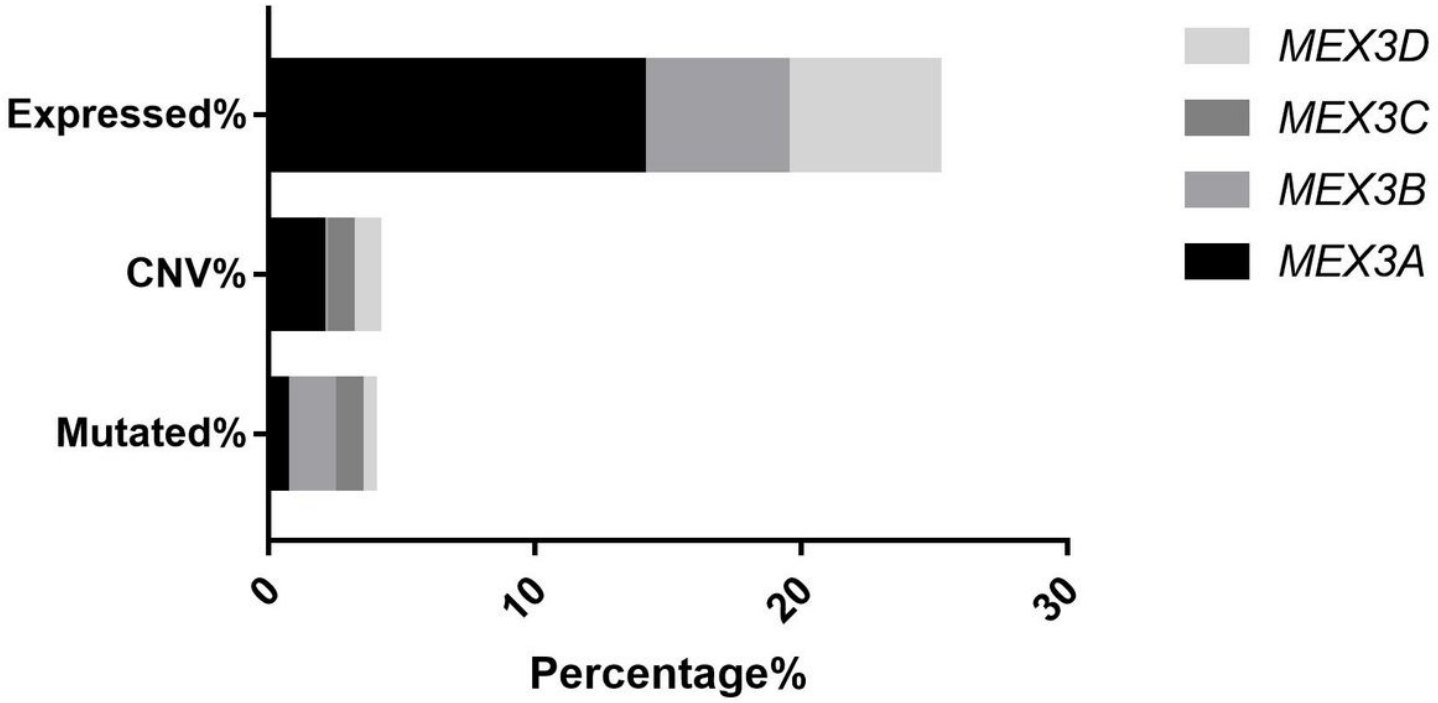

Figure 1

The MEX3 mutation percentage of different mutation types in lung cancer based on COSMIC database. Abbreviation: MEX3, muscle excess3；CNV, copy number variation.

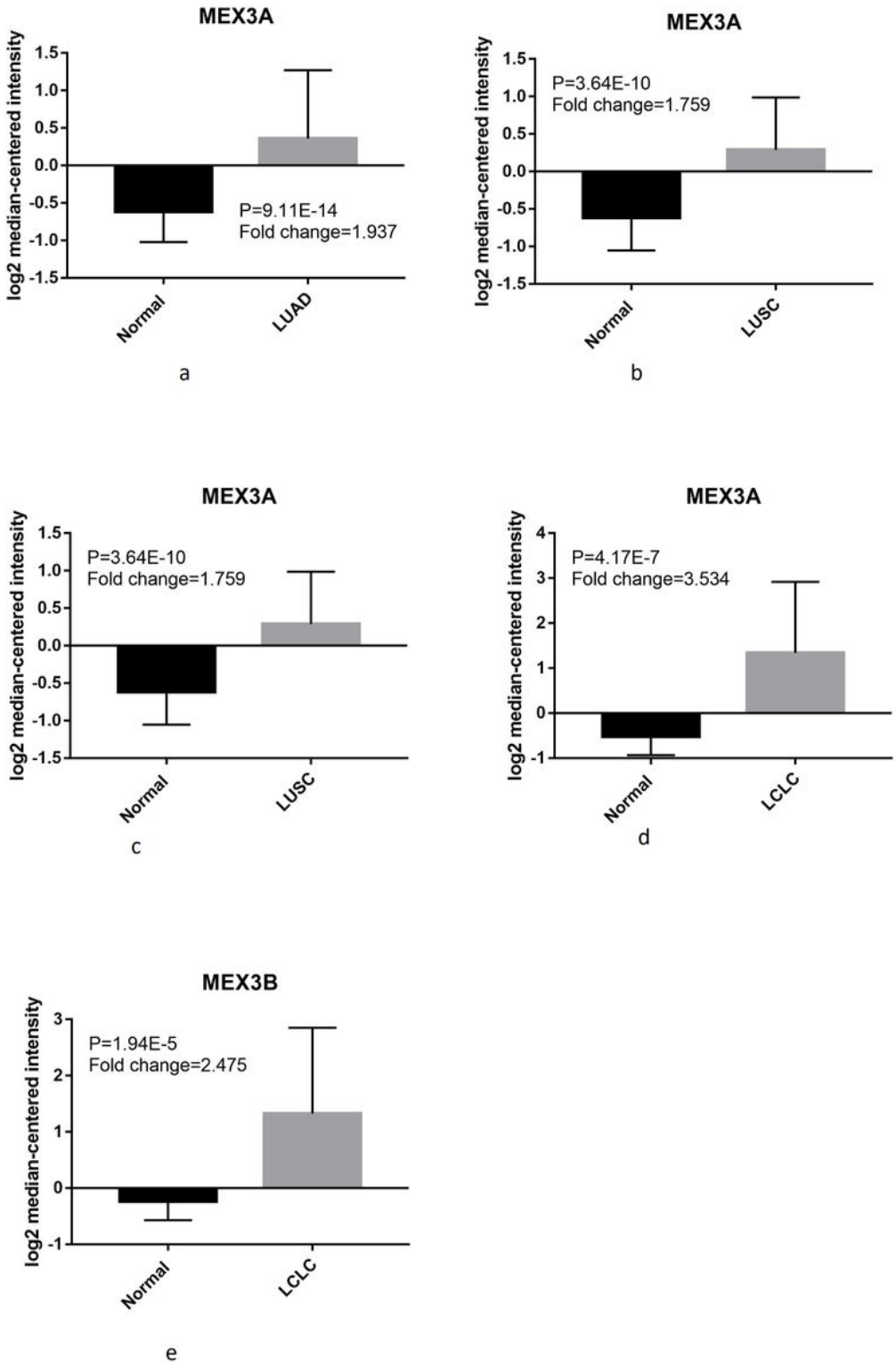

Figure 2

The analysis of MEX3 in lung cancer from Oncomine analysis. Column bar graph derived from gene expression data in Oncomine comparing expression levels of MEX3 in normal (left plot) and cancer (right plot) tissue and plotted using Graphpad Prism 7 software. Y-axis represents Mean with Standard Deviation (M±SD). (a) Comparison of MEX3A mRNA expression in LUAD. (b) Comparison of MEX3D mRNA expression in LUAD. (c) Comparison of MEX3A mRNA expression in LUSC. (d) Comparison of

MEX3A mRNA expression in LCLC. (e) Comparison of MEX3B mRNA expression in LCLC. Abbreviation: MEX3, muscle excess 3. Lung adenocarcinoma (LUAD), Lung squamous cell lung carcinoma (LUSC), Large cell lung carcinoma (LCLC).

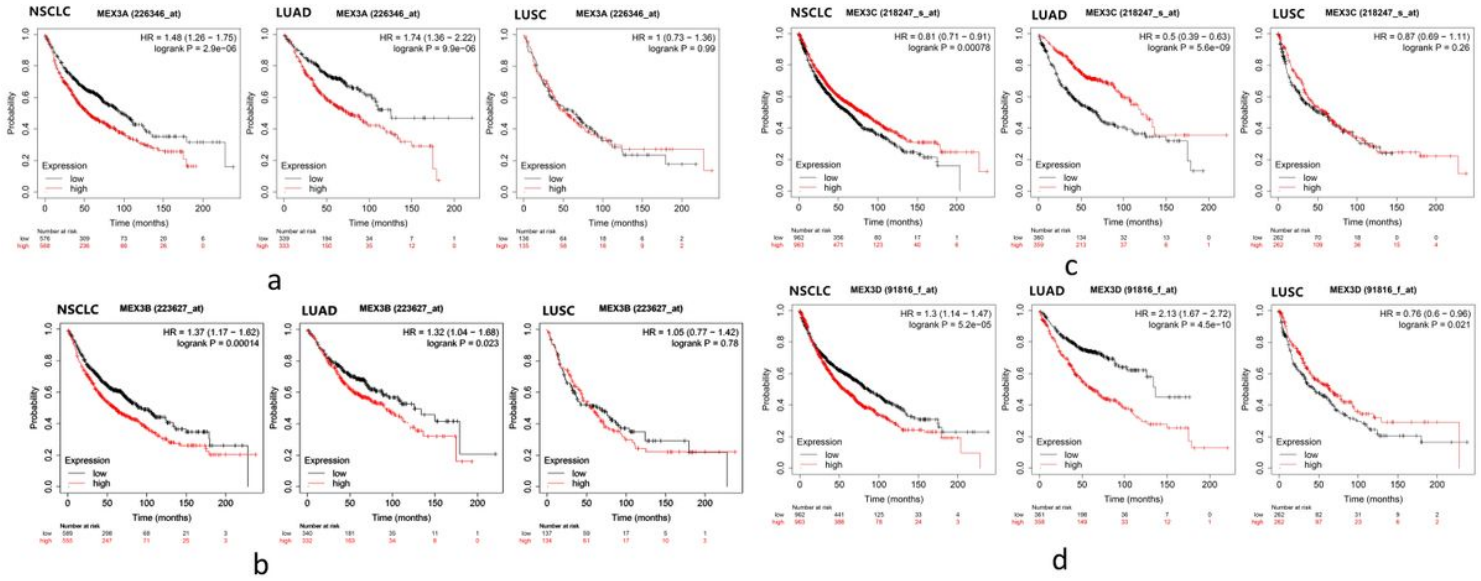

Figure 3

a The prognostic value of MEX3A expression. Survival curves were plotted for all NSCLC patients (n=1144). Survival curves were plotted for LUAD patients (n=672). Survival curves were plotted for LUSC patients (n=271). Data was analyzed using Kaplan-Meier Plotter. Patients with expression above the median are indicated in red line, and patients with expressions below the median in black line. Abbreviation: HR, hazard ratio; CI, confidence interval. b The prognostic value of MEX3B expression. Survival curves were plotted for all NSCLC patients (n=1924). Survival curves were plotted for LUAD patients (n=672). Survival curves were plotted for LUSC patients (n=271). Data was analyzed using Kaplan-Meier Plotter. Patients with expression above the median are indicated in red line, and patients with expressions below the median in black line. Abbreviation: HR, hazard ratio; CI, confidence interval. c The prognostic value of MEX3C expression. Survival curves were plotted for all NSCLC patients (n=1925). Survival curves were plotted for LUAD patients (n=719). Survival curves were plotted for LUSC patients (n=524). Data was analyzed using Kaplan-Meier Plotter. Patients with expression above the median are indicated in red line, and patients with expressions below the median in black line. Abbreviation: HR, hazard ratio; CI, confidence interval. d The prognostic value of MEX3D expression. Survival curves were plotted for all NSCLC patients (n=1925). Survival curves were plotted for LUAD patients (n=719). Survival curves were plotted for LUSC patients (n=524). Data was analyzed using Kaplan-Meier Plotter. Patients with expression above the median are indicated in red line, and patients with expressions below the median in black line. Abbreviation: HR, hazard ratio; CI, confidence interval.

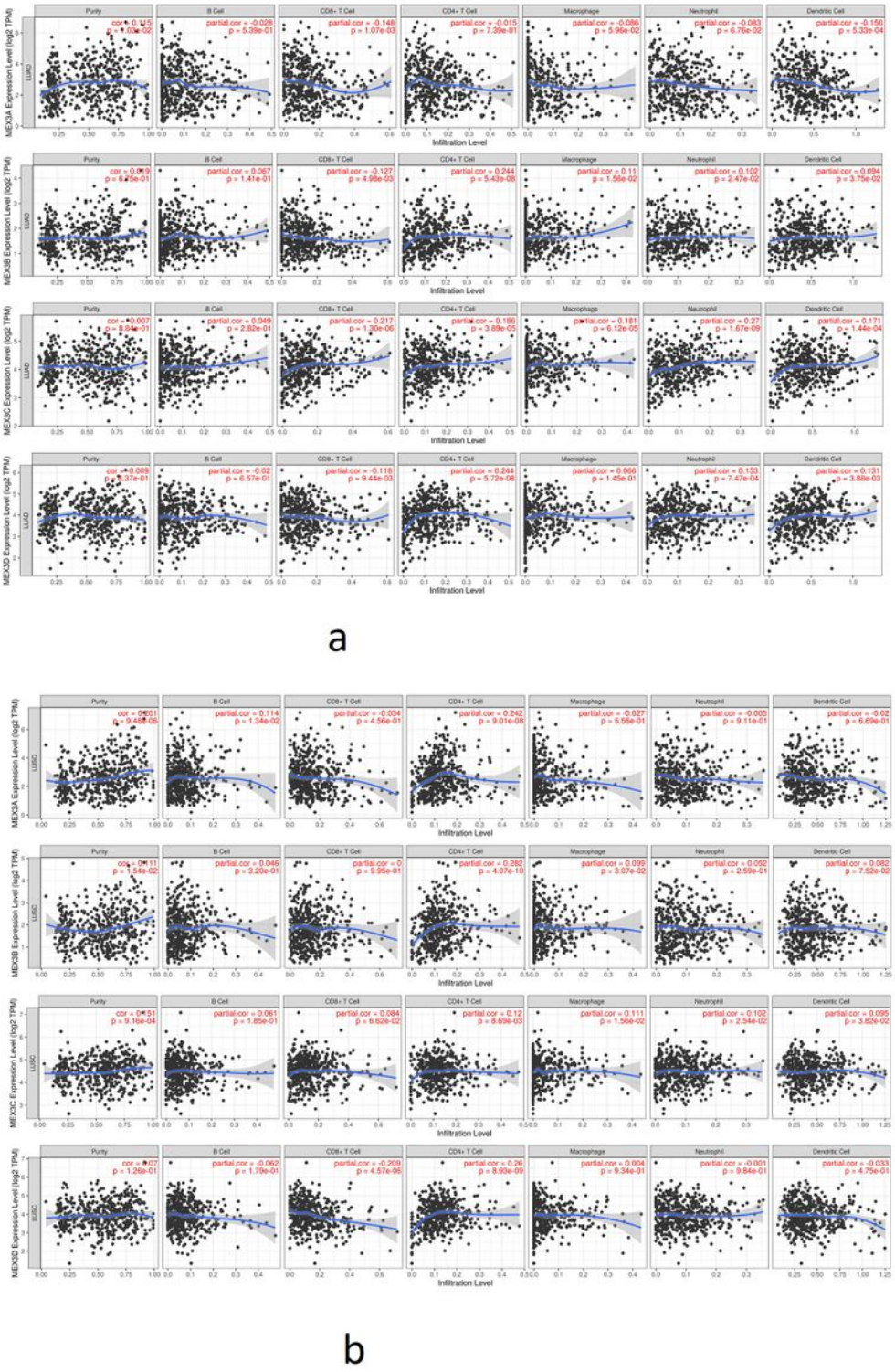

Figure 4

a Correlation between MEX3 and immune cells in LUAD. b Correlation between MEX3 and immune cells in LUSC.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalFile.zip