

New Phenomenon

CLINT1 is a subtype-specific biomarker and a downstream effector of p53-R273H in lung adenocarcinoma migration

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Lung cancer is the leading cause of cancer-related deaths worldwide. In 2020, approximately 1.8 million deaths were attributed to lung cancer, underscoring its status as the most lethal malignancy [1]. In China, lung cancer mortality exhibits pronounced geographical and sex-specific disparities, with particularly unfavorable trends observed in rural western regions [2]. Metastasis is the primary cause of death in patients with advanced disease. Lung cancer is broadly classified into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), with NSCLC accounting for approximately 85% of all cases. NSCLC is further subdivided into lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and large cell carcinoma. LUAD is the most prevalent subtype of NSCLC, particularly among women and non-smokers, and represents a major contributor to lung cancer mortality. The pathogenesis of LUAD is closely associated with diverse genetic mutations and epigenetic alterations. Tumor-associated mutations can influence both oncogenicity and immunogenicity, exerting context-dependent pro- and anti-tumor effects [3]. Several molecular alterations, such as EGFR mutations, ALK rearrangements, and KRAS mutations, are closely linked to LUAD initiation and progression and have become the basis for targeted therapies. Despite advances in understanding LUAD molecular mechanisms, the functional roles of many molecules remain poorly characterized.

The p53 protein is a critical tumor suppressor that regulates the cell cycle, maintains genomic stability, and induces apoptosis [4]. *TP53* is the most frequently mutated gene in NSCLC, occurring in over 50% of cases overall and in approximately 39% to 46% of adenocarcinomas. Most *TP53* mutations occur within the DNA-binding domain, with the R273H mutation standing out as a representative DNA contact mutant and one of the most prevalent variants, accounting for approximately 3.1% of all p53 mutations in human cancers [5,6]. Notably, the R273H mutation confers unique gain-of-function (GOF) traits, prominently enhancing cell migra-

tion, invasion, and metastasis, which are key phenotypes driving LUAD progression [7]. Distinct from other p53 mutants, R273H exerts oncogenic effects by directly binding to cellular proteins and dysregulating cancer-associated transcriptional networks. This renders it a pivotal target for deciphering mutant p53-driven malignant progression in LUAD. This mutation not only abolishes the tumor-suppressive function of wild-type p53 but also activates tumor-promoting pathways, enhancing cell migration, invasion, and drug resistance, thereby contributing to lung cancer progression and poor prognosis. Consequently, identifying key molecules associated with the R273H mutation holds significant research and clinical value in both LUAD research and clinical practice.

Clathrin interactor 1 (CLINT1), also known as EpsinR (EPNR), is an adaptor protein containing an Epsin N-terminal homology (ENTH) domain [8]. It directly recognizes the three-helix bundle domain of the mouse SNARE protein Vti1b and interacts with other SNARE proteins, playing a critical role in vesicular transport between the Golgi apparatus and endosomes, as well as in maintaining vesicle homeostasis and proper protein trafficking pathways. Aberrant CLINT1 expression has been shown to cause improper intracellular protein localization, thereby disrupting normal cellular functions. Studies suggest that CLINT1 is involved in processes such as metastasis and chemoresistance in various tumors and can interact with the cancer-related protein nonreceptor tyrosine kinase 2 [9]. However, the functional role of CLINT1 in lung cancer, particularly its differential effects in LUAD and LUSC, remains largely unexplored.

To investigate the subtype-specific biological functions of CLINT1 in NSCLC, we systematically assessed its expression patterns and clinical relevance. Analysis of 33 cancer types using the TIMER database revealed that CLINT1 expression was upregulated in several malignancies, including cholangiocarcinoma, esophageal carcinoma, LUAD, and stomach adenocarcinoma (Figure 1A).

Notably, CLINT1 was markedly upregulated in LUAD, whereas no significant increase was observed in LUSC. Validation using the UALCAN database confirmed that CLINT1 mRNA levels were significantly elevated in LUAD tissues (Figure 1B, top) but not in LUSC (Figure 1B, bottom). Consistent with the transcriptomic data, CLINT1 protein expression was significantly higher in LUAD (Figure 1C, top), with no significant difference detected in LUSC (Figure 1C, bottom). Stage-wise analysis indicated that CLINT1 expression was significantly elevated in LUAD stages I–III compared with normal tissues, whereas stage IV included only a single case (all $P < 0.05$), limiting statistical power (Figure 1D, top). Similarly, grade-wise analysis demonstrated significantly higher CLINT1

expression in grades I–III LUAD samples than in normal samples (all $P < 0.05$) (Figure 1D, bottom). These findings indicate that CLINT1 is specifically upregulated in LUAD at both the mRNA and protein levels but not in LUSC.

Given the consistent elevation of CLINT1 in LUAD, its prognostic significance was evaluated. Kaplan-Meier (KM) analysis showed that high CLINT1 expression was significantly associated with poorer overall survival (OS) (Figure 1E, left) and first-progression (FP) survival in LUAD ($P < 0.001$) (Figure 1F, left), with a hazard ratio > 1 . In contrast, CLINT1 expression showed no significant differences in LUSC (Figure 1E,F, right; $P > 0.05$, HR < 1). These results indicate that high CLINT1 expression correlates with poor

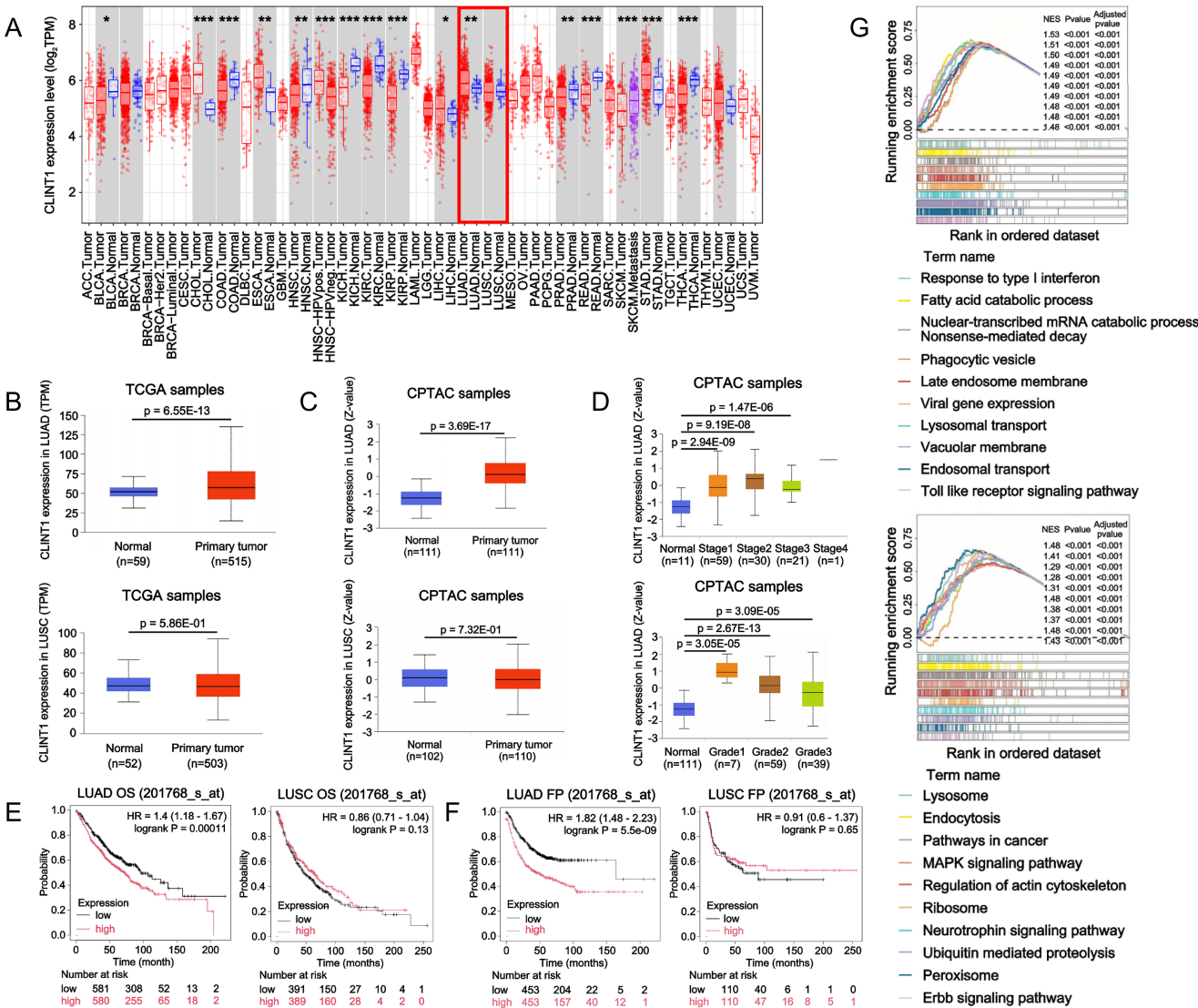


Figure 1. CLINT1 expression, prognostic significance, and functional enrichment in lung cancer (A) TIMER database analysis of CLINT1 expression across 33 cancer types, showing marked upregulation in LUAD but not in LUSC. (B) UALCAN (TCGA) validation of CLINT1 mRNA expression levels in LUAD and LUSC compared with normal tissues. (C) CPTAC analysis of CLINT1 protein expression, confirming significant upregulation in LUAD but not in LUSC. (D) Stage-wise and grade-wise expression analysis of CLINT1 in LUAD samples from the CPTAC dataset. (E) Kaplan-Meier overall survival (OS) analysis of CLINT1 expression in LUAD and LUSC patients. (F) Kaplan-Meier first-progression survival (FP) analysis of CLINT1 expression in LUAD and LUSC patients. (G) GSEA enrichment plots of the top GO biological processes (left) and KEGG pathways (right) positively associated with high CLINT1 expression in LUAD. In the box plots (A–D), the central line indicates the median, the box edges represent the interquartile range (25th–75th percentiles), and the whiskers extend to the minimum and maximum values. Statistical differences were analyzed using the Wilcoxon rank-sum test for TIMER data (A) and Student's t -test for UALCAN/CPTAC data (B–D). For survival analysis (E,F), P -values were calculated using the Log-rank test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

prognosis in LUAD but not in LUSC. Therefore, we aimed to understand the biological functions of CLINT1 in LUAD and whether it is closely associated with the occurrence and development of cancer. To address this question, the TCGA-LUAD dataset was stratified into high- and low-expression groups based on CLINT1 levels. Differential analysis and GO/KEGG enrichment identified 3619 differentially enriched GO terms and 154 KEGG pathways, revealing functional associations with CLINT1 expression. The top enriched GO biological process terms included response to type I interferon (NES = 1.53), fatty acid catabolic process (NES = 1.51), nuclear-transcribed mRNA catabolic process nonsense-mediated decay (NES = 1.50), and Phagocytic Vesicle

(NES = 1.49) (Figure 1G, top). The top KEGG pathways included lysosome (NES = 1.48), endocytosis (NES = 1.41), cancer pathways (NES = 1.29), MAPK signaling (NES = 1.28), and regulation of the actin cytoskeleton (NES = 1.31) (Figure 1G, bottom). These GSEA results suggest that CLINT1 plays a pivotal role in immune responses, vesicular transport, metabolic processes, and cancer-related signaling pathways, highlighting its potential in regulating the tumor microenvironment and cancer progression.

Given that CLINT1 was highly expressed in LUAD and associated with poor prognosis, we next investigated its functional role in LUAD cells. In H1299 and H358 cells, CLINT1 overexpression (Figure 2A) significantly enhanced cell migration (Figure 2B),

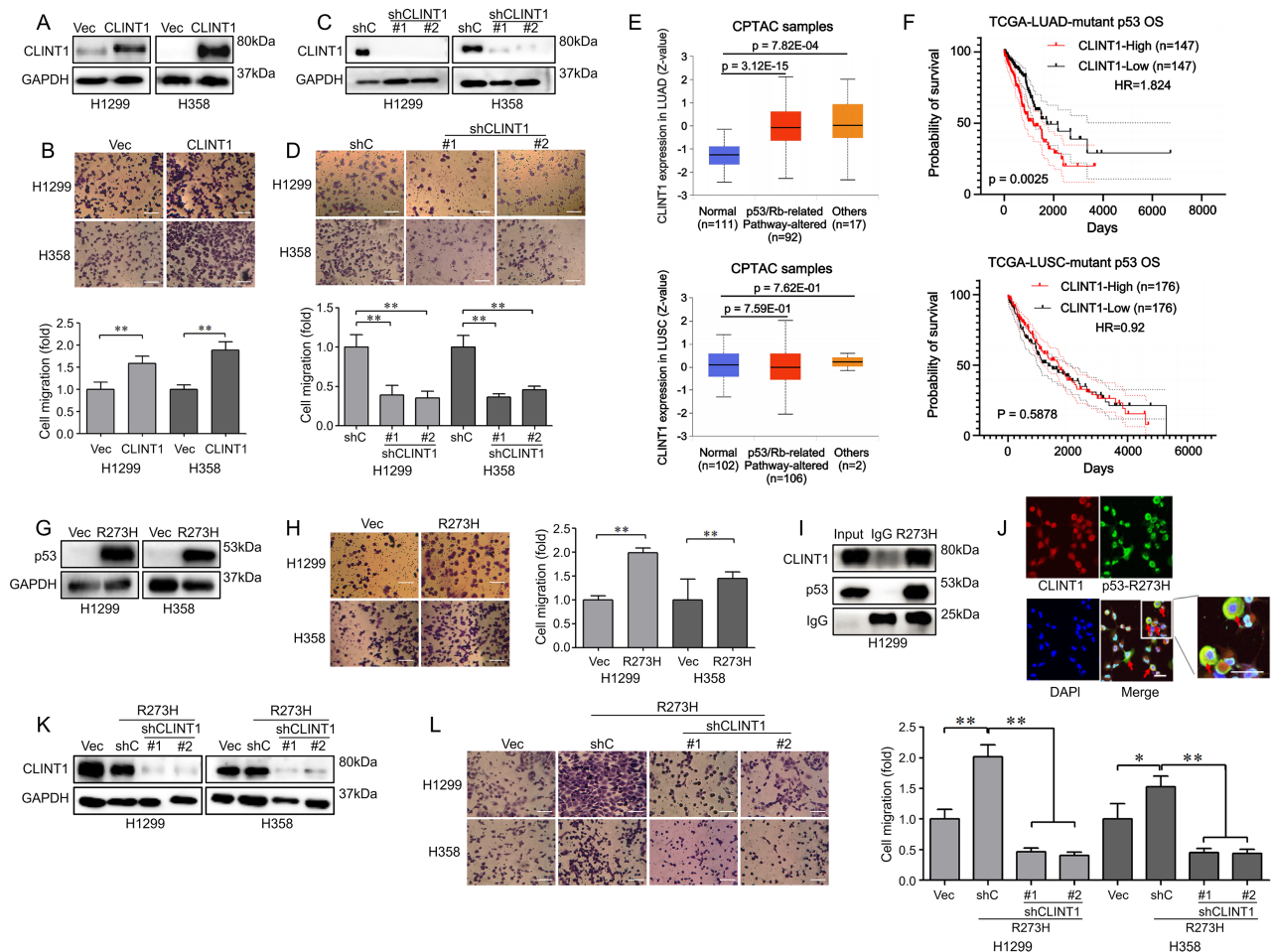


Figure 2. CLINT1 promotes LUAD cell migration and mediates p53-R273H-driven progression (A) Western blot analysis confirmed CLINT1 overexpression in H1299 and H358 cells using an anti-CLINT1 antibody (10470-1-AP, 1:1000; Proteintech, Wuhan, China), with GAPDH (60004-1-Ig, 1:50,000; Proteintech) as the loading control. (B) Transwell migration assays showing enhanced migration upon CLINT1 overexpression. (C) Western blotting was performed to validate *CLINT1* knockdown in H1299 and H358 cells using an anti-CLINT1 antibody (10470-1-AP, 1:1000; Proteintech), with GAPDH (60004-1-Ig, 1:50,000; Proteintech) as the loading control. (D) Transwell assays showing suppressed migration upon *CLINT1* knockdown. (E) CPTAC samples showing enrichment of CLINT1 expression in p53/Rb-related pathways in LUAD but not in LUSC. (F) Kaplan-Meier survival analysis of LUAD and LUSC patients with p53 mutations stratified by CLINT1 expression. High CLINT1 expression predicts poor survival in LUAD but not in LUSC. (G) Western blotting confirmed p53-R273H overexpression in H1299 and H358 cells using an anti-p53 antibody (10442, 1:1000; Proteintech), with GAPDH (60004-1-Ig, 1:50,000; Proteintech) as the loading control. (H) Transwell assays showing enhanced migration upon p53-R273H overexpression. (I) Co-immunoprecipitation analysis demonstrated the physical interaction between CLINT1 and p53-R273H. The antibodies against p53 (60283-2-Ig, 1:750; Proteintech), CLINT1 (10470-1-AP, 1:750; Proteintech), and control IgG (30000-0-AP, 1:750; Proteintech) were used. (J) Immunofluorescence staining showing cytoplasmic co-localization of CLINT1 (red) and p53-R273H (green), with nuclei stained by DAPI (blue). (K) Western blot analysis confirming effective *CLINT1* knockdown in cells overexpressing p53-R273H. (L) Transwell assays showing that *CLINT1* knockdown abrogates p53-R273H-induced migration. Data are presented as the mean $\pm$ SD from three independent experiments (B,D,H,L). For the box plot (E), whiskers represent the minimum and maximum values. Statistical significance was determined using Student's *t*-test (B,D,E,H,L) or the Log-rank test (F). **P* < 0.05, ***P* < 0.01. Scale bar = 100 μ m.

whereas *CLINT1* knockdown (Figure 2C) markedly suppressed migration (Figure 2D). These results demonstrate that CLINT1 promotes LUAD cell migration and confirm that CLINT1 itself can serve as a key molecule regulating LUAD migration, and its pro-migratory function is independent of the presence of p53-R273H. Given that *TP53* is mutated in over half of lung cancers, we examined whether CLINT1 function is influenced by p53 mutation status. While CLINT1 maintains a fundamental role in cell migration, subsequent analyses revealed that mutant p53-R273H exploits this core migratory regulator to amplify its oncogenic effects. Consistently, CPTAC analysis showed that CLINT1 expression in LUAD was enriched in p53/Rb-related pathways (Figure 2E, top), a pattern not observed in LUSC (Figure 2E, bottom). To assess the impact of p53 mutation on the prognostic value of CLINT1, LUAD and LUSC datasets from TCGA were stratified by p53 status. KM analysis revealed that in LUAD patients harboring p53 mutations, high CLINT1 expression was associated with significantly poorer survival, indicating a synergistic effect between CLINT1 and mutant p53 (Figure 2F, top). In contrast, no such effect was observed in p53-mutant LUSC patients (Figure 2F, bottom).

It is well established that over 30% of *TP53* mutations cluster within six DNA-binding domain hotspots, among which R273H has been a major focus of ongoing research [10]. To further clarify this relationship between CLINT1 and mutant p53, we specifically investigated the hotspot mutant p53-R273H. Overexpression of p53-R273H in H1299 and H358 cells, confirmed by western blot analysis (Figure 2G), markedly promoted cell migration (Figure 2H). Co-immunoprecipitation assay demonstrated a direct interaction between CLINT1 and p53-R273H (Figure 2I), and immunofluorescence staining revealed their cytoplasmic co-localization (Figure 2J). Importantly, silencing of *CLINT1* in p53-R273H-overexpressing cells (Figure 2K) abolished the migration-enhancing effect induced by p53-R273H (Figure 2L), directly verifying that the pro-migratory phenotype driven by p53-R273H is dependent on CLINT1. This functional dependency clarifies the distinct roles of CLINT1 under p53-null versus p53-R273H conditions and provides compelling evidence that CLINT1 serves as a critical downstream effector of p53-R273H-driven gain-of-function activity. Although CLINT1 contributes to basal migration in p53-null cells, this observation does not weaken our conclusion. Rather, it highlights a dual functional role for CLINT1: it is required for LUAD cell motility irrespective of p53 status, and it becomes indispensable when p53-R273H amplifies migratory behavior through gain-of-function mechanisms. Notably, CLINT1 depletion not only reduced baseline migration but also eliminated the additional migratory advantage conferred by p53-R273H, underscoring its essential role in mediating mutant p53 oncogenicity.

In summary, this study identifies CLINT1 as a LUAD-specific oncogenic driver that cooperates with mutant p53 to promote cancer cell migration and progression. In contrast, CLINT1 expression and functional relevance in LUSC are minimal, indicating that CLINT1 is unlikely to be a key pathogenic factor in this

subtype. Therefore, CLINT1 holds promise as a LUAD-specific biomarker for metastasis prediction and as a potential therapeutic target. Future studies should further explore the upstream regulators of CLINT1, its functional relationship with the *TP53* signaling axis, and its potential role in modulating sensitivity to immunotherapy, ultimately facilitating its clinical translation in precision oncology.

Data Availability

Data can be accessed upon reasonable request from the corresponding authors.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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