CENPF driven lung adenocarcinoma and lung squamous cell carcinoma with immune infiltrates

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Research article

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Abstract

Background: CENPF (centromere protein F) is a critical gene that associates with the centromere-kinetochore complex and plays an important role in the tumor development. However, the associations of CENPF expression and tumor infiltrating lymphocytes in lung cancer remain unknown.

Methods: CENPF expression and prognostic factor was analyzed via the Gene Expression Profiling Interactive Analysis (GEPIA) site. The correlation between CENPF and cancer immune infiltrates was investigated via and Tumor Immune Estimation Resource (TIMER) site. Further, correlations between CENPF expression and gene marker sets of immune infiltrates were analyzed by TIMER.

Results: The TCGA database of Lung adenocarcinoma (LUAD) and Lung squamous cell carcinoma (LUSC) patients showed that high CENPF expression was associated with poorer overall survival (OS HR=1.5, P=0.01) and disease-free survival (DFS HR=1.4, P=0.027) in LUAD. Specifically, high CENPF expression have no correlated with worse OS (OS HR=0.78, P=0.071) and DFS (DFS HR=1, P=0.87) in LUSC. CENPF expression was positively correlated with infiltrating levels of B cells, macrophage in LUAD, B cells, and CD8+ T cells, macrophages, neutrophils, and dendritic cells (DCs) in LUSC. CENPF expression showed strong correlations with diverse immune marker sets in LUAD, and LUSC. After down-regulating the expression of CENPF, the proliferative capacity of Lung adenocarcinoma and Lung squamous cell carcinoma cells was inhibited.

Conclusions: This report suggest that CENPF is high expression, correlated with poor prognosis and immune infiltrating levels of, including those of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and DCs in in LUAD and LUSC. In addition, CENPF expression is potentially closely related to the proliferation and metastasis of lung cancer cells. These studies suggest that CENPF can be used as a new prognostic target for determining prognosis and immune infiltration in Lung adenocarcinoma and Lung squamous cell carcinoma.

Background

Lung cancer is a malignant tumor with a high incidence in the world, Chemotherapy resistance, metastasis and rapid proliferation and are important biological characteristics leading to poor prognosis [1]. Except for prostate cancer in males and breast cancer in females, lung cancer is the second most commonly diagnosed cancer in men and women[2]. Due to the biological role of immune-related mechanisms in tumor disease, more immunotherapy strategies are considered to be a promising direction for the cancer treatment[3]. Immunotherapy displayed effective antitumor effects in non-small-cell lung carcinoma (NSCLC),mainly including programmed death ligand-1 (PD-L1) inhibitors, programmed death-1 (PD-1) and cytotoxic T lymphocyte associated antigen 4 (CTLA4)[4, 5]. In addition, Immunotherapy that has been approved for use, such as anti-PD-L1 showed a partial response in advanced lung cancer[6]. Therefore, further researches are needed to develop treatments for lung cancer, including the identification of prognostic factors for lung cancer Immunotherapy.

Centromere protein F (CENPF) located on chromosome 1q41, is a protein encoding gene acts as part of the centromere-kinetochore complex and a component of the nuclear matrix during G2 of interphase[7]. The express of CENPF accumulates during the cell cycle, reaches peak levels in the G2/M phase, and then declined upon completion of mitosis in a cell cycle-dependent manner via chromosome segregation regulating[8]. Previous studies indicate that CENPF is one gene that is strongly associated with aggressive prostate cancer, and that its expression is positively correlated with metastasis tumor cells as well as plays a critical role in metabolic of prostate cancer cells[8, 9]. Moreover, CENPF also significantly plays a role in cancer chemotherapeutic sensitivity. The previous findings demonstrate that the CENPF plays a critical role in cancer progression.

In the current study, we aim to analysis CENPF expression and correlation with prognosis of lung cancer patients in databases including GEPIA and TIMER. Moreover, we evaluated the correlation of CENPF with tumor-infiltrating immune cells in the Lung adenocarcinoma and Lung squamous cell carcinoma microenvironments via TIMER. And CENPF could promote the proliferation of Lung adenocarcinoma and Lung squamous cell carcinoma. The findings of this report reveal the important role of CENPF in lung adenocarcinoma and lung squamous cell carcinoma and provide a potential relationship and potential mechanism between CENPF and tumor immune interactions.

Methods

Cell culture

The A549 and H1299 cell line were purchased from China Center for Type Culture Collection (CCTCC, Wuhan, China). The two cells were cultured in DMEM (Gibco, NY, USA) supplemented with 10% FBS in a 37 °C, 5% CO₂ incubator.

TIMER Database Analysis

TIMER(<https://cistrome.shinyapps.io/timer/>) is a visible analysis resource base on immune infiltrates across multiple cancer types. The TIMER was used to estimate the abundance of immune infiltrates database including diverse cancer types from Public database(TCGA). We analyzed CENPF expression in Lung adenocarcinoma(LUAD) and Lung squamous cell carcinoma(LUSC) and the correlation of CENPF expression with the enrichment of immune infiltrates, including B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells, via gene modules.

Gene expression and survival analysis in GEPIA

GEPIA (Gene Expression Profiling Interactive Analysis) was used to analysis the CENPF expression and survival. GEPIA is an public database web that analysis the RNA sequencing expression including the

samples from TCGA and the GTEx projects. GEPIA was used to analysis over survival (OS) and disease free survival (DFS), based on CENPF expression with the Kaplan-Meier in LUAD and LUSC.

Wound healing analysis

The migration ability of NSCLC cells was evaluated using the scratch assay. After sh-Control and sh-CENPF transfection, cells were seeded in six-well plastic plates at a density of 5×10^5 cells/well. After 24 hours, the cells reached 90%–100% monolayer confluence. A straight scratch was artificially created in the cell monolayers with a sterile 200 μ L pipette tip. Cell debris was removed with PBS three times. The cell growth medium was FBS free and cultured for 48 hours at 37°C. Scratch wound widths were measured under the microscope, and the relative wide of wound closure was determined by comparing to sh-Control cells.

Transwell invasion assay

The effect of CENPF on NSCLC cell invasion was evaluated by Transwell invasion assay. 2×10^4 cells were transfected with sh-Control and sh-CENPF for 48 hours and then added into the Transwell upper chamber with Matrigel and DMEM(1:8) (BD, Matrigel™) polycarbonate membrane (8.0 μ m; Corning Incorporated, Corning, NY, USA). A total of 700 μ L medium containing 20% FBS was added to the lower Transwell chamber. After incubation for 24 hours, cells on the lower surface of the polycarbonate membrane were fixed with 4% paraformaldehyde and stained

CCK-8 assay

cells were seeded at a density of 3,000cells/well in 96-well plates. After treatment with 10 μ L CCK-8 in 100 μ L complete culture medium, absorbance was measured at 450 nm using a enzyme-labeled instrument. Each experiment was performed in triplicate.

Statistical Analysis

Statistical significance was tested using two-tailed Student's *t*-test and chi-squared test. Survival analyses were performed using Kaplan–Meier plots. Statistical analysis was performed using the SPSS22.0, with **P*<0.05, ** *P*<0.05.

Results

CENPF was downregulated in LUAD and LUSC

To evaluate the CENPF expression in TCGA, we analyzed CENPF expression base on the RNA-seq data of kinds of malignancies cancers. The differential expression between the tumor and normal tissues for CENPF in all TCGA tumors is shown in Figure.1A. CENPF expression was significantly higher in BLCA (bladder urothelial carcinoma), CHOL (choleangiocarcinoma) ,COAD (colon adenocarcinoma), ESCA(Esophageal carcinoma),HNSC(head and neck cancer),KICH (kidney chromophobe), KIRC (kidney

renal clear cell carcinoma), KIRP (Kidney renal papillary cell carcinoma), LIHC (liver hepatocellular carcinoma), LUAD (lung adenocarcinoma), LUSC (Lung squamous cell carcinoma) compared with adjacent or normal tissues. In addition, the express level of CENPF was significantly higher in LUAD (lung adenocarcinoma), LUSC (Lung squamous cell carcinoma) is shown in Figure.1B, C.

Prognostic role of CENPF in LUAD and LUSC

Further, In order to elucidate the Prognostic role of CENPF in LUAD and LUSC. When CENPF expression level was divided into top 50% versus lower 50%, we found that higher CENPF (top 50% vs bottom 50%) expression was significantly associated with worsened OS and DFS in LUAD (Figure 2A, B). We then explored whether CENPF expression was associated with survival in LUSC, we found that higher CENPF expression was significantly associated with improved OS and DFS (Figure 2C&D).

Prognostic role of immune Infiltration Level in LUAD and LUSC

To explore the prognostic role of immune infiltration level in LUAD and LUSC. The B cells, CD8⁺ T cells, CD4⁺ T cells, macrophage, neutrophils, and dendritic cells (DCs) was analyzed in the prognostic role. Accordingly, increased infiltrating levels of B cells, macrophage was significantly associated with improved prognosis in LUAD (Figure 3A). We then explored whether immune infiltration level was associated with survival in LUSC cohort and found that higher B cells, and CD8⁺ T cells, macrophages, neutrophils, and dendritic cells (DCs) infiltrating levels was significantly associated with improved survival (Figure 3B).

Association of CENPF with cancer immunity in LUAD and LUSC

We therefore hypothesized that immunity in LUAD and LUSC could be linked to CENPF expression. Cases with higher CENPF expression showed significantly increased infiltration of B cells, CD8⁺ T cells in LUAD (Figure 4A) and increased infiltration of B cells, and CD8⁺ T cells, macrophages, neutrophils, and dendritic cells in LUSC (Figure 4B). We analyzed correlation between CENPF expression and tumor immune infiltration level abundance indicated by decreased tumor purity. We found that higher CENPF expression was significantly associated with increased purity only in LUSC (Figure 4C). We then illustrated whether CENPF copy number between tumor immune infiltration level abundance. We found that CENPF copy number was significantly associated with increased tumor immune infiltration level (B cells, and CD4⁺ T cells, macrophages, neutrophils, and dendritic cells) in LUAD (Figure 4D). In the LUSC, CENPF copy number was significantly associated with increased tumor immune infiltration level (B cells, CD8⁺ T and CD4⁺ T cells, macrophages, neutrophils, and dendritic cells) in LUAD (Figure 4E).

CENPF and cancer immunity marker in LUAD and LUSC

As higher PD-L1, PD1, CTLA4 was associated with worsened prognosis in lung cancers, we hypothesized that our findings were due to increased cancer immunity with concomitant increased PD-L1, PD1, CTLA4 expression in LUAC and LUSC. Specifically, PD-L1 expression was associated with increased CD8+ cells, neutrophils, and B cells in LUAD and LUSC (Figure 5A, B). In LUAD cohort, we found that *CENPF* expression was significantly correlated with increased PD-L1($r=0.113$, $P=0.010$) but not with PD-1 and CTLA4 expression ($P=0.081$, $P=0.343$). Also, *CENPF* expression was significantly correlated with increased PD1($r=0.1148$, $P=0$) and CTLA4($r=-0.15$, $P=0$) but not with PD-L1 ($P=0.755$) in LUSC cohort.

CENPF knockdown suppresses the proliferation, migration and invasion of NSCLC cells

We next determined the effects of CENPF knockdown on the proliferation potential of NSCLC cells in vitro. Our CCK8 assay results showed that CENPF knockdown group inhibited the growth abilities of H1299 and A549 cells than that of control group (Figure 6A). In addition, the results of colony formation assays showed that CENPF knockdown also inhibited the clone formation abilities of H1299 and A549 cells (Figure 5B). In order to find whether CENPF is related to the cancer metastasis in NSCLC cells, H1299 and A549 cells were seeded in the culture palte for cell scratch assay to detect cell migration ability. The results indicated that CENPF knockdown group inhibited the migration of H1299 and A549 cells (Figure 6C). Further, the transwell migration and invasion assay was used to analyze the function of CENPF in NSCLC cells migration and invasion. It was also demonstrated that CENPF knockdown inhibited the migration and invasion of H1299 and A549 cell (Figure 6D).

Discussion

CENPF is a component of the nuclear matrix during the G2 phase of interphase. CENPF localization suggests that it is important in chromosome segregation during mitosis [10]. It localizes to the intracellular bridge and the spindle in the cell cycle, respectively. Although the biological function and molecular mechanism of CENPF have not been studied in depth, it is known that CENPF is up-regulated and is associated with poor prognosis of prostate cancer and hepatocellular carcinoma[11, 12]. In addition, CENPF might act as mitochondria and other cellular cargoes by attaching them during both polymerization and depolymerization of microtubule[13]. Previous study demonstrated that CENPF express highly, may be as an key regulator in lung cancer progress[14–16]. Here, we conclude that changes in CENPF expression levels are associated with prognosis of lung cancer from the perspective of immune infiltration. High expression level of CENPF correlates to the poorer prognosis in LUSC and LUAD. Further analysis showed that in LUAD and LUSC, tumor immune infiltration levels and different immunological markers (PDL1.PD1, CTLA4) were significantly associated with CENPF expression levels.

Therefore, our research provides insights into the potential role of CENPF in tumor immunology and its use as a biomarker for cancer.

In present study, we measured CENPF expression and survival rate based on TCGA data from GEPIA. The level of CENPF expression in LUAD and LUSC based in TCGA data underlying unclear mechanisms to different biological properties. Furthermore, the data from TCGA showed high level of CENPF expression was correlated with poorer prognosis in LUAD, LUSC. Another intriguing finding of this study is that CENPF expression is correlated with diverse immune infiltration levels in lung cancer. Moreover, the correlation between CENPF expression and the marker genes of immune cells implicate the role of CENPF in tumor immunotherapy in LUAD and LUSC. The tumor microenvironment(TME) plays a key role in the occurrence and development of tumors, and immune infiltration is one of the most important features[17, 18]. In addition, the latest research found that tumor immune cell infiltration significantly affects the prognosis and efficacy of immunotherapy, mainly including tumor macrophages and neutrophils infiltrating[19, 20]. Therefore, it is particularly important to identify new immunotherapeutic targets in lung cancer by studying gene networks and tumor immunity[21, 22].

Conclusion:

Taken together, high CENPF expression correlates with poorer prognosis and increased immune infiltration levels of LUAD and LUSC, especially in in non-small-cell lung carcinoma (NSCLC). In addition, in NSCLC cell lines A549 and H1299, CENPF knockdown potentially contributes to the inhibition of tumor proliferation. Therefore, CENPF may play an important role in tumor immune cell infiltration and may serve as a prognostic biomarker for lung cancer patients.

Notes

Lei Li and Pengchao Zheng contributed equally to this work.

Abbreviations

CENPF

centromere protein F

TCGA

the cancer genome atlas

LUAD

Lung Adenocarcinoma

LUSC

Lung squamous cell carcinoma

NSCLC

non-small-cell lung carcinoma

PD-L1

programmed death ligand-1
CCK-8
Cell counting kit

Declarations

Ethics approval and consent to participate

This study was reviewed and approved by the Medical Ethics Committee of the Department of Cardio-Thoracic Surgery, Second People's Hospital of Jinmen, Jinmen, China.

Consent for publication

All authors have agreed to publish this manuscript.

Availability of data and material

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

Competing interests

The authors declare that they have no competing interests.

Funding

No

Authors' contributions

LL contributed to the experiment design, and data analysis. PZ contributed to the experiment implementation, LL and PZ contributed to manuscript draft and data analysis. All authors read and approved the final manuscript

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Disclosure

The authors report no conflicts of interest in this work.

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Figures

Figure 1

CENPF expression levels in different types of human cancers. (A) Human CENPF expression levels in different tumor types from TCGA database were determined by TIMER (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). (B) Human CENPF expression levels in Lung adenocarcinoma(LUAD) from TCGA database were determined by GEPIA (* $P < 0.05$). (C) Human CENPF expression levels in Lung squamous cell carcinoma(LUSC) from TCGA database were determined by GEPIA (* $P < 0.05$).

Figure 2

Prognostic role of CENPF in LUAD and LUSC (A) Kaplan-Meier over survival (OS) curves comparing the high and low expression of CENPF in LUAD. (B) Kaplan-Meier disease free survival(DFS) curves comparing the high and low expression of CENPF in LUAD. (C) Kaplan-Meier over survival(OS) curves comparing the high and low expression of CENPF in LUSC. (D) Kaplan-Meier disease free survival (DFS) curves comparing the high and low expression of CENPF in LUSC.

Figure 3

Prognostic roles of immune-related factors in LUAD and LUSC (A) Kaplan-Meier over survival (OS) in patients of B cells, CD4+cell, CD8+cell, macrophages, neutrophils, and dendritic cells (DCs) count in LUAD.(B) Kaplan-Meier over survival (OS) in patients of B cells, CD4+cell, CD8+cell, macrophages, neutrophils, and dendritic cells (DCs) count in LUSC.

Figure 4

Correlation of CENPF expression with immune infiltration level in LUAD and LUSC (A) The correlation of CENPF expression is significantly with infiltrating levels of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells in LUAD. (B) The correlation of CENPF expression is significantly with infiltrating levels of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells in LUSC. (C) The correlation of CENPF expression and tumor purity of LUAD and LUSC. (D, E) The correlation of CENPF copy number with infiltrating levels of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells in LUAD and LUSC.

Figure 5

Correlation analysis between CENPF with immune marker (A) The correlation between expressions of CENPF and PD-L1 (CD274), PD-1 (PDCD1), CTLA4 in LUAD. (B) The correlation between expressions of CENPF and PD-L1 (CD274), PD-1 (PDCD1), CTLA4 in LUSC

Figure 6

CENPF Knockdown inhibited proliferation, migration, invasion ability of non-small-cell lung carcinoma (NSCLC) (A) Growth curves between sh-Control and sh-CENPF by CCK-8 assay. (B) Representative images of the colony formation assay. (C) Cell scratch assay analysis the sh-Control and sh-CENPF cell migration ability in A549 and H1299. (D) Transwell migration, and invasion assays with U-87 and U-251 cells. The results are shown as the mean $\pm$ SD of at three independent experiments.