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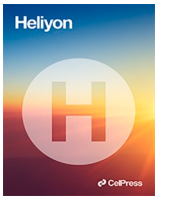

Research article

Exploring the prognostic and therapeutic value of HIF1A in lung adenocarcinoma

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ABSTRACT

Lung adenocarcinoma (LUAD) remains a challenge within the realm of non-small cell lung cancer (NSCLC), demanding innovative diagnostic and therapeutic solutions. In this study, we systematically detected the correlation between the expression of hypoxia-induced factor 1A (HIF1A) and the clinical characteristics of LUAD, alongside lung squamous cell carcinoma (LUSC). Our bioinformatic analysis reveals that HIF1A mRNA expression is significantly upregulated in both LUAD and LUSC samples compared to non-tumorous lung tissues. The overexpression is positively correlated with increased copy number variation and negatively associated with promoter methylation. However, meta-analysis and survival analyses revealed a pronounced association between elevated HIF1A expression and poor clinical outcome specifically within the LUAD subset, with no such correlation evident in LUSC. Additionally, we explored the interplay between HIF1A expression, leukocyte infiltration, and the presence of immunosuppressive markers, revealing HIF1A's suppressive role in cytotoxicity against cancer cells. Furthermore, we performed in silico prediction to explore the correlations between HIF1A and its interacting proteins, associated pathways, glycolysis, and m^6A modification, and the feasibility of targeting HIF1A with specific drugs. In summary, our study revealed the prognostic significance and therapeutic potential of HIF1A in LUAD.

1. Background

Lung cancer is one of the most predominant cancer types worldwide. A majority of patients progress to metastatic and malignant stages at the time of diagnosis, posing significant challenges in terms of both treatment and the prevention of reoccurrence [1]. Lung cancer can be categorized into two primary clinical groups: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) [2]. NSCLC, in particular, accounts for over 80 % of all lung cancer cases, with its three major subtypes, namely lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and large cell lung carcinoma (LCLC), holding prominence within this category [3–5]. Among these subtypes, LUAD commonly originates from alveolar cells, is more frequent in non-smokers, has frequent genetic mutations, and responds well to targeted therapy [6,7]. LUAD emerges as the most frequent NSCLC, contributing to approximately 50 % of all lung cancer-related deaths [8]. By comparison, LUSC develops from squamous cells, is strongly linked to smoking, has fewer genetic mutations, and is treated primarily with traditional therapies such as surgery, radiation therapy, and chemotherapy [9]. The molecular mechanisms and tumor microenvironment contributing to the carcinogenesis of LUAD and LUSC also exhibit significant differences

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[10–13].

Enhancing the clinical outcome of NSCLC relies heavily on the development of novel diagnosis and treatment strategies [14]. For instance, assessment of epidermal growth factor receptor (EGFR) mutation types has become the cornerstone of molecular characterization before initiating NSCLC treatment, and it has demonstrated substantial benefits, as evidenced by the improved long-term patient survival rate [15]. Furthermore, mutations in the p53 gene have been demonstrated to play important roles in chemotherapy resistance in lung cancer, attributable to their impact on transcriptional function and cell cycle regulation [16,17].

The intricate interplay within the tumor microenvironment, shaped by factors such as tumor proliferation, inadequate oxygen supply, and disrupted angiogenesis, plays a pivotal role in regulating tumor progression [18,19]. Hypoxia-inducible factor 1α, encoded by the HIF1A gene, undergoes stabilization within the hypoxic tumor microenvironment, assuming a central role as a master regulator in adapting to hypoxia. This critical function ultimately fosters tumor progression [20,21], encompassing the activation of non-coding RNAs [20,22]. In the context of lung cancer, HIF1A has been demonstrated to promote tumor proliferation by activating downstream AKT and ERK signaling pathways [23]. Additionally, it stimulates angiogenesis through the direct upregulation of VEGFA expression [24].

Although the therapeutic significance of HIF1A in LUAD and LUSC has been partially revealed by *in vivo* antagonist administration [25], the correlation between HIF1A expression and the prognosis of LUAD and LUSC patients with various clinical features remains elusive. Additionally, the relationship between HIF1A expression and the immune infiltration status, its interacting proteins, and possible targeting drugs have not been comprehensively explored. The limited comprehensive analysis of HIF1A across subtypes of lung cancer may restrict its clinical application as a potential druggable target for precise medicine.

In the current study, we started with a systematic exploration of HIF1A expression within LUAD and LUSC. Leveraging an array of bioinformatic analyses, we delve into HIF1A's expression patterns, genome characteristics, and prognostic value. Our finding unveils a striking elevation in HIF1A expression levels and concurrent copy number variation (CNV), alongside a decrease in promoter methylation, not only in LUAD but also in LUSC. However, it is noteworthy that a significant correlation between HIF1A expression and clinical outcome was discernible exclusively in LUAD. This observation underscores the potential of HIF1A as a valuable biomarker for predicting poor prognosis in LUAD patients, whereas its relevance in the context of LUSC remains less pronounced. Subsequently, we evaluated the association between HIF1A expression or methylation levels with tumor-infiltrating lymphocytes (TILs) in LUAD. This assessment provides further evidence supporting the oncogenic impact of HIF1A and its potential role in regulating tumor microenvironment. Lastly, we delved into the realm of HIF1A-interacting proteins, pathway associations, and their implications for drug resistance in LUAD. Our predictions highlight HIF1A's predominant influence on metabolism-related pathways, aligning with its established biological functions and known role in modulating chemotherapy drug turnover. In conclusion, the multifaceted investigation collectively underscores the oncological significance of HIF1A in LUAD and supports its potential as a candidate marker and therapeutic target.

2. Materials and methods

2.1. mRNA expression analysis

HIF1A mRNA expression data were obtained from The Cancer Genome Atlas (TCGA) [26] (https://portal.gdc.cancer.gov/) and Student's *t*-tests were performed to compare the gene expression levels in tumors and adjacent non-tumorous tissues. We further conducted additional expression analyses using online resources, specifically, UALCAN [27] (http://ualcan.path.uab.edu/), GEPIA platform [28] (http://gepia.cancer-pku.cn/), and GSCA online toolbox [29] (http://bioinfo.life.hust.edu.cn/GSCA/). These analyses were based on the TCGA-LUAD and TCGA-LUSC data sets, which are accessible from the TCGA database.

2.2. Analysis of expression and methylation regarding different characteristics

UALCAN was applied to analyze the differential HIF1A expression and promoter methylation in total samples or samples from different groups with distinct clinical parameters in LUAD or LUSC. Statistical difference between groups was assessed using Students's *t*-test.

2.3. Survival analysis

The survival analysis of patients with LUAD or LUSC was performed using the GEPIA platform with the TCGA-LUAC or TCGA-LUSC data set.

2.4. Gene expression across various CNV groups

HIF1A expression level analysis in different CNV groups was performed using the cBioportal database [30,31] (https://www.cbioportal.org/). Additionally, correlation analysis between HIF1A CNV with mRNA level was conducted using LinkedOmics [32] (http://www.linkedomics.org/).

2.5. Immune cell infiltration analysis

The correlation between HIF1A expression and methylation levels with tumor infiltration cells (TILs) in LUAD was determined by Spearman correlation with the GSCA database [29].

2.6. Expression analysis in different subtypes

HIF1A expression difference among different tumor subtypes was assessed by the integrated tool TISIDB [33] (http://cis.hku.hk/TISIDB/).

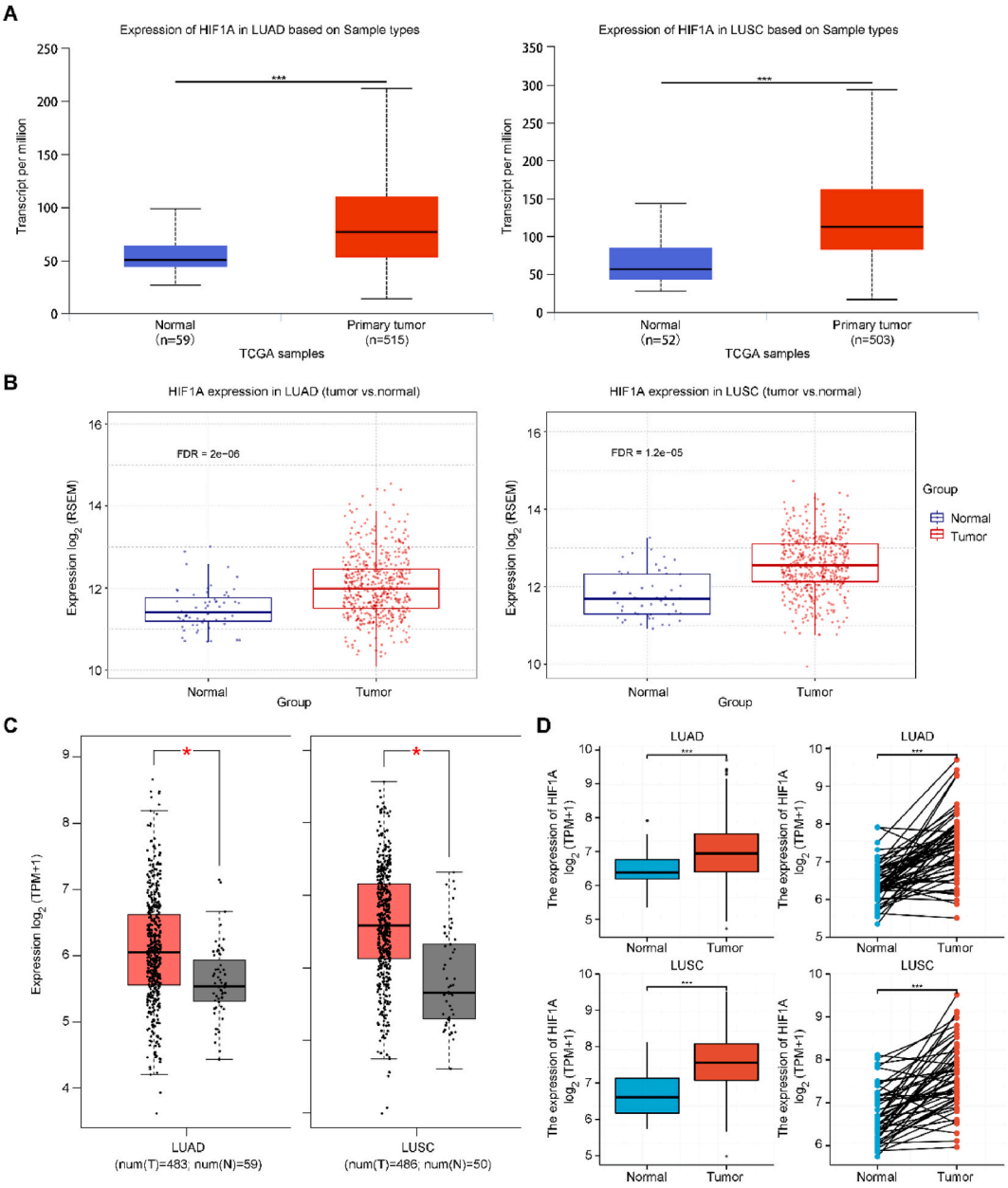

Fig. 1. Expression level analyses of HIF1A in LUAD and LUSC. A. Overall expression of HIF1A in LUAD and LUSC compared to adjacent controls using the UALCAN database. B. Expression analysis of HIF1A in LUAD and LUSC using the GSCA online platform. C. Comparison of HIF1A mRNA level in tumor or control samples from LUAD and LUSC patients as displayed in GEPIA. D. Unpaired (left) and paired (right) analysis of HIF1A expression level in LUAD or LUSC patients compared to adjacent controls using TCGA data sets.

2.7. Correlation of drug sensitivity with gene expression

The correlation between drug sensitivity in CTRP catalogs and HIF1A expression was performed using the "drug" module of GSCA [29]. The drug-target relationship network related to HIF1A was generated using TISIDB [33] (http://cis.hku.hk/TISIDB/).

2.8. Meta-analysis

Meta-analysis regarding HIF1A in LUAD and LUSC patients was conducted using the online platform Lung Cancer Explorer [34] (https://lce.biohpc.swmed.edu/lungcancer/). The studies included in the analysis are listed in the relevant results.

2.9. Analysis of candidate interacting proteins with HIF1A

Enrichment of the HIF1A-interacting protein set and the relationship network was achieved using the GeneMANIA database [35] (http://genemania.org/).

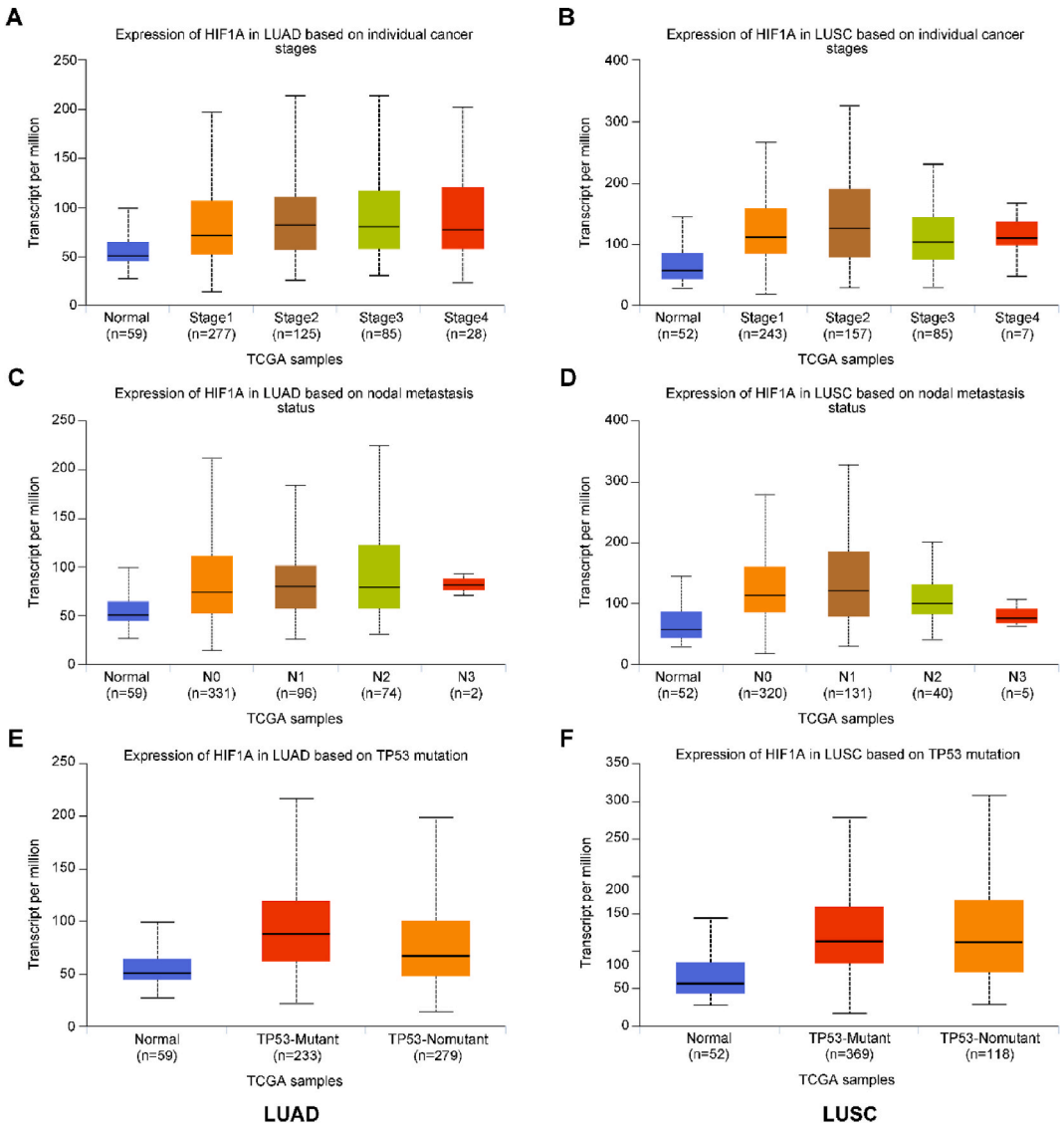

Fig. 2. Expression of HIF1A in LUAD or LUSC patients classified by different clinical parameters. A-F. mRNA expression level analysis of HIF1A by UALCAN in different groups of LUAD (A, C, E) or LUSC patients (B, D, F) divided by cancer stages (A, B), nodal metastasis statuses (C, D), and TP53 mutation (E, F). Statistical significance analysis results between the two groups in each graph are shown in Table S1.

2.10. Gene set co-expression analysis

R (v4.2.1) was utilized to analyze the correlation between HIF1A and immune checkpoint encoding genes, glycolysis-related genes, and m^6A modification-related genes from the TCGA-LUAD data sets. All the data were visualized by the R package "ggplot2".

2.11. Statistical analysis

The comparison of two quantitative data sets was conducted using paired or unpaired Student's *t*-test. Data were presented as mean ± SD. Bioinformatic correlations were calculated using Pearson's correlated coefficient, and group differences were analyzed using the Wilcoxon test. Through all figures, $P < 0.05$ was considered statistically significant. Significance levels are represented as follows: $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), and $P < 0.0001$ (****).

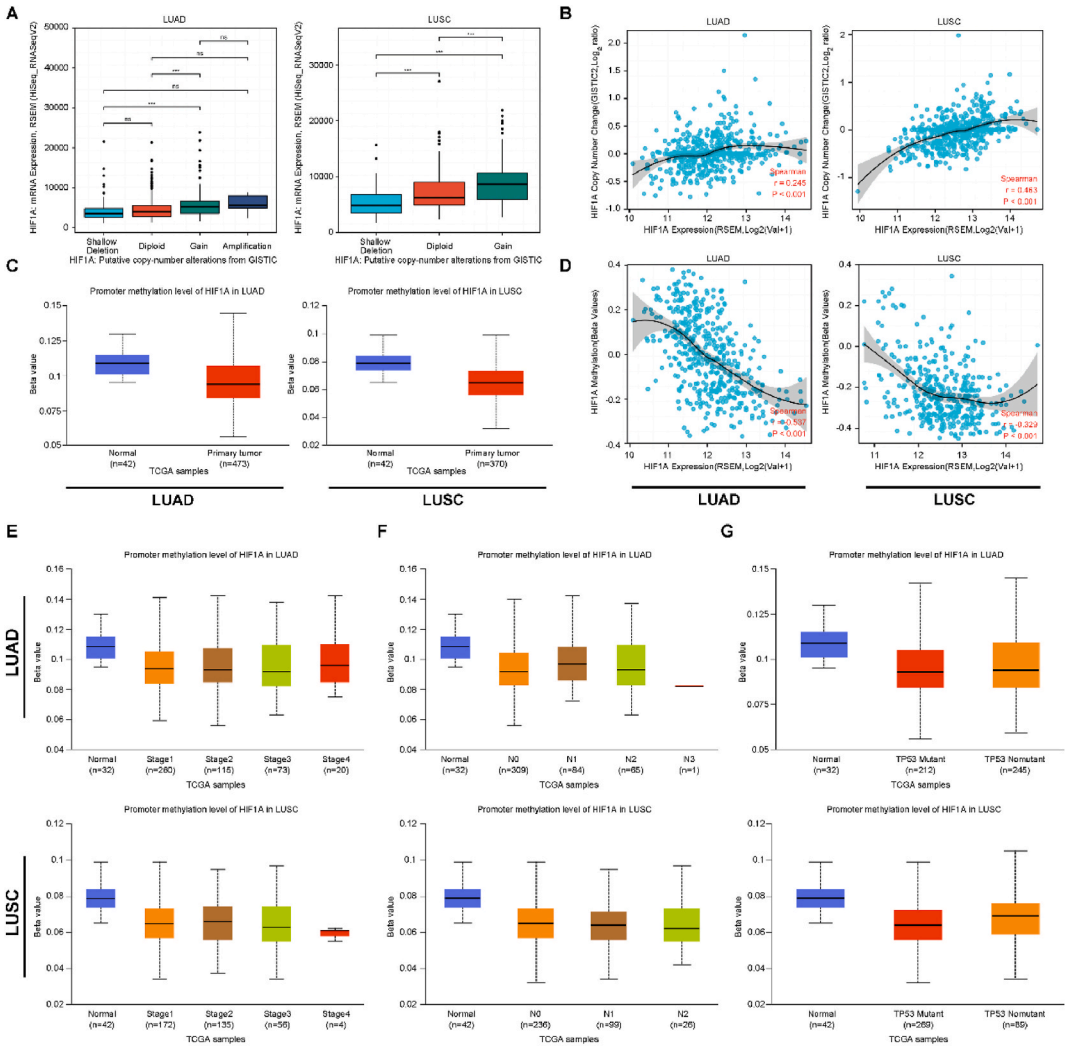

Fig. 3. Analysis of CNV and promoter methylation of HIF1A in LUAD and LUSC. A. mRNA expression level of HIF1A regarding different CNV classifications in LUAD and LUSC samples. B. Correlation analysis of HIF1A CNV with HIF1A expression level using LinkedOmics. C. Promoter methylation of HIF1A in LUAD and LUSC compared to adjacent controls using UALCAN. D. Correlation analysis of HIF1A promoter methylation with HIF1A expression level using LinkedOmics. E-G. Promoter methylation level analysis of HIF1A by UALCAN in different groups of LUAD or LUSC patients divided by cancer stages, nodal metastasis statuses, and TP53 mutation. Statistical significance analysis results between the two groups in each graph of E-G are shown in Table S2.

3. Results

3.1. Upregulation of HIF1A in LUAD and LUSC

To assess HIF1A expression across different subtypes of NSCLC, we employed various pan-cancer bioinformatic methods from different platforms and resources. Initially, we analyzed HIF1A expression in LUAD and LUSC using UALCAN, which revealed a significant increase in its mRNA levels in both subtypes (Fig. 1A). This finding was consistently corroborated through investigations using the GSCA (Fig. 1B) and GEPIA (Fig. 1C) platforms. To further validate the elevated expression of HIF1A in LUAD and LUSC, we obtained mRNA expression data from TCGA and conducted in-silico analyses. The data consistently supported the notion of HIF1A overexpression in these two subtypes of NSCLC (Fig. 1D). Taken together, these results collectively affirm that HIF1A expression is markedly higher in LUAD and LUSC tissues compared to their normal counterparts.

3.2. HIF1A expression in LUAD and LUSC patients with varied clinical characteristics

To gain a comprehensive understanding of the clinical significance and potential impact of HIF1A in the progression of LUAD and LUSC, we leveraged the UALCAN database to investigate HIF1A expression across different clinical parameters. Our analysis didn't reveal any significant differences in HIF1A expression based on cancer stages in either LUAD or LUSC (Fig. 2A and B). However, a noteworthy observation was made regarding LUSC patients suffering from more severe nodal metastasis, as they exhibited decreased HIF1A expression (Fig. 2D), in contrast to LUAD patients (Fig. 2C). Furthermore, we found a positive correlation between TP53 mutation and HIF1A expression, primarily in LUAD (Fig. 2E and F). Additionally, LUAD patients with longer periods of smoking history displayed higher HIF1A mRNA levels compared to non-smokers or those with shorter smoking for shorter histories, while this smoking period-dependent difference was not observed in LUSC patients (Figs. S1A–B). Notably, HIF1A expression appeared relatively lower in Asian patients, and the difference was not statistically significant compared to control for both LUAD and LUSC (Figs. S1A–B). Patients of different ages or genders exhibited limited effects on the expression level of HIF1A in both subtypes (Figs. S1A–B). In summary, our

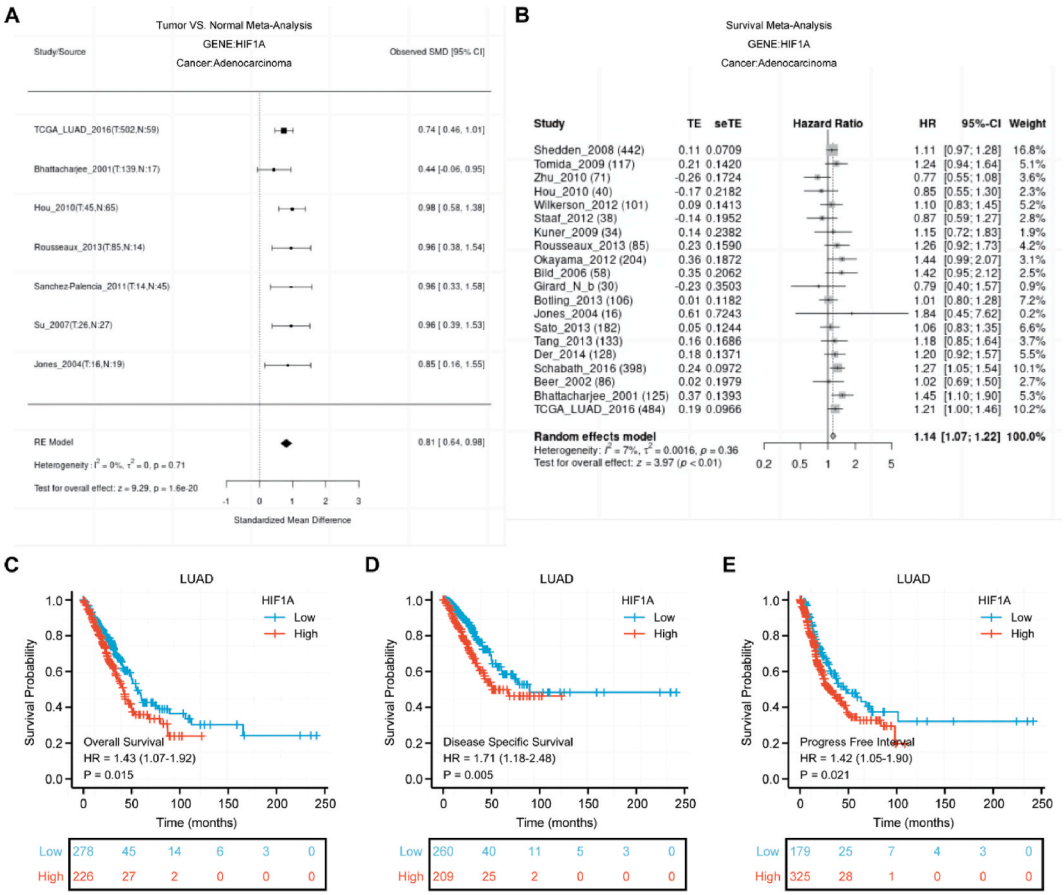

Fig. 4. Correlation between HIF1A expression and the prognosis of LUAD patients. A. Meta-analysis of HIF1A expression in LUAD patient tumor samples compared to normal samples. B. Meta-analysis of correlation between HIF1A mRNA level with patient survival. C-E. OS (C), DFS (D), and PFS (E) rates of LUAD patients with high or low HIF1A expressions analyzed by GEPIA.

analysis has revealed associations between HIF1A and different clinical parameters, which might benefit precise medicine using HIF1A as a target.

3.3. CNV and promoter methylation of HIF1A in LUAD and LUSC

To explore the underlying causes of HIF1A upregulation in LUAD and LUSC, we investigated the CNV and promoter methylation of HIF1A, representing regulatory mechanisms operating at the genetic and epigenetic levels. As anticipated, analysis results from cBioPortal indicated that HIF1A expression levels were significantly higher in tumors with an increased copy number of HIF1A compared to those with HIF1A deletions in both LUAD and LUSC (Fig. 3A). Spearman correlation analysis using LinkedOmics further affirmed the positive correlation between HIF1A CNV and its expression level in the two subtypes (Fig. 3B). On the other hand, HIF1A promoter methylation demonstrated a decrease in LUAD and LUSC patients and exhibited a negative correlation with its expression

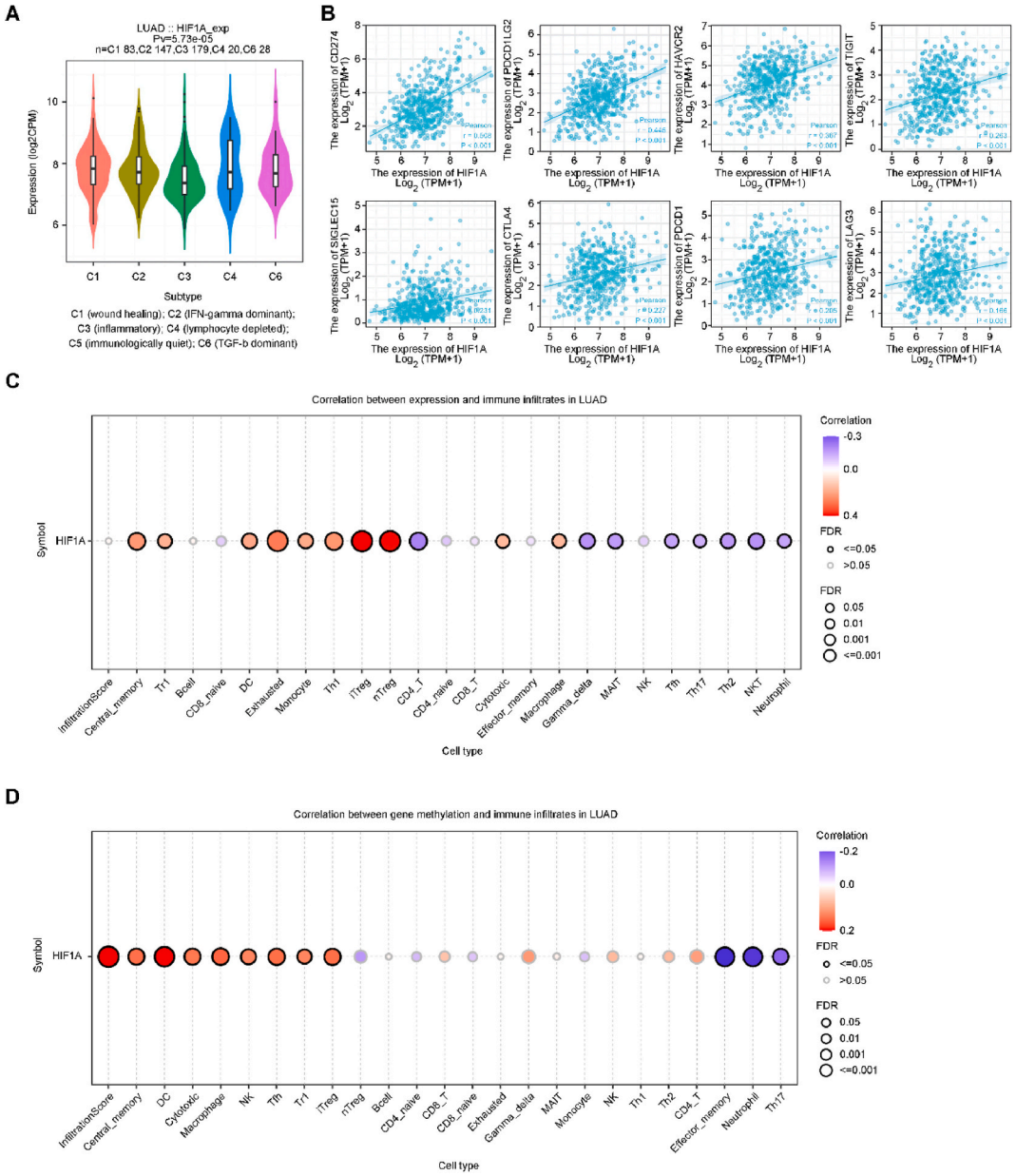

Fig. 5. Association of HIF1A expression with immune subtype, checkpoint gene levels, and TILs in LUAD. A. Violin plots showing the expression distribution of HIF1A in each tumor subtype in LUAD analyzed by TISIDB. B. Pearson correlation analysis of HIF1A expression with expression of eight immune checkpoint coding genes. C. Correlation between HIF1A expression and immune infiltrates in LUAD from GSCA database. D. Correlation between HIF1A promoter methylation and immune infiltrates in LUAD from GSCA database.

(Fig. 3C and D), aligning with its established biological function.

We further explored the impact of various clinical parameters on HIF1A methylation. In the case of LUAD and LUSC, cancer stages and nodal metastasis status exhibited limited influence on HIF1A methylation (Fig. 3E and F). However, patients with TP53 mutations showed a significant decrease in methylation in LUSC but not LUAD (Fig. 3G). Smoking was associated with decreased HIF1A promoter methylation in both subtypes, with long-term smoking having an effect only in LUAD patients, not in LUSC patients (Figs. S1C–D). Moreover, we observed higher levels of HIF1A methylation in Asian patients compared to other racial groups, which mirrored the differences in expression levels among different races (Figs. S1C–D). Once again, as predicted, patients' age or gender had minimal effects on promoter methylation of HIF1A in both subtypes (Figs. S1C–D).

3.4. Prognosis significance of HIF1A in LUAD and LUSC

To assess the correlation between HIF1A expression and the clinical outcome of patients with LUAD and LUSC, we conducted a meta-analysis using the Lung Cancer Explorer platform. Seven patient cohorts in total were included for the comparison of HIF1A expression in normal lung tissues against LUAD or LUSC tumors. The standardized mean difference (SMD) of HIF1A between tumor and normal samples was 0.81 [95 % CI 0.64–0.98] and 1.49 [95 % CI 1.17–1.81] in patients with LUAD and LUSC, respectively (Fig. 4A, S2A). Regarding overall survival (OS), LUAD patients with lower HIF1A expression exhibited significantly better clinical outcomes, as indicated by the hazard ratio integrated from 16 studies (Fig. 4B). However, we didn't observe any significant difference in patient survival among LUSC patients with varying levels of HIF1A expression (Fig. S2B). In summary, our meta-analysis results indicated that HIF1A expression was negatively correlated with the survival of LUAD patients, while this association is not significant in LUSC patients.

We further delved into the survival correlation with HIF1A expression in LUAD and LUSC using two independent databases. Firstly, our analysis of data using the GEPIA database showed that the OS was negatively correlated with HIF1A expression in LUAD when categorized by median grouping, but no such correlation was observed in LUSC (Figs. S3A–B). Similarly, Disease-free survival (DFS) exhibited a negative association with HIF1A levels in LUAD when categorized by quartiles, but this association was not evident in LUSC (Figs. S3A–B). We then performed an analysis using data sets from TCGA for both LUAD and LUSC. The results indicated that OS, DFS, and progress-free survival (PFS) of LUAD patients all displayed significant negative correlations with high HIF1A expression (Fig. 4C–E), while none of these survival indexes showed significant correlation in LUSC patients (Figs. S2C–E).

To gain a better view of the prognosis value of HIF1A in patients with different clinical parameters, we analyzed the survival index of LUAD patients across various categories, including cancer stages, metastasis statuses, pathology stages, smoking behaviors, ages, and genders. The results showed that HIF1A exhibited significant correlations with unfavored clinical outcomes in LUAD patients with mild TNM stages and pathological stages in all survival indexes, while no significant correlation was observed in patients with more advanced disease stages (Figure S4A-C, S5A). In addition, the prognosis of aged patients (>65 years old), displayed a significant association with HIF1A expression (Fig. S5B). The prognosis of patients of both genders exhibits a partial correlation with HIF1A levels (Fig. S5C). The effect of smoking on the prognosis value of HIF1A expression in LUAD was relatively intricate. OS and DFS were significantly correlated with HIF1A in smokers but not in non-smokers, while PFS was only significantly correlated in non-smokers or those with light/moderate smoking (Figs. S5D–E). In conclusion, our analyses of patient survival data suggested that HIF1A expression strongly correlated with poor prognosis in LUAD patients, regardless of most clinical characteristics, while its prognosis value in LUSC is limited. Thus, we focused on HIF1A's diagnostic and therapeutic values in LUAD in the subsequent analyses.

3.5. Correlation of HIF1A with TILs and immune subtypes in LUAD

Given the significant prognosis value of HIF1A in LUAD but not LUSC, we examined its impact on immune cell infiltration and immune subtypes. HIF1A displayed decreased expression in subtype C3 (inflammatory) suggesting that its expression inhibited the inflammatory response in LUAD (Fig. 5A), which may be associated with its tumor-progressing effect. Next, we analyzed the correlation between HIF1A expression and the expression levels of immune checkpoint coding genes from the TCGA-LUAD data set. HIF1A showed a positive correlation with the expression of all immune checkpoints, indicating the shaping effect of HIF1A on tumor microenvironment and immune cell characteristics (Fig. 5B).

To gain more insight into the correlation between HIF1A and the infiltration of specific immune cell types, we utilized the GSCA platform to perform a more detailed analysis. HIF1A expression was found to decrease the infiltration of cytotoxic and inflammatory cells, including gamma-delta T cells, mucosal-associated invariant T (MAIT) cells, invariant natural killer T (iNKT) cells, neutrophils, and $CD4^{+}$Th17 cells, which was consistent with the immune subtype analysis and its oncogenic function (Fig. 5C and S6A). On the other hand, iTreg, nTreg, Th1 cells, exhausted T cells, and monocytes were increased with higher HIF1A expression levels (Fig. 5C and S6B).

We also examined the associations between HIF1A methylation and TILs, revealing that HIF1A promoter methylation is positively linked to the infiltration of multiple immune cell types, such as dendritic cells (DCs), cytotoxic cells, and NK cells (Fig. 5D and S7A), and negatively associated with the infiltration of effector memory cells, neutrophils, and Th17 cells (Fig. 5D and S7B).

These findings indicated a complex relationship between the epigenetic regulation of HIF1A and LUAD microenvironment. Overall, these analyses suggested that HIF1A expression generally exhibits an immune-suppressive effect, especially suppressing inflammatory and cytotoxic responses, on TILs and the microenvironment in LUAD.

3.6. Mapping the interacting proteins of HIF1A in LUAD

Given the prognostic value and potential oncogenic function of HIF1A, we investigated HIFA-interacting proteins in LUAD and their associated biological functions using the GeneMANIA database. Multiple proteins linked to hypoxia response and angiogenesis, including ARNT, ARNT2, HIF3A, HIF1AN, VHL, and EPO, as well as glycolysis-related enzymes such as PFKFB3 and ENO1, were predicted as potential partners of HIF1A (Fig. 6A). These interacting proteins were clustered into functional pathways primarily related to hypoxia cell response and carbohydrate metabolism, notably glycolysis (Fig. 6B).

It's worth noting that these pathways are interconnected, as glycolysis is typically activated in a hypoxia environment to generate ATP through anaerobic respiration. Additionally, HDAC2 and UBC were predicted as molecular partners of HIF1A, suggesting that HIF1A overexpression in LUAD may be involved in the regulation of epigenetic and post-translational modifications (Fig. 6A). In conclusion, the overexpression of HIF1A predominantly influences cellular responses to hypoxia and the activation of glycolysis in LUAD.

3.7. Correlations of HIF1A expression with glycolysis genes and m^6A modification-related genes in LUAD

To further confirm the impact of HIF1A overexpression on the activation of glycolysis LUAD, we assessed the correlation between HIF1A mRNA levels and individual glycolysis genes using data from the TCGA-LUAD data set. Remarkably, all 32 analyzed genes displayed a significant positive correlation with HIF1A expression (Fig. S8). These results demonstrated the promotive role of HIF1A expression in augmenting glycolysis in LUAD.

m^6A mRNA modification has been demonstrated to play a role in the oncogenesis of LUAD [36]. Utilizing the same data set, we investigated the association between HIF1A expression and the expression of 23 m^6A modification-related genes. Remarkably, 20 out of all the analyzed genes exhibited a significant positive correlation with HIF1A mRNA level (Figure S9A-B). Notably, both FTO and ALKBH5, two well-known m^6A erasers were significantly increased with high HIF1A levels, while only one writer, METTL14, displayed a significant association (Fig. S9A). These results revealed the close connection between HIF1A and m^6A modification in LUAD and indicated that HIF1A overexpression may accelerate the dynamic changes of m^6A modification.

3.8. Correlation between HIF1A and drug efficacy in LUAD

In addition to the analysis of the HIF1A's prognostic value as well as its cellular and molecular effects, we also uncovered the association between HIF1A expression and sensitivity of chemotherapeutic agents from the CTRP catalogs using the GSCA platform. Remarkably, we observed significant positive correlations with high confidence across all the analyzed agents, suggesting that HIF1A overexpression and hypoxia microenvironment might hinder the effectiveness of chemotherapy treatment in LUAD (Fig. 7A). This effect of HIF1A may be attributed to its regulation of LUAD cell metabolism, as metabolic factors in LUAD have also been indicated to regulate drug resistance [37].

Furthermore, we analyzed the drugs targeting HIF1A and their related target network to explore the feasibility of HIF1A-targeting therapy against LUAD using the TISIDB database (Fig. 7B). We identified five small molecules from Drugbank that may affect HIF1A, including one (DB06082, PX-478) that specifically targets HIF1A, and another (DB08687, FG-2216) that targets only hypoxia response proteins (HIF1A and EGLN1). Taken together, targeting HIF1A for LUAD is theoretically practicable, however, considering its effect on

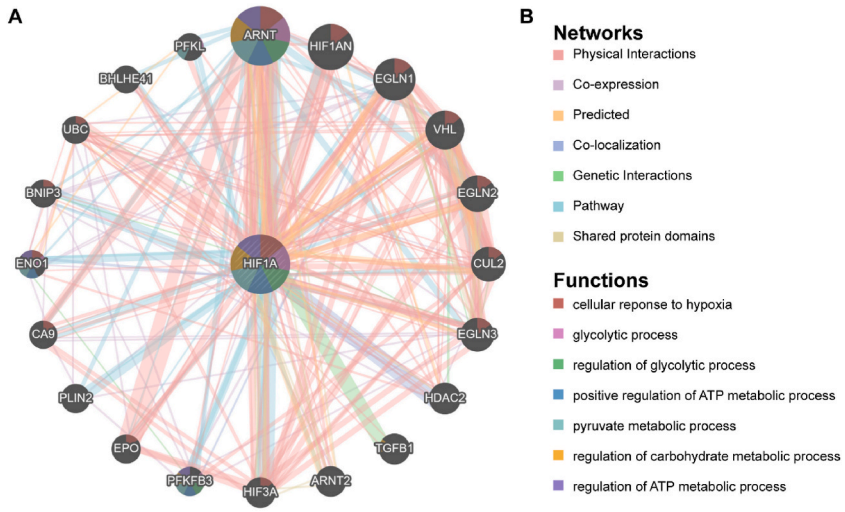

Fig. 6. The networks and functions of candidate HIF1A-interacting proteins. A. plot indicating the interactions between HIF1A and its interacting candidate proteins. B. GeneMANIA analysis results showing the relationships and functional annotations of the HIF1A-interacting proteins.

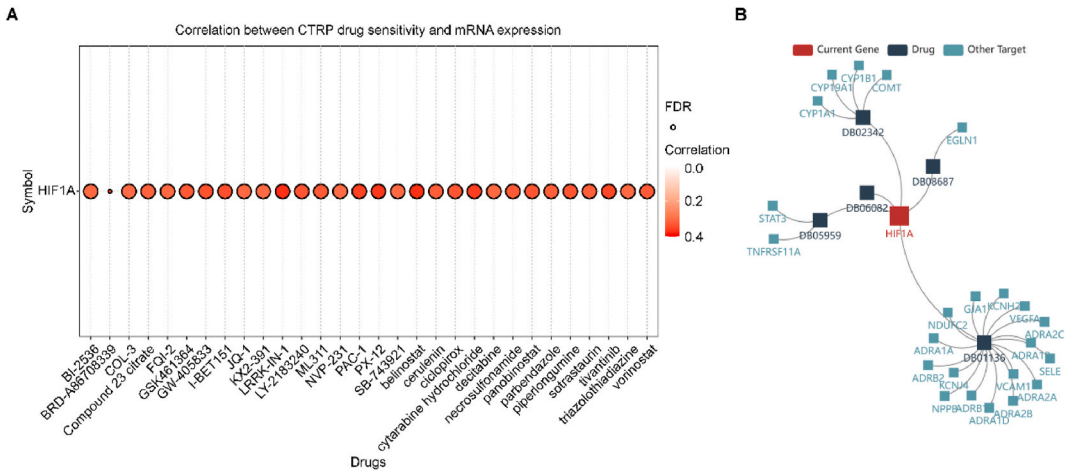

Fig. 7. Correlation between HIF1A and drug efficacy in LUAD. A. Summary plot indicating the overall significant correlation between HIF1A expression and the sensitivity of specified drugs using the CTRP catalog in LUAD. B. Targeting network of drug molecules from Drugbank that target HIF1A, and their other targets obtained from TISDB. DB01136: Carvedilol, DB02342: 2-Methoxyestradiol, DB05959: ENMD-1198, DB06082: PX-478, DB08687: FG-2216.

sensitizing chemotherapy agents, bench works *in vivo* are necessary to validate the feasibility and assess the potential clinical benefit of this approach.

4. Discussion

In the present study, we systematically explored the prognostic and therapeutic significance of HIF1A in LUAD and LUSC and conducted extensive bioinformatic analyses. Utilizing multiple databases, we assessed HIF1A expression in LUAD and LUSC, examining its correlation with patient survival with various clinical parameters. Our finding revealed significant upregulation of HIF1A in both LUAD and LUSC, especially in patients with a history of heavy smoking, however, it was significantly associated with poor clinical outcomes exclusively in LUAD, not in LUSC, even though HIF1A may play an oncogenic role in LUSC [38]. This discrepancy may result from the differential impact of hypoxia on LUAD and LUSC cells [39]. However, the underlying mechanism remains unclear and needs further characterization.

As expected, HIF1A CNV showed a positive correlation, while promoter methylation exhibited a negative correlation with its expression. Moreover, we delved into the interplay between HIF1A expression, CNV, and methylation along with immune cell infiltration, immune subtype, and checkpoint gene expression, uncovering an oncogenic role of HIF1A in shaping the tumor microenvironment and promoting immunosuppression. Additionally, we predicted the interacting proteins with HIF1A, evaluated their biological functions, and identified a positive correlation between HIF1A expression and genes related to glycolysis and m^6A modification. Moreover, our study revealed that HIF1A expression impaired the sensitivity of multiple chemotherapy agents, emphasizing its potential as a therapeutic target. Druggable by small molecule inhibitors, HIF1A presents an attractive candidate for combined treatment strategies, offering promising prospects for clinical applications against LUAD.

The heterogeneity with NSCLC, especially LUAD, underscores the need to identify novel biomarkers for disease diagnosis and prognosis analysis [40]. We systematically evaluated and confirmed the prognostic value of HIF1A in LUAD, but not in LUSC, through leveraging data mining and analyses across diverse databases. Our findings revealed that metastasis, tumor progression, as well as patient gender and age, had limited effect on HIF1A upregulation in LUAD, while smoking displayed a significant influence on HIF1A expression, suggesting its potential involvement in stimulating cellular hypoxia-induced pathways. Interestingly, we observed a correlation between TP53 mutation and increased HIF1A expression, implying potential crosstalk between DNA repair, apoptosis, and hypoxia response in LUAD. Indeed, TP53 mutations have been linked to HIF1A overexpression in multiple scenarios, possibly due to the frequent exposure of cancer cells to hypoxia, which stimulates HIF1A expression [41–43].

The tumor environment and TILs dynamically regulate the progression of tumorigenesis [44,45]. As a coding gene of hypoxia-induced factor, HIF1A expression reflects the cellular response to the hypoxia microenvironment and may, in turn, shape the microenvironment and immune cell components [46–48]. To gain a full view of the effect of HIF1A on TILs, we investigated the correlation of mRNA expression, CNV, and promoter methylation with the infiltration of different immune cell types. Our analyses consistently revealed significant correlations, indicating increased infiltration of oncogenic immune cells such as Tregs, exhausted T cells, and Th1 cells, as well as decreased infiltration of inflammatory cells like Th17 cells and neutrophils. Additionally, immune subtype-specific expression analysis of HIF1A showed downregulation in type C3 (inflammatory subtype), which aligns with the infiltration analysis and suggests that the hypoxia environment suppresses inflammation in tumors, probably due to reduced levels of reactive oxygen species. Moreover, HIF1A displays significant co-expression with eight immunosuppressive checkpoint coding genes, indicating the relationship between tumor hypoxia and the immune state. This finding is consistent with the infiltration of

immunosuppressive cells. Nevertheless, further validation of these associations, especially through clinical and animal experiments, is essential to provide evidence about the effect of HIF1A on the shaping of tumor immunity in LUAD.

The enrichment of the HIF1A-interacting protein network suggested a strong relationship with hypoxia induction, angiogenesis, and anaerobic respiration activity. This finding aligns with HIF1A's well-known oxygen-related biology function and previous reports on its effect on metabolism related to NSCLC [49–51]. The association between HIF1A and glycolysis is further confirmed by its co-expression with all 32 glycolysis-related genes analyzed. m^6A mRNA modification has been widely studied for its involvement in LUAD development and regulation [36,52–54]. We identified a correlation between HIF1A expression and 20 m^6A modification-related genes, including the two erasers FTO and ALKBH5, as well as one writer, METTL14. Notably, five members of the m^6A YTF family readers also correlated with HIF1A expression. Thus, the influence of HIF1A upregulation on cellular m^6A modification and its consequence require further experimental validation, including assessments of the overall cellular level in LUAD and differences in the modification-expression relationship between LUAD and adjacent non-tumorous tissues.

HIF family transcription factors are essential in mediating the oncogenic effect of hypoxia in various cancers and promoting anti-cancer drug resistance, making them promising druggable targets for cancer treatment [55]. Consistently, our analysis uncovers a positive correlation between HIF1A expression and sensitivity of multiple chemotherapy drugs. Thus, targeting HIF1A with our screened chemicals alone or in conjunction with existing drugs may improve the efficacy of anti-LUAD treatments, although further functional verification of these chemicals is required.

It is worth noting that our bioinformatic analyses are based on limited sample numbers. Some of the marginal differences may become statistically evident by the inclusion of a larger cohort. In addition, these conclusions must be consolidated by *in vitro* cell biology assay and *in vivo* animal model experiments.

5. Conclusions

In summary, through comprehensive data mining and bioinformatic analyses, we characterized the prognostic and therapeutic value of HIF1A in LUAD patients with varying characteristics. We also explored the potential oncogenic mechanisms of HIF1A by investigating its association with immune cell infiltration, protein interaction networks, and co-expression with immune checkpoint coding genes, glycolysis genes, and m^6A-related genes. Overall, our results highlight the value of HIF1A as a diagnosis and prognosis marker for LUAD and underscore the potential of HIF1A-targeting therapies in the treatment of this lethal disease.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Publicly available datasets were analyzed in this study. This data can be found at https://portal.gdc.cancer.gov/, http://ualcan.path.uab.edu/, http://gepia.cancer-pku.cn/, http://bioinfo.life.hust.edu.cn/GSCA/, https://www.cbioportal.org/, http://www.linkedomics.org/, http://cis.hku.hk/TISIDB/, http://cis.hku.hk/TISIDB/, https://lce.biohpc.swmed.edu/lungcancer/, and http://genemania.org/. All data generated or analyzed during this study are included in this published article.

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CRediT authorship contribution statement

Zhimin Lu: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yanyu Bi:** Writing – review & editing, Validation, Investigation, Data curation. **Jialu Jiang:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Xuming Yao:** Writing – review & editing, Validation, Methodology, Investigation. **Guoxin Hou:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37739.

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