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RAPID COMMUNICATION

PLAU plays a functional role in driving lung squamous cell carcinoma metastasis

The high mortality of patients with lung squamous cell carcinoma (LUSC) results from metastasis rather than primary tumors,[1] whereas the molecular pathogenesis of cancer metastasis remains poorly understood. *PLAU* encodes urokinase-type plasminogen activator (uPA) which is closely related to tumor diagnosis, treatment target, and prognosis of patients.[2,3] We previously predicted that *PLAU* is a key gene negatively associated with overall survival by integrated multidimensional analyses of The Cancer Genome Atlas (TCGA) datasets from 504 samples of LUSC tumor tissues and 46 samples of adjacent non-tumorous lung tissues.[4] However, whether the role of *PLAU* contributes to the metastasis of LUSC remains unclear. Here, we analyzed the association between uPA levels and LUSC and found that uPA levels were associated with pathological variations in LUSC patients. Furthermore, we found that overexpression or interference of *PLAU* resulted in significant changes in the migration abilities and metastasis-associated gene expression in lung and LUSC cells, respectively. These findings uncover a key role for *PLAU* in LUSC metastasis and provide a potential novel early diagnosis and therapeutic target for metastatic LUSC intervention. We demonstrated that *PLAU* (uPA) is vital to LUSC metastasis; thus, it could be a promising biomarker for this disease, which contributes to the prevention of LUSC metastasis by encouraging the development of diagnostic and therapeutic strategies that target the identified *PLAU* (uPA)-associated genes and pathways.

To investigate the expression of uPA in human lung cancer, tumor tissues from 100 LUSC patients were collected using a cancer tissue microarray, and their uPA expression in tumor tissues was analyzed. The intensity was scored as 0 (negative immunostaining, −), 1 (weak immunostaining, +), 2 (moderate immunostaining, ++), and 3 (strong immunostaining, +++), and the percentages of the weak, moderate, and strong groups are presented in Figure 1A–C and Table S1 with representative images. Meanwhile, the percentage of positive cells in the tumor stroma was documented with the scoring criteria as follows: 0 (<5%), 1 (6%–25%), 2 (26%–50%), 3 (51%–75%), and 4 (>75%).[5] Positive uPA expression was found in most tumor tissues of LUSC (Table S2).

Mouse subcutaneous transplanted tumor models of SK-MES-1, NCI–H226, and NCI–H520 human LUSC cells were constructed, and the expression of uPA protein in tumor and normal tissues was detected by immunohistochemistry (IHC). As shown in Figure 1D, the expression of uPA in SK-MES-1, NCI–H226, and NCI–H520 mouse tumor tissues was higher than that in normal lung tissues. Although the expression of uPA in mouse tumor tissues of cell SK-MES-1 is lower than that of NCI–H226 and NCI–H520, our previous work showed that the mRNA transcription of uPA in SK-MES-1 is higher than those of pulmonary bronchial epithelial cell 16-HBE-T using quantitative polymerase chain reaction (qPCR), which is consistent with the current research conclusions.[4] Thus, we chose SK-MES-1 and 16-HBE-T to carry out further research.

To explore the effect of uPA overexpression on the migration ability of 16-HBE-T cells, we set up three experimental groups (control, p-PLAU-NC, and p-PLAU groups) after *PLAU* overexpression plasmid was successfully constructed (Fig. S1) and verified their migration ability using scratch experiments. After scratching, photographs were taken at 0 h and 24 h. Figure 1E shows that the migration distance of the p-PLAU group was significantly larger than those of the control group or p-PLAU-NC group, and there was no significant difference between the control and p-PLAU-NC groups. The migration area of the three groups was calculated using ImageJ, and Figure 1G shows that the cell migration area of the p-PLAU group was significantly larger than that of the control group or p-PLAU-NC group. However, there was no significant difference between the control and p-PLAU-NC groups, indicating that the overexpression of *PLAU* promoted the migration of 16-HBE-T cells. The CCK-8 assay (Fig. 1I) showed that cell activity in the p-PLAU group was significantly higher than that in the p-PLAU group.

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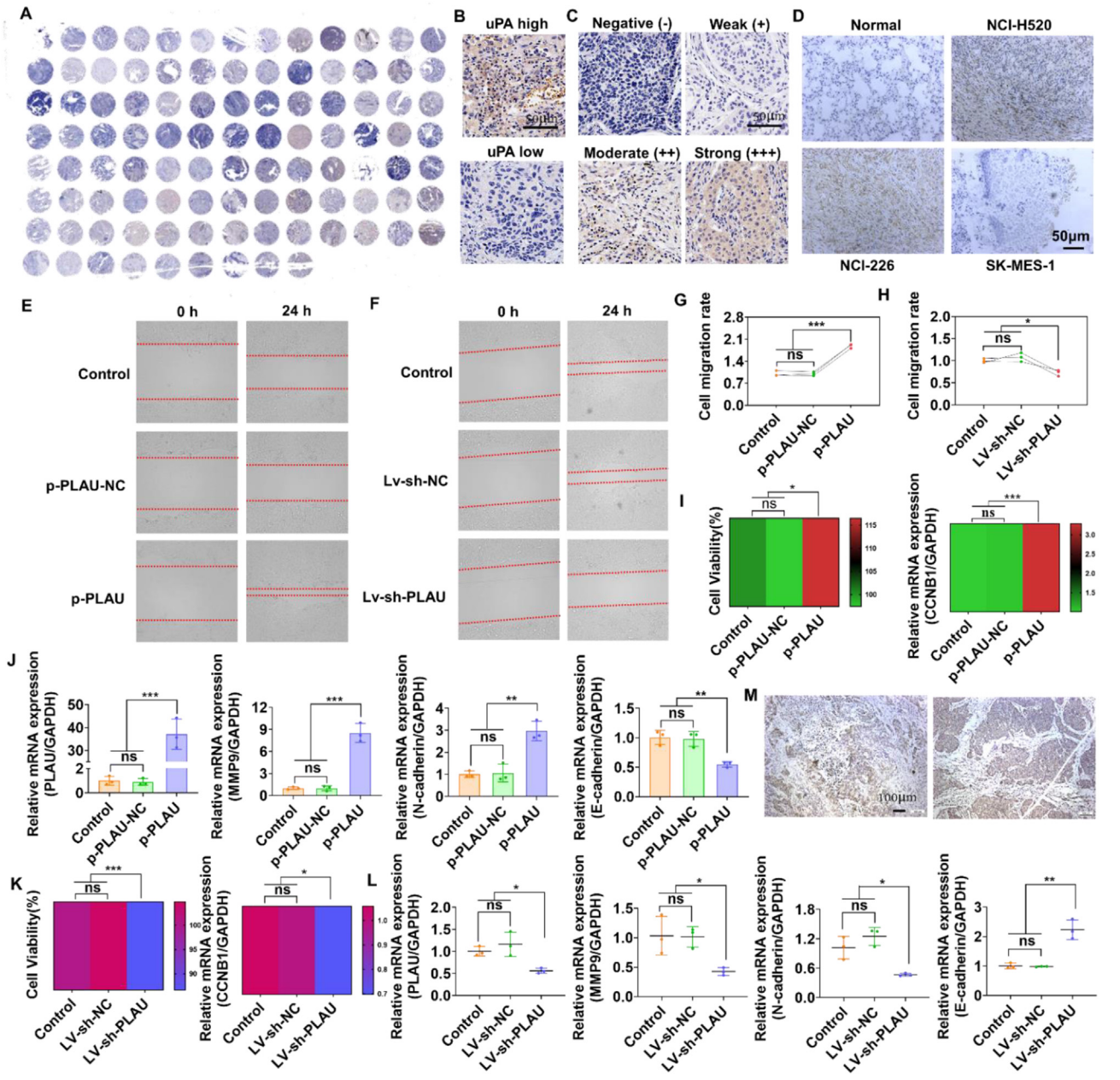

Figure 1 PLAU is identified as having a functional role in lung squamous cell carcinoma (LUSC) metastasis. **(A)** Expression of uPA in LUSC patients by immunohistochemistry slide of the tissue microarray. **(B)** Representative immunohistochemical staining for uPA in LUSC patients. **(C)** Elevated expression of uPA is a positive marker in tumor cells of most LUSC patients. IHC analysis of uPA expression in LUSC tissues. **(D)** The expression of uPA protein in normal lung tissue, and cell line derived xenografts tumor models constructed by NCI—H520, NCI—H226, and SK-MES-1, respectively (magnification = 200× , Scale = 50 μm). "Normal" indicates normal mouse lung tissue. **(E)** Scratch results of the control group, p-PLAU-NC group, and p-PLAU group at 0 h and 24 h, respectively. **(F)** Scratch results of the control group, LV-sh-NC group, and LV-sh-PLAU group at 0 h and 24 h, respectively. **(G)** Comparison of cell migration rates in the control group, p-PLAU-NC group, and p-PLAU group. **(H)** Comparison of cell migration rates in the control group, LV-sh-NC group, and LV-sh-PLAU group. ns, no significant difference. **(I)** The CCK-8 experiment detects the effect of uPA overexpression on the activity of 16-HBE-T cells (left). mRNA expression levels of CCNB1 in 16-HBE-T cells after uPA overexpression (right). ns, no significant difference. **(J)** Detection of PLAU, MMP9, N-cadherin, and E-cadherin mRNA expression levels in 16-HBE-T cells respectively after uPA overexpression. ns, no significant difference. **(K)** The CCK-8 experiment detects the effect of uPA inhibition on the activity of SK-MES-1 cells (left). qPCR was used to detect the mRNA expression of uPA in the SK-MES-1 cells of each group (right). ns, no significant difference. **(L)** The effect of inhibiting the expression of uPA on the PLAU, MMP9, N-cadherin, and E-cadherin mRNA expression in SK-MES-1 cells. **(M)** Detection of LUSC slices in clinical LUSC patients. ns, no significant difference. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Overexpression of *PLAU* significantly increased the mRNA expression of CCNB1 in 16-HBE-T cells (Fig. 1I), and CCNB1 was related to the cell cycle, indicating that overexpression of *PLAU* could enhance the proliferation of 16-HBE-T cells.

After overexpressing *PLAU*, we examined the changes in the mRNA expression of MMP9, N-cadherin, and E-cadherin in 16-HBE-T cells using qPCR to understand the molecular mechanisms of migration. The expression of MMP9 is related to the malignant progression of tumors, including their migration, invasion, and metastasis. N-cadherin enhances the migration and invasion of tumor cells and its increased expression is an important step in the metastasis and progression of epithelial carcinoma. Loss of E-cadherin expression in humans is associated with tumor development and poor prognosis. Figure 1J showed that the mRNA expression levels of MMP9 and N-cadherin in the p-PLAU group were significantly higher than those in the control and p-PLAU-NC groups. However, E-cadherin mRNA expression in the p-PLAU group was significantly lower than that in the control and p-PLAU-NC groups. These results suggest that the biological functions of 16-HBE-T cells may be altered by *PLAU* overexpression.

After *PLAU* was knocked down, we observed that the migration distance of SK-MES-1 cells in the LV-sh-PLAU group was significantly less than that of the control or LV-sh-NC groups in the scratch experimental analysis, indicating that the migration ability of SK-MES-1 cells decreased significantly after *PLAU* was inhibited (Fig. 1F). Three experimental groups (control, LV-sh-NC, and LV-sh-PLAU) were subjected to the CCK-8 test to illustrate that interference with *PLAU* expression could inhibit the proliferation ability of SK-MES-1 cells (Fig. 1K). The effect of uPA inhibition on SK-MES-1 cell proliferation was also analyzed on CCNB1 using qRT-PCR. CCNB1 expression in the LV-sh-PLAU group was significantly lower than that in the control group or LV-sh-NC group (Fig. 1K), supporting this conclusion. The mRNA expression levels of MMP9, N-cadherin, and E-cadherin were detected using qPCR in SK-MES-1 cells (Fig. 1L). Opposite gene expression results with uPA overexpression were obtained, in which the mRNA expression of MMP9 and N-cadherin in SK-MES-1 cells in the LV-sh-PLAU group was significantly lower than that in the control group or LV-sh-NC group, whereas the expression of E-cadherin increased significantly after treatment with *PLAU*, compared with the respective controls (Fig. 1L).

Formalin-fixed, paraffin-embedded tissues from two LUSC patients were selected randomly. Informed consent was obtained from the patients. The included LUSC patients were all stage III and treated according to standard clinical recommendations with either chemotherapy or radiation and developed lymph node metastasis. We performed IHC analysis and found positive expression of uPA in the tumor sections of two clinical cases (Fig. 1M), which further supports the association of uPA with cancer metastasis in LUSC patients. To date, the application of early markers in the diagnosis and treatment of LUSC in clinical settings is very limited, especially for metastatic LUSC. The high mortality rate of LUSC is partly due to the lack of early biomarker detection. Therefore, the identification of key molecules involved in LUSC is required to improve clinical diagnosis and treatment outcomes. The uPA findings of the present study have the potential to improve early diagnosis of LUSC. In addition, LUSC may have led to metastasis and needs to be considered to evaluate the therapeutic effect of LUSC, including the monitoring of several characteristic cytokines. However, in this study, the new findings of uPA provide an improved understanding of the molecular mechanisms of carcinogenesis and progression of LUSC and assist with the identification of potential therapeutic targets of LUSC.

Collectively, we carried out extensive investigations on the contribution of PLAU (uPA) to LUSC metastasis. Multiple databases, including tumor microarrays and clinical tumor samples, were applied to support the value of PLAU (uPA) as a novel biomarker in LUSC. *PLAU* overexpression significantly enhanced the migration and proliferation of 16-HBE-T cells, whereas PLAU interference inhibited the migration and proliferation of SK-MES-1 cells. Our findings uncover a key role for PLAU in LUSC metastasis and provide potential novel early diagnosis and therapeutic targets for metastatic LUSC intervention.

Conflict of interests

The authors declare no potential conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.04.010.

References

1. Satpathy S, Krug K, Jean Beltran PM, et al. A proteogenomic portrait of lung squamous cell carcinoma. *Cell*. 2021;184(16):4348—4371.e40.
2. Urban P, Vuaroqueaux V, Labuhn M, et al. Increased expression of urokinase-type plasminogen activator mRNA determines adverse prognosis in ErbB2-positive primary breast cancer. *J Clin Oncol*. 2006;24(26):4245—4253.
3. Salden M, Splinter TA, Peters HA, et al. The urokinase-type plasminogen activator system in resected non-small-cell lung cancer. Rotterdam Oncology Thoracic Study Group. *Ann Oncol*. 2000;11(3):327—332.
4. Ning P, Wu Z, Hu A, et al. Integrated genomic analyses of lung squamous cell carcinoma for identification of a possible competitive endogenous RNA network by means of TCGA datasets. *PeerJ*. 2018;6:e4254.

5. Cheng Y, Mo F, Li Q, et al. Targeting CXCR2 inhibits the progression of lung cancer and promotes therapeutic effect of cisplatin. *Mol Cancer*. 2021;20(1):62.

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