OnLine Journal of Biological Sciences

Research Article

Decoding Lung Cancer: PLAU as a Critical Driver and Diagnostic Marker

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Article history
Received January 16, 2025
Revised: March 08, 2025
Accepted: March 12, 2025

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Abstract: Lung cancer remains a leading cause of cancer-related mortality, and understanding its underlying molecular mechanisms is critical for developing targeted therapies. We developed a novel tool called the Transcriptome-TCGA Integration (TTI), which combines in vitro transcriptome data with clinical datasets from the Cancer Genome Atlas (TCGA). TTI allows for the identification of key genes and pathways involved in lung cancer, providing valuable insights into tumor biology. The TTI tool successfully identified PLAU as a key driver of lung cancer progression, promoting NF-κB pathway activation and EMT. Inhibition of PLAU led to decreased NF-κB activity, reduced cell viability, and diminished migration and invasion capabilities in lung cancer cells. Furthermore, cfDNA analysis revealed that PLAU inhibition lowered cfDNA-derived PLAU levels, offering a potential diagnostic marker for tumor dynamics. TTI is a versatile tool that not only elucidates critical molecular pathways in lung cancer but also holds significant potential for application across other cancer types. By integrating AI and machine learning, the TTI tool can be further developed into an AI-driven platform for precision oncology, enabling predictive treatment models and cross-cancer analyses. Our study demonstrates the utility of TTI in advancing cancer research and personalized medicine, with implications for future therapeutic developments in multiple cancer contexts.

Keywords: TCGA, Transcriptome, PLAU, Lung Cancer; cfDNA

Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide, with approximately 1 million deaths annually. Despite advancements in treatment, its prognosis remains poor, with a 5-year survival rate of only 15% (Sabari et al.,2017, Schild et al., 2015). Molecularly targeted therapies, such as those targeting EGFR, ALK, and PD-1/PD-L1, have shown promise, yet challenges persist in identifying reliable biomarkers for early diagnosis and effective therapeutic targeting. While some biomarkers have been identified, their clinical utility has been limited due to factors such as poor specificity, late detection, or limited therapeutic implications.

Various biomarkers, including KRAS mutations, EGFR mutations, and PD-L1 expression, have been extensively studied in non-small cell lung cancer (NSCLC) (Riely et al., 2009). However, these biomarkers often show limited predictive power or are specific only to certain subtypes, highlighting the need for more universally applicable biomarkers. For instance, EGFR mutations are common in Asian populations but are less frequent in other demographics. Similarly, while PD-L1 expression is useful for guiding immunotherapy, it does not provide consistent predictive value across all patient populations (Steuer et al., 2016).

Recent research has turned towards extracellular matrix (ECM)-degrading enzymes, like PLAU (urokinase plasminogen activator), as potential novel biomarkers for cancer. PLAU plays a critical role in ECM degradation, cell migration, and invasion key processes in cancer metastasis. Unlike other biomarkers, PLAU is involved in fundamental cellular processes central to cancer biology, particularly epithelial-mesenchymal transition (EMT), a

process that enhances metastatic potential in various cancers, including lung cancer (Connor et al., 2019, Tang et al., 2013). Furthermore, PLAU overexpression has been linked to the activation of the NF-κB signaling pathway, a crucial regulator of inflammation, cell survival, and cancer progression. Dysregulated NF-κB contributes to tumor growth, metastasis, and resistance to chemotherapy, particularly in NSCLC (Baldwin, 2001; Gu et al.,2018; Karin and Greten, 2005). The relationship between PLAU and NF-κB signaling suggests that PLAU has significant potential as not only a diagnostic marker but also a therapeutic target.

While previous studies, such as Tang et al. (2013), have highlighted the role of PLAU in breast cancer, its involvement in lung cancer remains underexplored. This study introduces a novel approach by integrating transcriptomic data with TCGA to identify genes that are commonly upregulated in lung cancer, with PLAU emerging as a key candidate. Through this innovative use of the Transcriptome-TCGA Integration (TTI) tool, we expand our understanding of PLAU's role in lung cancer progression and its potential as a critical driver of metastasis. Additionally, we explore the potential of cfDNA analysis to identify key genetic signatures related to PLAU expression, which could offer a novel, non-invasive approach for early lung cancer detection and monitoring (Cisneros-Villanueva et al., 2022). This work not only reinforces PLAU's potential as a diagnostic and therapeutic target but also lays the groundwork for developing new therapeutic strategies and cfDNA-based diagnostic tools to improve the prognosis of lung cancer patients.

Materials and Methods

Cell Lines and Culture: MRC5 and A549 cell lines were cultured in recommended media with 10% FBS and 1% penicillin-streptomycin (HyClone™, Corning™) at 37°C, 5% CO_2. Cells (<5 passages) were sourced from the Korean Cell Line Bank.

Transcriptome Analysis: Total RNA was extracted using TRIzol reagent, and cDNA was synthesized using a High Capacity cDNA Reverse Transcription Kit (Takara). RNA quality (RIN >7.0) was assessed via NanoDrop and Bioanalyzer. Sequencing was performed on an Illumina NovaSeq 6000 with ~30M reads/sample.

TCGA Data Analysis: TCGA data were analyzed using Python, with a fold change >1.5 and $p<0.05$ considered significant.

CRISPR/MSCV Transfection: PLAU-targeting CRISPR plasmids or control vectors were transfected using Lipofectamine 3000. Puromycin (1 μg/mL) selection was applied for 72 hours post-transfection.

Western Blot: Protein lysates were analyzed via SDS-PAGE and PVDF transfer. Antibodies targeting PLAU, IκBα, p-IκBα, NF-κB, p-NF-κB p65, and actin were used, followed by HRP-conjugated secondary antibodies.

RT-qPCR: cDNA synthesized from TRIzol-extracted RNA was used for RT-qPCR with SYBR Green. Gene expression was quantified using the 2^-ΔΔCt method with GAPDH as a control.

Cell Proliferation: Cells were counted at 2, 4, and 6 days using a Vi-CELL™ XR Cell Counter with Trypan blue staining.

Migration and Invasion Assays: Migration was assessed using transwell inserts with serum-free media (upper chamber) and FBS-supplemented media (lower chamber). Invasion was evaluated using Matrigel™-coated transwells. Migrated/invaded cells were stained with crystal violet and counted.

Aprotinin Treatment: Aprotinin (10 μg/mL) was applied to A549 and MRC5 cells for 24 hours in standard conditions.

Clonogenic Assay: Cells were seeded into 6-well plates and cultured for two weeks. Colonies were fixed, stained with crystal violet, and counted.

cfDNA Extraction and Analysis: cfDNA was collected from media, extracted with QIAamp DNA Blood Mini Kit (Qiagen), and quantified using a TapeStation.

PCR: PCR was conducted on genomic DNA using TaKaRa Taq™ polymerase. Products were analyzed on 1.5% agarose gels stained with Gel-Red.

Statistical Analysis: Experiments were performed in triplicate, and data are presented as mean ± SEM. Statistical significance was assessed using Student's t-test or Pearson correlation ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

Results

Figure 1 shows development of the Transcriptome-TCGA Integration (TTI) Tool for Identifying Key Genes in Lung Cancer.

Workflow for the development and establishment of the TTI tool. Transcriptomic data from in vitro lung cancer (A549) and normal lung (MRC5) cell lines were integrated with clinical data from lung adenocarcinoma and normal tissues in TCGA. The TTI tool identified genes with a fold change greater than 1.5 between tumor and normal tissues.

Despite significant advances in cancer research, the molecular mechanisms underlying lung adenocarcinoma progression remain poorly understood. Identifying key

genetic drivers is critical for developing targeted therapies (Li et al., 2023). To address this, we conducted a transcriptome analysis comparing normal lung cells (MRC5) and adenocarcinoma cells (A549), integrating these results with data from The Cancer Genome Atlas (TCGA), which included 59 normal and 83 adenocarcinoma tissue samples (Supplementary Data 1). Using the Transcriptome-TCGA Integration (TTI) method, we identified genes that were consistently upregulated or downregulated in tumor samples. The use of Bowtie2 for genome-wide alignment and Python scripts for filtering TCGA data (with a fold change >1.5) ensured that we identified genes of significant clinical relevance. This integrated approach provides valuable insights into the molecular pathways that drive lung cancer progression and could potentially reveal new biomarkers for diagnosis and therapeutic targets.

Figure 2. Identification of PLAU Overexpression in Tumor Tissue via TTI and GSEA Analysis of Upregulated Genes in Lung Cancer.

(A) Venn diagram showing overlap of TCGA and transcriptomic data, identifying commonly upregulated and downregulated genes in lung adenocarcinoma

(B) GEPIA database confirms significant upregulation of PLAU in lung adenocarcinoma patients ($P<0.05$)

(C) Kaplan-Meier curves show higher PLAU expression correlates with poorer overall survival, suggesting its prognostic potential

(D) Pathway enrichment analysis of 140 upregulated genes reveals cancer-related pathways, supporting PLAU's role in lung cancer progression

Using TTI, we identified genes consistently upregulated or downregulated in tumors compared to normal tissues. Upregulated genes included BIK, GDF15, PLAU, and PMAIP1, while downregulated genes included FOSB, SELENOP, and METTL7A (Fig. 2A). Among these, PLAU (urokinase-type plasminogen activator, uPA) was the most upregulated gene in TCGA and transcriptome data. PLAU is involved in plasminogen activation, cell migration, invasiveness, and tumor metastasis (Tang et al., 2013).

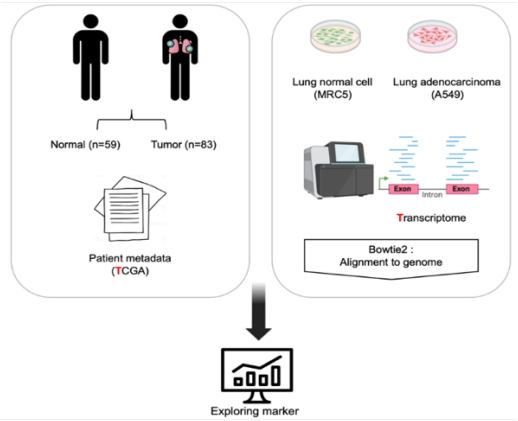

Fig. 1: Establishment of the Transcriptome-TCGA Integration (TTI) Tool

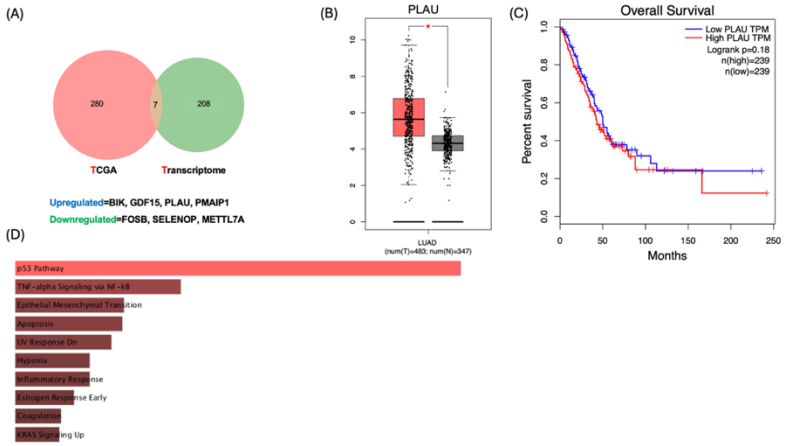

Fig. 2: Analysis of PLAU Expression and its Clinical Relevance in Lung Adenocarcinoma

PLAU expression was significantly higher in tumors compared to normal tissues in GEPIA analysis (Fig. 2B). Kaplan-Meier survival analysis revealed that higher PLAU expression was associated with worse overall survival (OS) in lung adenocarcinoma patients (Fig. 2C). Gene set enrichment analysis (GSEA) further showed enrichment of TNF-alpha signaling via NF-kB and epithelial-mesenchymal transition pathways in tumor tissues, suggesting that PLAU promotes tumorigenesis through these mechanisms (Fig. 2D).

Figure 3. CRISPR-mediated Knockout of PLAU and Validation by Western Blot and RT-qPCR

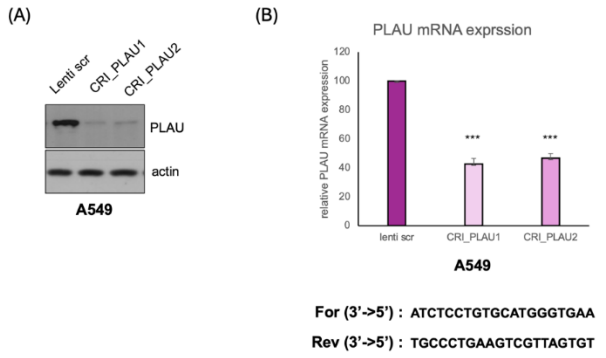

Fig. 3: Validation of PLAU Knockout via CRISPR-Cas9

(A) Western blot showing the expression levels of PLAU in A549 cells with PLAU CRISPR-Cas9 knockout (KO) compared to control cells

(B) Quantitative RT-PCR analysis assessing the mRNA levels of PLAU in A549 cells with PLAU CRISPR-Cas9 knockout compared to control($P<0.001$)

To investigate the functional role of PLAU in lung adenocarcinoma cells, we employed CRISPR/Cas9 technology to knock out the PLAU gene in A549 cells. The guide RNA (gRNA) sequences targeting exon regions of the PLAU gene were designed and cloned into a CRISPR/Cas9 plasmid. Following transient transfection of A549 cells with the CRISPR/Cas9 construct, clonal populations were isolated and expanded for further analysis.

As expected, PLAU protein expression was completely abolished in the knockout cells, while the control cells exhibited strong PLAU protein bands (Fig. 3A). Actin was used as a loading control to ensure equal protein loading across samples. Similarly, to confirm the efficiency of PLAU knockout at the transcriptional level, we performed reverse transcription quantitative PCR (RT-qPCR). RT-qPCR was then conducted using PLAU-specific primers and normalized to the expression of the housekeeping gene GAPDH. The results demonstrated a significant reduction in PLAU mRNA expression in the knockout cells compared to the control, with an approximate >50% decrease in transcript levels (Fig. 3B). Together, these data demonstrate the successful knockout of PLAU at both the mRNA and protein levels, confirming the efficiency of CRISPR/Cas9-mediated gene editing in A549 cells. The knockout cells can now be utilized to further explore the role of PLAU in lung cancer progression and its associated signaling pathways.

Figure 4. PLAU Knockout Reduces NF-κB Pathway Activation and Suppresses Epithelial to Mesenchymal Transition (EMT):

(A) Western blot showing decreased p-IKBα and p-NF-κB p65 levels in PLAU-knockout A549 cells, indicating reduced NF-κB pathway activation

(B) Bar graph showing reduced TNF-α, IL-6, and IL-8 mRNA expression in PLAU-knockout A549 cells compared to controls (**$P<0.01$, ***$P<0.001$)

(C) Line graph showing decreased proliferation of PLAU-knockout A549 cells compared to controls (***$P<0.001$).
(D) Representative images and bar graph showing reduced migration in PLAU-knockout A549 cells (***$P<0.001$, scale bar = 200 μm)

(D) Representative images and bar graph showing reduced invasion in PLAU-knockout A549 cells (***$P<0.001$, scale bar = 200 μm)

Gene Set Enrichment Analysis (GSEA) revealed that TNF-alpha signaling via NF-κB and EMT pathways were significantly upregulated in lung adenocarcinoma compared to normal tissues (Fig. 2D). Based on this, we hypothesized that PLAU modulates these pathways. In PLAU-knockout A549 cells, Western blot showed reduced phosphorylation of IκBα and NF-κB p65, indicating suppressed NF-κB activation (Fig. 4A). RT-qPCR revealed significantly lower TNF-alpha, IL-6, and IL-8 mRNA levels in PLAU-knockout cells, suggesting reduced inflammatory responses (Fig. 4B). PLAU knockout also reduced cell growth to levels similar to normal lung fibroblast MRC5 cells (Fig. 4C) and significantly decreased migration and invasion, key EMT processes (Figs. 4D, 4E). These results highlight PLAU's role in promoting NF-κB activation, inflammation, and EMT, contributing to lung adenocarcinoma progression. Targeting PLAU may offer a novel therapeutic strategy.

Figure 5. PLAU Overexpression Rescues Cell Viability and Inhibition by Aprotinin Reduces NF-κB Pathway Activation.

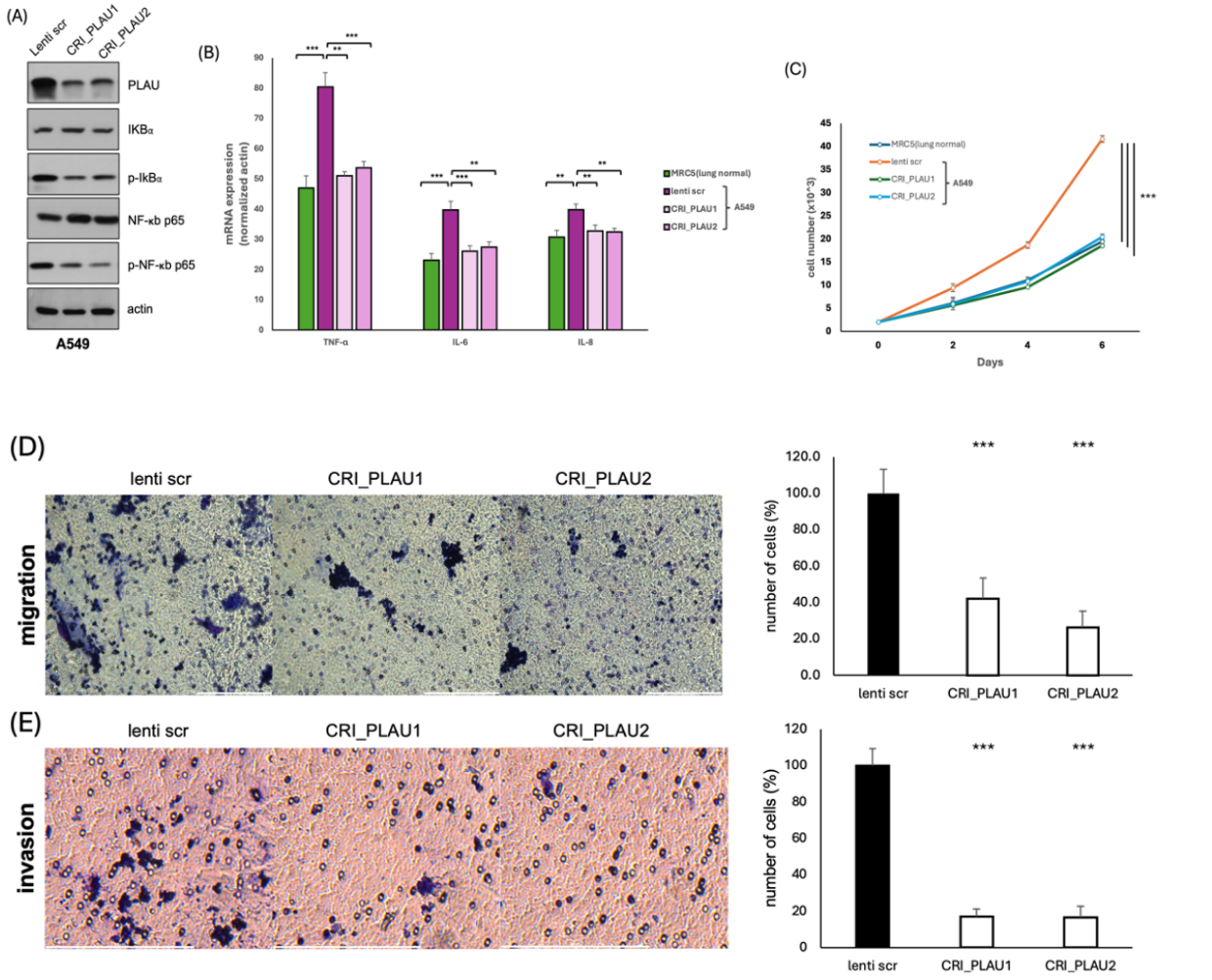

Fig. 4: Functional Analysis of PLAU Knockout in A549 Cells

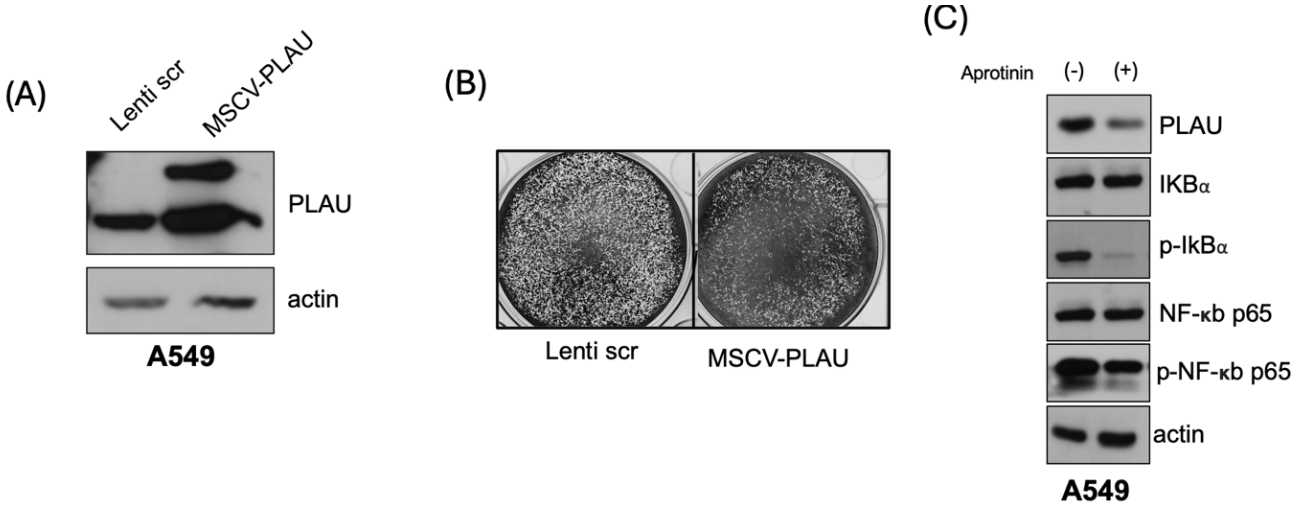

Fig. 5: Analysis of PLAU Overexpression and Aprotinin Treatment in A549 Cells

(A) Western blot showing PLAU overexpression in A549 cells, confirming successful transduction and increased PLAU protein levels
(B) Clonogenic assay showing enhanced cell viability and proliferation upon PLAU overexpression
(C) Western blot demonstrating reduced phosphorylation of IκBα and NF-κB p65 in aprotinin-treated A549 cells, indicating inhibition of the NF-κB pathway

To further investigate the role of PLAU in lung cancer, we performed a gain-of-function study by overexpressing PLAU in A549 cells using a vector designed for PLAU transduction (Fig. 5A). This approach allowed us to assess whether restoring PLAU expression in knockout cells could rescue the reduced cell viability observed in the previous experiments. As expected, overexpression of PLAU successfully restored the decreased cell viability caused by PLAU knockout, confirming the critical role of PLAU in promoting lung cancer cell survival (Fig. 5B). These results suggest that targeting PLAU expression could provide a therapeutic strategy for enhancing lung cancer cell survival and overcoming resistance to conventional therapies.

Furthermore, to explore the therapeutic potential of targeting PLAU, we treated A549 cells with aprotinin, a known inhibitor of PLAU. PLAU is expressed as uPA, which plays a key role in the conversion of plasminogen to plasmin (Tang et al., 2013). Plasmin is an enzyme that degrades the extracellular matrix and promotes the invasion and metastasis of cancer cells (Andreasen et al., 2000). Aprotinin inhibits the activity of uPA, blocking plasmin production and extracellular matrix degradation, thereby reducing the mobility and invasion ability of cancer cells (Chazaud et al., 2002). Upon treatment with aprotinin, we observed a significant reduction in NF-κB pathway activity, as indicated by the decreased phosphorylation levels of NF-κB pathway-related proteins (Fig. 5C). These findings suggest that inhibiting PLAU using aprotinin can effectively suppress the NF-κB pathway, further supporting the idea that PLAU plays a key role in modulating this signaling cascade in lung cancer.

Figure 6. Aprotinin Treatment Reduces cfDNA-Derived PLAU Levels in A549 Cells.

(A) Workflow of cfDNA extraction and analysis from normal lung (MRC5) and lung adenocarcinoma (A549) cell lines.
(B) Fragment size distribution of cfDNA in MRC5, A549 and aprotinin-treated A549
(C) Agarose gel electrophoresis of cfDNA from A549 and MRC5 cells showing increased DNA fragments in A549 cells compared to MRC5, with a reduction after aprotinin

Cell-free DNA (cfDNA) has emerged as a promising biomarker for cancer diagnosis due to its ability to reflect tumor dynamics and genetic alterations. (Diehl et al., 2008, Wan et al., 2017) Building on our previously established in vitro cfDNA extraction method (Cho, 2024), we analyzed cfDNA from three groups: MRC5 (normal lung fibroblasts), A549 (lung adenocarcinoma cells), and A549 cells treated with aprotinin, a PLAU inhibitor.

cfDNA was extracted and quantified using PLAU-specific primers via PCR. While the total cfDNA levels were similar across the groups, the PLAU-derived cfDNA was significantly reduced in aprotinin-treated A549 cells, with levels comparable to those in MRC5 cells (Figs. 6A, 6C). Notably, consistent with prior studies (Udomruk et al., 2021), cfDNA fragments from A549 cells were smaller than those from MRC5 cells (Fig. 6B), reflecting characteristic tumor-derived cfDNA size differences.

These findings suggest that PLAU inhibition via aprotinin not only reduces intracellular PLAU expression but also decreases tumor-specific cfDNA levels, supporting the hypothesis that cfDNA reflects tumor dynamics. This highlights the potential of targeting PLAU to modulate tumor activity and provides valuable insights into tumor suppression mechanisms.

Figure 7. The role of PLAU, which can be released as cfDNA, in activating the NF-kB pathway in lung cancer.

This figure (Fig. 7) illustrates the role of PLAU in activating the NF-κB pathway in lung cancer and its potential as a circulating biomarker. PLAU, which can be released into the bloodstream as cell-free DNA (cfDNA), plays a crucial role in promoting inflammation and cancer progression through its activation of the NF-κB signaling pathway. In our study, we demonstrate that PLAU overexpression in lung adenocarcinoma cells leads to enhanced NF-κB activation, contributing to processes like the TNF-α and IL-6 inflammatory response, epithelial-to-mesenchymal transition (EMT), and cancer cell growth. Moreover, the release of PLAU as cfDNA into circulation suggests its potential as a non-invasive biomarker for monitoring tumor activity and treatment efficacy. Our findings underscore the dual role of PLAU in both promoting tumor progression and as a promising diagnostic tool, highlighting its therapeutic potential in lung cancer and possibly other cancer types.

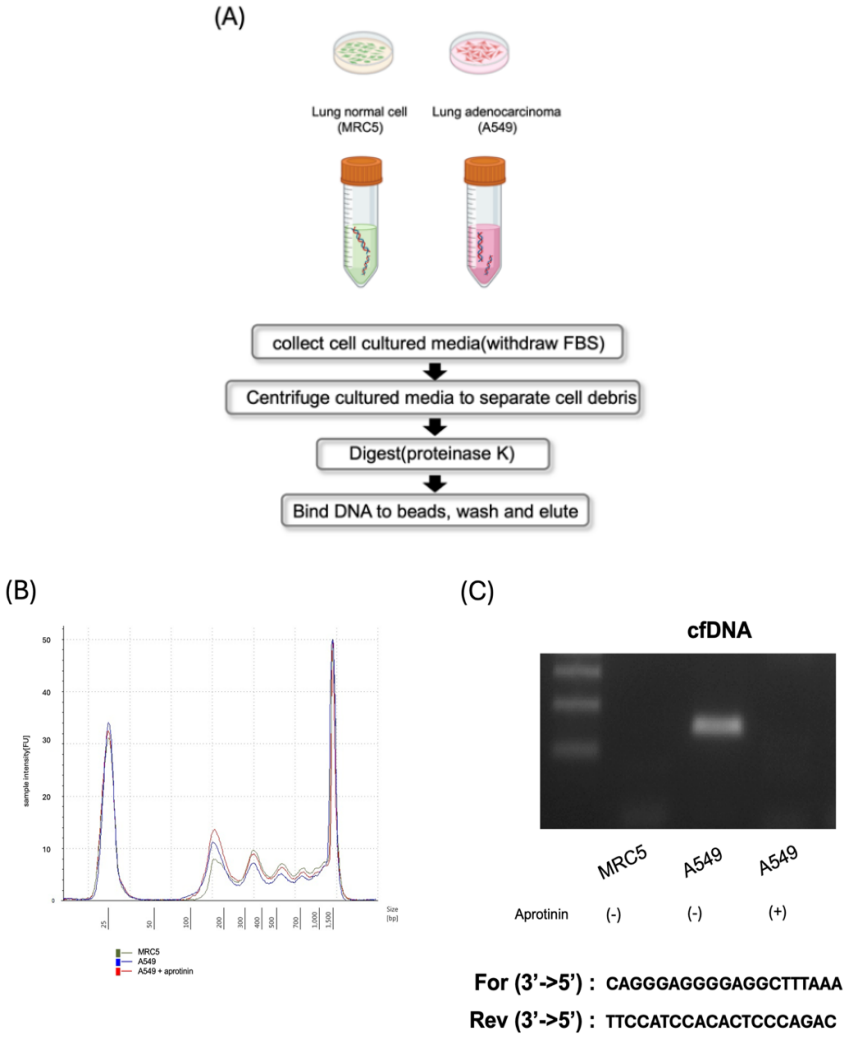

Fig. 6: Electrophoresis of cfDNA-Derived PLAU Levels in A549 and MRC5 Cells

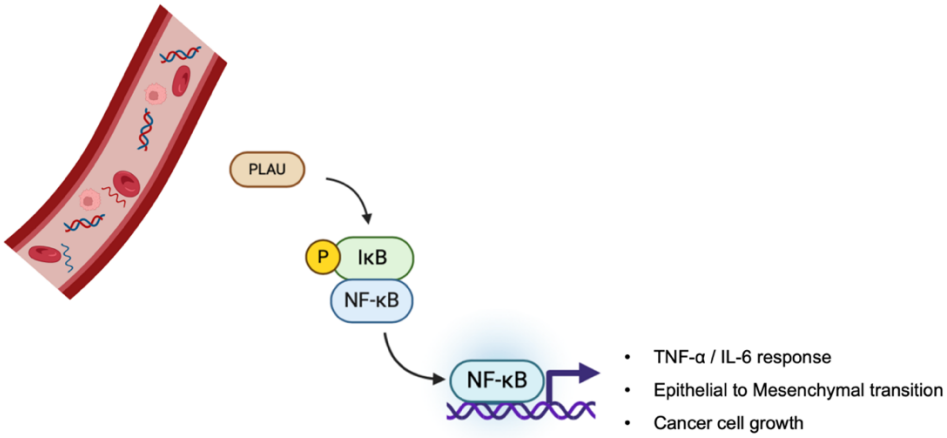

Fig. 7: The role of PLAU, which can be released as cfDNA, in activating the NF-kB pathway in lung cancer

Discussion

In this study, we identified PLAU as a key player in lung adenocarcinoma progression, highlighting its role in activating the NF-κB pathway, driving inflammation, and promoting epithelial-to-mesenchymal transition (EMT). These findings are consistent with prior research that emphasizes the importance of inflammation and EMT in cancer progression (Ricciardi et al., 2015). However, it is important to address the novelty of our approach, especially in terms of using integrated transcriptomic and clinical datasets.

Historically, biomarkers such as KRAS, EGFR, and ALK have been well-established for lung adenocarcinoma diagnosis and treatment (Riely et al., 2009, Steuer et al., 2016). While these biomarkers are crucial for targeted therapies, they often face limitations in their applicability across different cancer subtypes. PLAU, on the other hand, plays a role in extracellular matrix degradation, cell migration, and inflammation, all of which are essential for tumor progression. Our study differentiates itself by using the Transcriptome-TCGA Integration (TTI) tool to integrate transcriptomic data from cell lines with clinical datasets from TCGA, allowing for a more comprehensive analysis of PLAU's role in cancer. This integration not only identifies PLAU as an upregulated gene in lung adenocarcinoma but also provides new insights into its involvement in inflammatory signaling and cancer progression, areas that were not fully addressed by earlier studies such as Tang et al. (2013).

While the role of PLAU in cancer has been discussed in previous studies, including the work of Tang et al., which highlighted its involvement in inflammation and metastasis, our study provides a deeper understanding by using the TTI tool to bridge the gap between basic research and clinical data. Through this integrated approach, we demonstrate that PLAU activates the NF-κB pathway, which in turn drives inflammatory responses and promotes tumorigenic processes such as EMT, cell migration, and invasion. This distinction underscores the utility of TTI in identifying clinically relevant genes and pathways, offering a more nuanced understanding of PLAU's role in cancer.

Additionally, we investigated the use of aprotinin, a known PLAU inhibitor, as a potential therapeutic agent (Chazaud et al., 2002). Our results show that aprotinin treatment reduces NF-κB activation, decreases inflammatory cytokine levels, and lowers tumor-specific cfDNA in lung adenocarcinoma cell lines. The use of cfDNA as a biomarker in cancer has gained significant attention due to its non-invasive nature and ability to reflect tumor activity and genetic alterations. While cfDNA has been used in cancer diagnostics and monitoring, our study further emphasizes its potential in tracking the effectiveness of PLAU-targeted therapies. The reduction in cfDNA-derived PLAU levels after aprotinin treatment suggests that cfDNA could serve as a valuable tool for monitoring treatment responses and assessing tumor dynamics.

However, while our findings provide promising insights into the therapeutic potential of PLAU inhibition, several limitations need to be addressed. First, all experiments were conducted in vitro, and further validation in vivo is necessary to confirm the therapeutic efficacy and safety of PLAU inhibitors like aprotinin. Additionally, aprotinin's non-specific inhibitory effects raise concerns regarding its broader therapeutic application, highlighting the need for more specific PLAU-targeted therapies. The development of such inhibitors will be crucial in minimizing potential side effects and improving the precision of PLAU-based treatments.

Another important consideration is the cross-cancer relevance of PLAU. While this study focuses on lung adenocarcinoma, PLAU's expression and functional role in other cancer types remain underexplored. Previous studies have shown that PLAU is upregulated in various cancers, including breast and colorectal cancers (Ding et al., 2022). A broader investigation into the role of PLAU across different cancer types will be essential in understanding its potential as a universal cancer biomarker. This is an area for future research, and we hope to expand our findings to include cross-cancer insights in subsequent studies.

Finally, while cfDNA has shown promise as a non-invasive biomarker, several challenges remain in its clinical application (Wan et al., 2017). The heterogeneity of cfDNA levels across different patients and cancer types, as well as the lack of standardized methods for cfDNA collection and analysis, pose significant hurdles to its widespread use. Further clinical studies are needed to establish the clinical validity of cfDNA as a biomarker for PLAU activity and its role in personalized medicine.

In conclusion, our study demonstrates that PLAU plays a critical role in lung adenocarcinoma progression by activating the NF-κB pathway, driving inflammation, and promoting EMT. We have shown that PLAU inhibition can reduce tumorigenic processes, including cell migration, invasion, and inflammatory signaling. Moreover, cfDNA analysis offers a promising avenue for non-invasive monitoring of PLAU activity and tumor dynamics. These findings lay the foundation for future research into PLAU-targeted therapies and cfDNA-based diagnostics, which could ultimately improve outcomes for patients with lung adenocarcinoma. Further research, including in vivo validation and the development of more specific PLAU inhibitors, will be crucial to advancing these approaches in clinical settings.

Conclusion

Our study identifies PLAU as a key driver of lung adenocarcinoma, acting both as a functional regulator and a potential biomarker. Using our Transcriptome-TCGA Integration (TTI) tool, we showed that PLAU is significantly upregulated in tumors and promotes NF-κB activation, EMT, and inflammation. PLAU knockout reduced cancer cell proliferation and invasion, while overexpression reversed these effects. Inhibition with aprotinin suppressed NF-κB signaling and lowered cfDNA-derived PLAU levels, supporting its therapeutic and diagnostic value.

TTI effectively integrates in vitro and clinical data, while cfDNA analysis enables non-invasive monitoring of tumor activity. Though further in vivo validation is needed, this study lays the foundation for PLAU-targeted therapies and highlights the broader potential of TTI for precision oncology.

Acknowledgment

The authors are grateful to our group for their helpful advice.

Funding Information

The authors have not received any financial support or funding to report.

Author's Contributions

Author S.C. participated in all experiments, coordinated the data analysis, and contributed to the writing of the manuscript. Author MGK as the corresponding author, supervised the overall project, provided critical revisions, and finalized the manuscript for submission.

Ethics

All experiments were conducted in accordance with relevant guidelines and regulations.

References

Andreasen, P. A., Egelund, R., & Petersen, H. H. (2000). The plasminogen activation system in tumor growth, invasion, and metastasis. *Cellular and Molecular Life Sciences (CMLS)*, *57*(1), 25–40. https://doi.org/10.1007/s000180050497

Baldwin, A. S. (2001). Control of oncogenesis and cancer therapy resistance by the transcription factor NF-κB. *Journal of Clinical Investigation*, *107*(3), 241–246. https://doi.org/10.1172/jci11991

Chazaud, B., Ricoux, R., Christov, C., Plonquet, A., Gherardi, R. K., & Barlovatz-Meimon, G. (2002). Promigratory Effect of Plasminogen Activator Inhibitor-1 on Invasive Breast Cancer Cell Populations. *The American Journal of Pathology*, *160*(1), 237–246. https://doi.org/10.1016/s0002-9440(10)64367-2

Cho, S. (2025). Investigation of lung cancer biomarkers through in vitro cell-free DNA. *National High School Journal of Science (NHSJS)*.

Cisneros-Villanueva, M., Hidalgo-Pérez, L., Rios-Romero, M., Cedro-Tanda, A., Ruiz-Villavicencio, C. A., Page, K., Hastings, R., Fernandez-Garcia, D., Allsopp, R., Fonseca-Montaño, M. A., Jimenez-Morales, S., Padilla-Palma, V., Shaw, J. A., & Hidalgo-Miranda, A. (2022). Cell-free DNA analysis in current cancer clinical trials: a review. *British Journal of Cancer*, *126*(3), 391–400. https://doi.org/10.1038/s41416-021-01696-0

Connor, A. A., Denroche, R. E., Jang, G. H., Lemire, M., Zhang, A., Chan-Seng-Yue, M., Wilson, G., Grant, R. C., Merico, D., Lungu, I., Bartlett, J. M. S., Chadwick, D., Liang, S.-B., Eagles, J., Mbabaali, F., Miller, J. K., Krzyzanowski, P., Armstrong, H., Luo, X., … Gallinger, S. (2019). Integration of Genomic and Transcriptional Features in Pancreatic Cancer Reveals Increased Cell Cycle Progression in Metastases. *Cancer Cell*, *35*(2), 267-282.e7. https://doi.org/10.1016/j.ccell.2018.12.010

Diehl, F., Schmidt, K., Choti, M. A., Romans, K., Goodman, S., Li, M., Thornton, K., Agrawal, N., Sokoll, L., Szabo, S. A., Kinzler, K. W., Vogelstein, B., & Diaz Jr, L. A. (2008). Circulating mutant DNA to assess tumor dynamics. *Nature Medicine*, *14*(9), 985–990. https://doi.org/10.1038/nm.1789

Ding, Y., Niu, W., Zheng, X., Zhou, C., Wang, G., Feng, Y., & Yu, B. (2022). Plasminogen activator, urokinase enhances the migration, invasion, and proliferation of colorectal cancer cells by activating the Src/ERK pathway. *Journal of Gastrointestinal Oncology*, *13*(6), 3100–3111. https://doi.org/10.21037/jgo-22-1215

Gu, L., Wang, Z., Zuo, J., Li, H., & Zha, L. (2018). Prognostic significance of NF-κB expression in non-small cell lung cancer: A meta-analysis. *PLOS ONE*, *13*(5), e0198223. https://doi.org/10.1371/journal.pone.0198223

Karin, M., & Greten, F. R. (2005). NF-κB: linking inflammation and immunity to cancer development and progression. *Nature Reviews Immunology*, *5*(10), 749–759. https://doi.org/10.1038/nri1703

Li, M. S. C., Mok, K. K. S., & Mok, T. S. K. (2023). Developments in targeted therapy & immunotherapy—how non-small cell lung cancer management will change in the next decade: a narrative review. *Annals of Translational Medicine*, *11*(10), 358–358. https://doi.org/10.21037/atm-22-4444

Ricciardi, M., Zanotto, M., Malpeli, G., Bassi, G., Perbellini, O., Chilosi, M., Bifari, F., & Krampera, M. (2015). Epithelial-to-mesenchymal transition (EMT) induced by inflammatory priming elicits mesenchymal stromal cell-like immune-modulatory properties in cancer cells. *British Journal of Cancer*, *112*(6), 1067–1075. https://doi.org/10.1038/bjc.2015.29

Riely, G. J., Marks, J., & Pao, W. (2009). KRAS Mutations in Non-Small Cell Lung Cancer. *Proceedings of the American Thoracic Society*, *6*(2), 201–205. https://doi.org/10.1513/pats.200809-107lc

Sabari, J. K., Lok, B. H., Laird, J. H., Poirier, J. T., & Rudin, C. M. (2017). Unravelling the biology of SCLC: implications for therapy. *Nature Reviews Clinical Oncology*, *14*(9), 549–561. https://doi.org/10.1038/nrclinonc.2017.71

Schild, S. E., Tan, A. D., Wampfler, J. A., Ross, H. J., Yang, P., & Sloan, J. A. (2015). A new scoring system for predicting survival in patients with non-small cell lung cancer. *Cancer Medicine*, *4*(9), 1334–1343. https://doi.org/10.1002/cam4.479

Steuer, C. E., Behera, M., Berry, L., Kim, S., Rossi, M., Sica, G., Owonikoko, T. K., Johnson, B. E., Kris, M. G., Bunn, P. A., Khuri, F. R., Garon, E. B., & Ramalingam, S. S. (2016). Role of race in oncogenic driver prevalence and outcomes in lung adenocarcinoma: Results from the Lung Cancer Mutation Consortium. *Cancer*, *122*(5), 766–772. https://doi.org/10.1002/cncr.29812

Tang, L., & Han, X. (2013). The urokinase plasminogen activator system in breast cancer invasion and metastasis. *Biomedicine & Pharmacotherapy*, *67*(2), 179–182. https://doi.org/10.1016/j.biopha.2012.10.003

Udomruk, S., Orrapin, S., Pruksakorn, D., & Chaiyawat, P. (2021). Size distribution of cell-free DNA in oncology. *Critical Reviews in Oncology/Hematology*, *166*, 103455. https://doi.org/10.1016/j.critrevonc.2021.103455

Wan, J. C. M., Massie, C., Garcia-Corbacho, J., Mouliere, F., Brenton, J. D., Caldas, C., Pacey, S., Baird, R., & Rosenfeld, N. (2017). Liquid biopsies come of age: towards implementation of circulating tumour DNA. *Nature Reviews Cancer*, *17*(4), 223–238. https://doi.org/10.1038/nrc.2017.7