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Correlation analysis between gene IGF2BP3 and the poor prognosis and tumor immune infiltration of Adenocarcinoma of the lung

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Abstract: *Adenocarcinoma of the lung (LUAD) is a common subtype of lung cancer with a poor prognosis and high incidence worldwide. In recent years, increasing evidence has demonstrated that IGF2BP3 plays an important role in the initiation and progression of many types of human cancer. However, the mechanism of IGF2BP3 in LUAD is still unclear.So we performed pancancer analysis of IGFBP3 expression and prognosis using The Cancer Genome Atlas (TCGA) and UALCAN data. Then, we identified the microRNA and lncRNA that lead to the overexpression of IGFBP3 by using a series of in silico analyses, including expression analysis, correlation analysis and survival expression. Finally, we use TIMER to analyze the correlation of IGF2BP3 expression level with immune cell infiltrating level or immune checkpoint expression level in LUAD. Our study proved that IGF2PB3, upstream lncRNA, and VIRMA can impact m6A modification in LUAD, thus impacting prognosis, and the VIRMA /LINC00665 /has-let-7c-5p /IGF2BP3 axis is also related to tumor immune cells in LUAD. However, these results should be validated by much more clinical trials in future.*

Keywords: *m6A modification; lnc-RNA; LUAD; immune cell infiltrating*

1. Introduction

Adenocarcinoma of the lung has been proven to be a cancer with strong metastatic capacity, and some studies have found that metastasis of LUAD starts even in T1 stage [1]. LUAD is also a cancer with a high mortality rate [2]. Many patients lose the opportunity for surgery, and even after surgery, the prognosis of LUAD is also poor [3]. Recently, many studies have identified that LUAD always has a high EGFR mutation rate [4]. Some research has proven that immunotherapy can effectively relieve LUAD [5], but an increasing number of studies have found that LUAD is resistant to immunotherapy targeted drugs [6]. On the other hand, immunotherapy is unsuitable for patients with EGFR gene mutations. Immunotherapy needs more in-depth research in the future.

Metabolic reprogramming is meaningful in many cancers [7], and many studies have shown that m6A RNA methylation broadly participates in the metabolism of tumor cells [8]. m6A methylation mainly occurs on adenine in the "RRAH" sequence, and its function is determined by the "writer", "eraser" and "reader" [9, 10]. m6A methylation has been proven to be closely related to the initiation and progression of LUAD [11, 12]. Recently, some studies have shown that m6A methylation is closely associated with tumor immunity and that m6A-related genes participate in immune responses in cancer [13]. However, which immune cells are specifically regulated by m6A-related genes is not clear, and more analysis is needed for verification.

One of the most important m6A-related genes in cancer is the family of IGF2BP (14). The IGF2BP family can recognize mRNAs modified to promote cancer progression [15], and IGF2BP is also correlated with immunity [16, 17]. In this study, we first used expression analysis and survival analysis for the IGF2BP family in multiple types of cancer and chose the most significant gene in the IGF2BP family. We determined that IGF2BP3 is the key m6A-related gene in LUAD and IGF2BP3 can impact tumor immunity in LUAD. Second, we performed expression analysis and survival analysis of the upstream noncoding RNAs (ncRNAs) associated with the regulation of IGF2BP3, including microRNAs and long

ISSN 2706-6819 Vol.6, Issue 5: 55-66, DOI: 10.25236/IJFM.2024.060509

noncoding RNAs (lncRNAs). Finally, we found the upstream lncRNAs. In addition, we introduced the correlation of IGF2BP3 with immune cell infiltration and immune cell and immune checkpoints in LUAD. In summary, our study suggests that IGF2BP3 and its upstream ncRNAs and VIRMA are closely related to prognosis through m6A modification, and the axis can impact tumor immunity in LUAD.

2. Methods

2.1 TGCA data download, process, and analysis

The mRNA expression data of LUAD were downloaded from TCGA database(https://genome - cancer.ucsc.edu/), after which these data were normalized and then differential expression analysis was processed for IGF2BP family using R package limma [18]. P value <0.05 was considered as statistically significant.

2.2 GEPIA database analysis

GEPIA (http://gepia.cancer-pku.cn/) is a tool for cancer and normal gene expression profiling and interactive analyses based on TCGA and The Genotype-Tissue Expression (GTEx) data [19]. GEPIA was used to determine IGF2BP3 and lncRNA expression in LUAD. P value < 0.05 was considered as statistically significant. GEPIA was also used to conduct survival analysis for IGF2BP3, lncRNA and microRNA. Log rank p value <0.05 was considered as statistically significant. In addition, expression correlation of IGF2BP3 with lncRNA and immune checkpoints or expression correlation of microRNA with IGF2BP3 was evaluated using GEPIA database. |R|>0.1 and p value < 0.05 were set as selection criteria for identifying as statistically significant.

2.3 UALCAN database analysis

UALCAN (UALCAN (uab.edu)) is another database for tumor and normal gene expression and survival analysis [20]. We used it for further verify the expression of IGF2BP3, lncRNA and microRNA in LUAD and normal tissue. p value < 0.05 was considered as statistically significant. We also used this to conduct analysis for IGF2BP3, LINC00655 and VIRMA. p value < 0.05 was considered as statistically significant.

2.4 RM2target database analysis

RM2target (m6a2target.canceromics.org) is a database for basic information of target genes. Upstream binding lncRNAs and miRNAs of IGF2BP3 were predicted by RM2target gene prediction. programs. And the database also used to determine the role of IGF2BP3 and VIRMA in m6A modification.

2.5 Starbase database analysis

StarBase (http://starbase.sysu.edu.cn/) is a database for exploring miRNA-related studies [21]. starBase was used to perform expression correlation analysis for miRNA-IGF2BP3, lncRNA-has-let-7c-5p and lncRNA-IGF2BP3 in LUAD. The expression level of has-let-7c-5p in LUAD and normal tissues was also analyzed by starBase. In addition, starBase was used to predict candidate lncRNAs that could potentially bind to miRNA.

2.6 SRAMP database analysis

SRAMP (www.cuilanb.cn/sramp) is a useful tool to predict m6A modification sites on the RNA sequences of interests [22]. We used SRAMP database to predict the modification points on LINC00655.

2.7 TIMER database analysis

TIMER(https://cistrome.shinyapps.io/timer/) is a web server for analysis of tumor-infiltrating immune cells [23]. We use TIMER to analyze the correlation of IGF2BP3 expression level with immune cell infiltrating level or immune checkpoint expression level in LUAD. p value <0.05 was considered as statistically significant.

ISSN 2706-6819 Vol.6, Issue 5: 55-66, DOI: 10.25236/IJFM.2024.060509

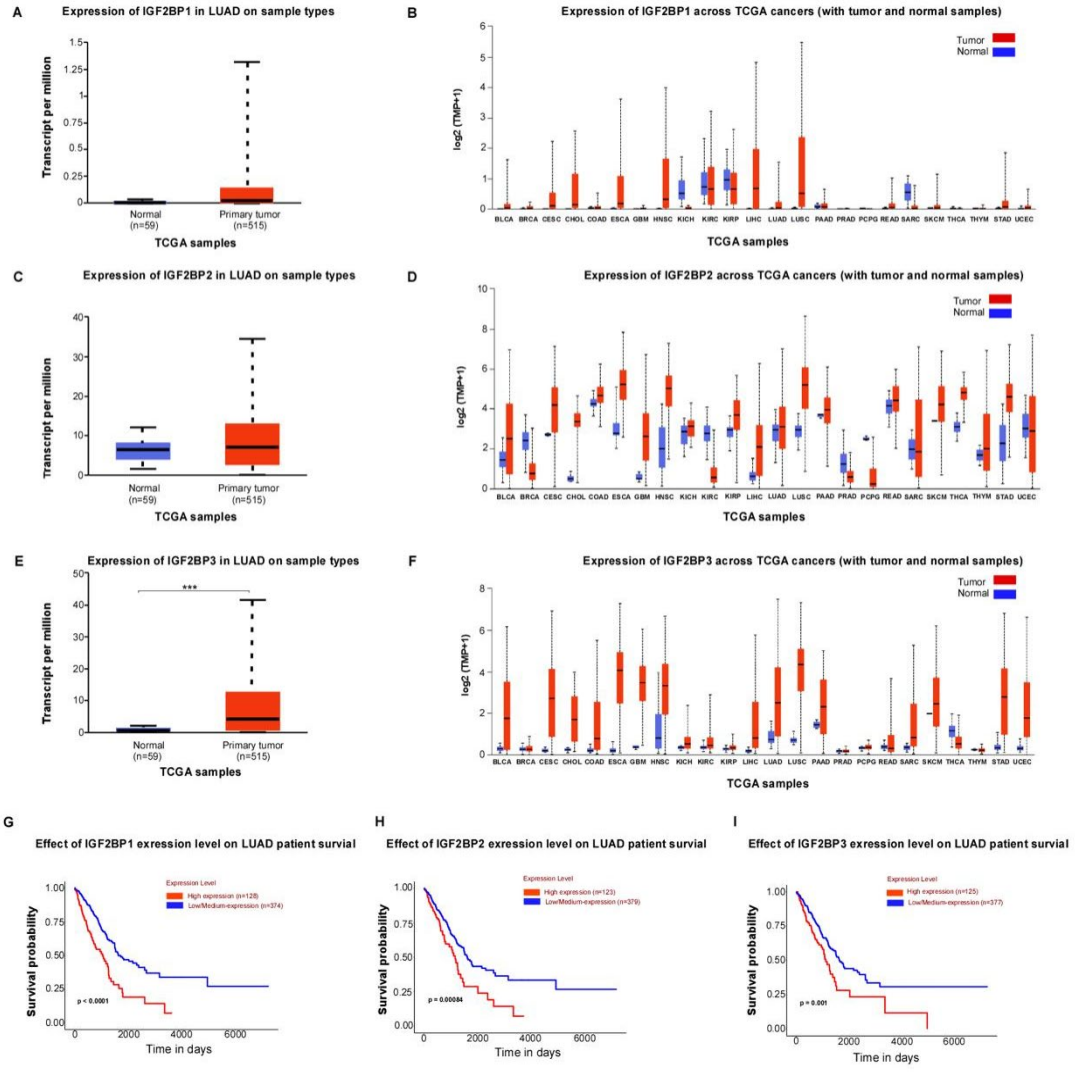
2.8 Statistical analysis

The statistical analysis in this study was automatically calculated by the online database mentioned above p value<0.05 or log rank p value<0.05 was considered as statistically significant.

3. Results

3.1 Pancancer analysis of IGF2BP expression

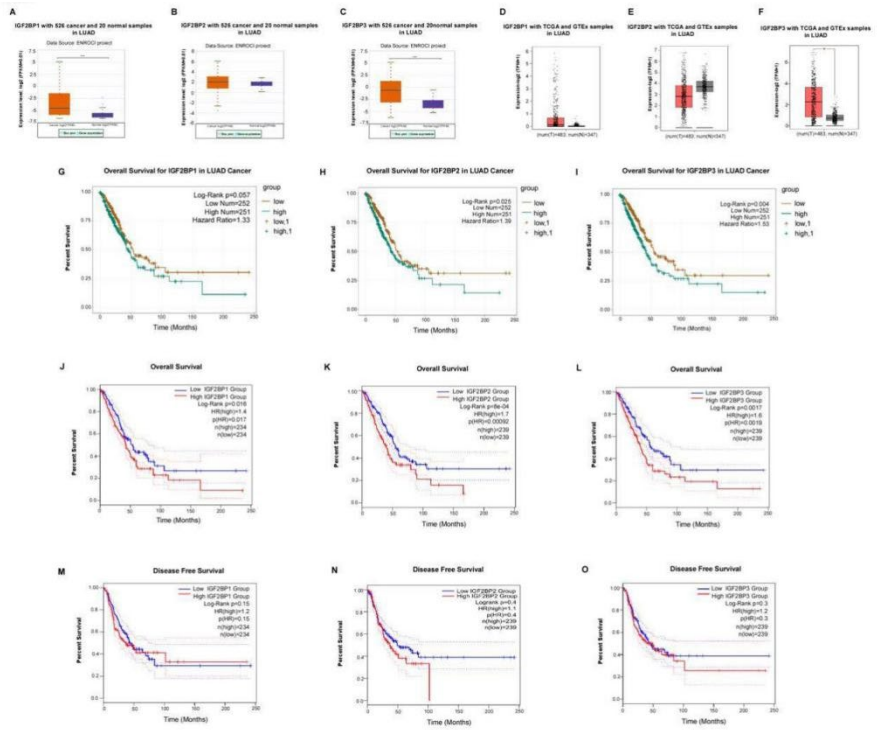
To choose the important gene in the IGF2BP family in patients with LUAD, we first analyzed the expression of the three most common genes in the IGF2BP family, namely, IGF2BP1, IGF2BP2 and IGF2BP3. As shown in Figure 1 A-F and Figure 2 A-F, compared to IGF2BP1 or IGF2BP2, IGF2BP3 was significantly upregulated in LUAD compared to normal samples. Further comparing these three genes of the IGF2BP family, we found that the expression of IGF2BP3 was more significantly different between normal samples and many cancers, including BLCA, CESC, CHOL, COAD, ESCA, GBM, HNSC, KICH, LIHC LUSC, PAAD SARC, SKCM, STAD and UCEC. We determined that IGF2BP3 could be the key gene of the IGF2BP family in patients with LUAD.



*(A, B): The expression of IGF2BP1 in 24 types of cancer. (C, D): The expression of. IGF2B2 in 24 types of cancer. (E, F): The expression of IGF2BP3 in 24 types of cancers. (G-I): Survival analysis of IGF2BP1, IGF2BP2 and IGF2BP3. Processed by TCGA and UALCAN database. *p value < 0.05; **p value < 0.01; ***p value < 0.001.*

Figure 1: Expression and survival analysis of IGF2BP1, 2, and 2 in 24 types of cancers. identified IGF2BP3 as an m6A-related gene in LUAD.

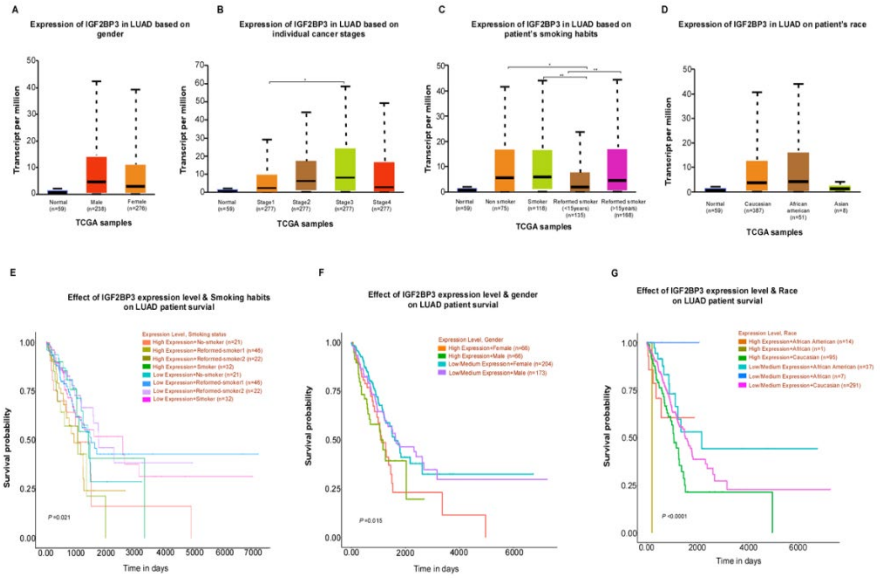
ISSN 2706-6819 Vol.6, Issue 5: 55-66, DOI: 10.25236/IJFM.2024.060509



*(A, D): The expression of IGF2BP1 in LUAD. (B, E): The expression of IGF2BP2 in LUAD. (C, F): The expression of IGF2BP3 in LUAD. (G, J, M): The overall and disease survival of IGF2BP1. (H, K, N): The overall and disease survival of IGF2BP2. (I, L, O): The overall and disease survival of IGF2BP3. *p value < 0.05; **p value < 0.01; ***p value < 0.001.*

Figure 2: Expression and survival analysis of IGF2BP1, 2, and 3 in LUAD

3.2 Analysis of the prognostic value of IGF2BP in LUAD



*(A): The expression of IGF2BP3 in LUAD based on sex. (B): The expression of IGF2BP3 in LUAD based on different cancer stages. (C): The expression of IGF2BP3 in LUAD based on different smoking habits. (D): The expression of IGF2BP3 in LUAD based on different races. (E-G): Survival analysis of IGF2BP3 in LUAD based on different smoking habits, sex and race. *p value < 0.05; **p value < 0.01; ***p value < 0.001.*

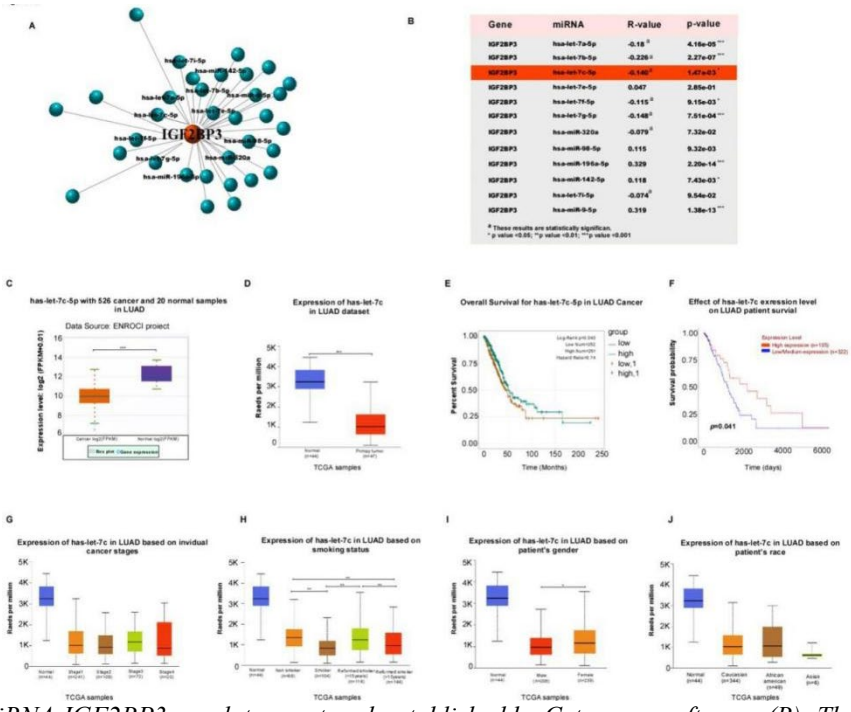
Figure 3: The expression and survival analysis of IGF2BP3 in LUAD based on sex, cancer stage, smoking habits and race.

ISSN 2706-6819 Vol.6, Issue 5: 55-66, DOI: 10.25236/IJFM.2024.060509

Next, we performed survival analysis for the IGF2BP family in LUAD. According to overall survival (OS), high expression of IGF2BP1, IGF2BP2 and IGF2BP3 in LUAD was associated with poor prognosis, and by comparison, IGF2BP3 had the most significant difference. (Figure 1 G-I and Figure 2 G-O) We further analyzed the expression of IGF2BP3 in patients with LUAD based on different sexes, individual cancer stages, smoking habits and races. We found that for sex, the expression of IGF2BP3 in male patients was higher than that in female patients. For cancer stages, the expression of IGF2BP3 was highest in patients with LUAD in stage 3. For race, the expression of IGF2BP3 was significantly higher in Caucasian and African patients, but smoking did not affect the expression of IGF2BP3 in patients with LUAD. (Figure 3 A-D) We also analyzed the prognostic value based on low or high expression levels of IGF2BP3 under different smoking habits, sexes and races. We found that female patients with high expression of IGF2BP3 and Caucasian patients with high expression of IGF2BP3 had the poorest prognosis. (Figure 3 E-G) Combining the expression and survival analyses, we deduced that IGF2BP3 may function as a key regulator in the carcinogenesis of LUAD.

3.3 Prediction and analysis of upstream miRNAs of IGF2BP3

ncRNAs are responsible for the regulation of gene expression [24]. To determine which ncRNAs can modulate IGF2BP3, we first predicted upstream miRNAs that could be related to IGF2BP3. We found 12 miRNAs and used Cytoscape software to establish a miRNA-IGF2BP3 regulatory network, as shown in Figure 4 A. According to the mechanism of action of miRNA in the regulation of target gene expression [25], we found a negative correlation between miRNA and IGF2BP3. Therefore, we performed expression correlation analysis, and the results are listed in Figure 4 B. We found that hsa-let-7c-5p was significantly negatively correlated with IGF2BP3 in LUAD. The other remaining miRNAs had no statistical expression relationship with IGF2BP3. Next, we determined the expression and prognostic analysis of hsa-let-7c-5p in LUAD. As shown in Figure 4 C-F, hsa-let-7c-5p was significantly downregulated in LUAD, and the downregulation of hsa-let-7c-5p caused poor prognosis in patients with LUAD. Finally, we also found that the expression of hsa-let-7c-5p was related to smoking and sex. The expression of hsa-let-7c-5p was upregulated in male patients and patients who smoke, but the expression levels in different cancer stages and different races were not significantly different (Figure 4 G-J). All the findings proved that hsa-let-7c-5p might be the potential regulatory miRNA of IGF2BP3 in LUAD.



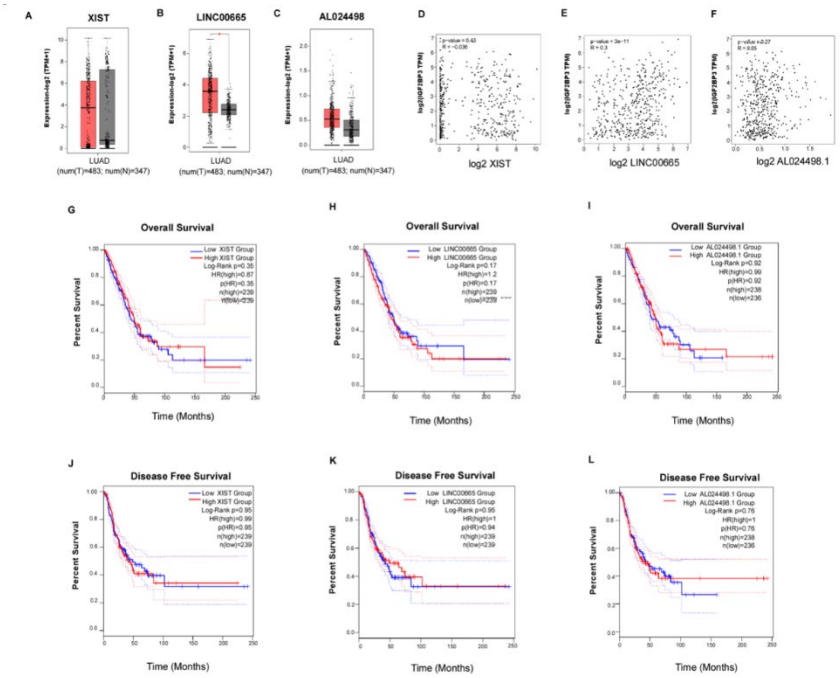
*(A): The miRNA-IGF2BP3 regulatory network established by Cytoscape software. (B): The expression correlation between predicted miRNAs and IGF2BP3 in HCC analyzed by the starBase database. (C, D): The expression of hsa-let-7c-5p in LUAD and normal samples. (E, F): Survival analysis of hsa-let-7c-5p in LUAD. (G-J): The expression of hsa-let-7c-5p under different cancer stages, smoking habits, gender and race in LUAD. *p value < 0.05; **p value < 0.01; ***p value < 0.001.*

Figure 4: Identification of hsa-let-7c-5p as a potential upstream miRNA of IGF2BP3 in LUAD

ISSN 2706-6819 Vol.6, Issue 5: 55-66, DOI: 10.25236/IJFM.2024.060509

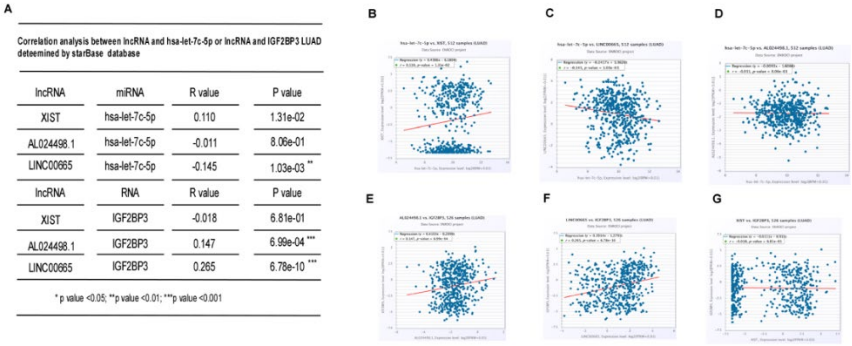
3.4 Prediction and analysis of lncRNA upstream of hsa-let-7c-5p

Then, we used the starBase database to predict the upstream lncRNA hsa-let-7c-5p, and we predicted a series of possible lncRNAs. Next, we used TCGA and GTEx to determine the expression levels of these lncRNAs in LUAD. We found that LINC00665 had the most significant difference between patients with LUAD and normal people, and the expression level of LINC00655 was upregulated in patients with LUAD (Figure 5 A-C). Subsequently, we analyzed the correlation between lncRNAs and IGF2BP3 by using starBase and found that LINC00665 was most significantly correlated with IGF2BP3 and was positively correlated with IGF2BP3 (Figure 5 D-F, Figure 6). Finally, we analyzed the prognostic values of the lncRNAs in LUAD, and there was no statistical significance between the expression level of lncRNAs and the prognosis of patients with LUAD (Figure 5 G-L). The competing endogenous RNA (ceRNA) hypothesis indicates that lncRNAs can increase mRNA expression by competitively binding to shared miRNAs [26]. There will be a negative correlation between lncRNAs and miRNAs but a positive correlation between lncRNAs and mRNAs. Combining the above series of analyses, we considered LINC00655 to be the most likely upstream lncRNA of the hsa-let-7c-5p/IGF2BP3 axis in LUAD.



*(A-C): The expression of XIST, LINC00655 and AL024498 compared with normal samples. (D-F): Correlation analysis between IGF2BP3 and XIST, LINC00655 and AL024498 in LUAD. (G-L): Overall and disease analysis of XIST, LINC00655 and AL024498 in LUAD. *p value < 0.05; **p value < 0.01; ***p value < 0.001.*

Figure 5: Expression survival and correlation analysis for lncRNA upstream of hsa-let-7c-5p in LUAD



Correlation analysis between lncRNA and hsa-let-7c-5p or lncRNA and IGF2BP3 LUAD deteemined by starBase database

lncRNA	miRNA	R value	P value
XIST	hsa-let-7c-5p	0.110	1.31e-02
AL024498.1	hsa-let-7c-5p	-0.011	8.06e-01
LINC00665	hsa-let-7c-5p	-0.145	1.03e-03 **
lncRNA	RNA	R value	P value
XIST	IGF2BP3	-0.018	6.81e-01
AL024498.1	IGF2BP3	0.147	6.99e-04 ***
LINC00665	IGF2BP3	0.265	6.78e-10 ***

* p value <0.05; **p value <0.01; ***p value <0.001

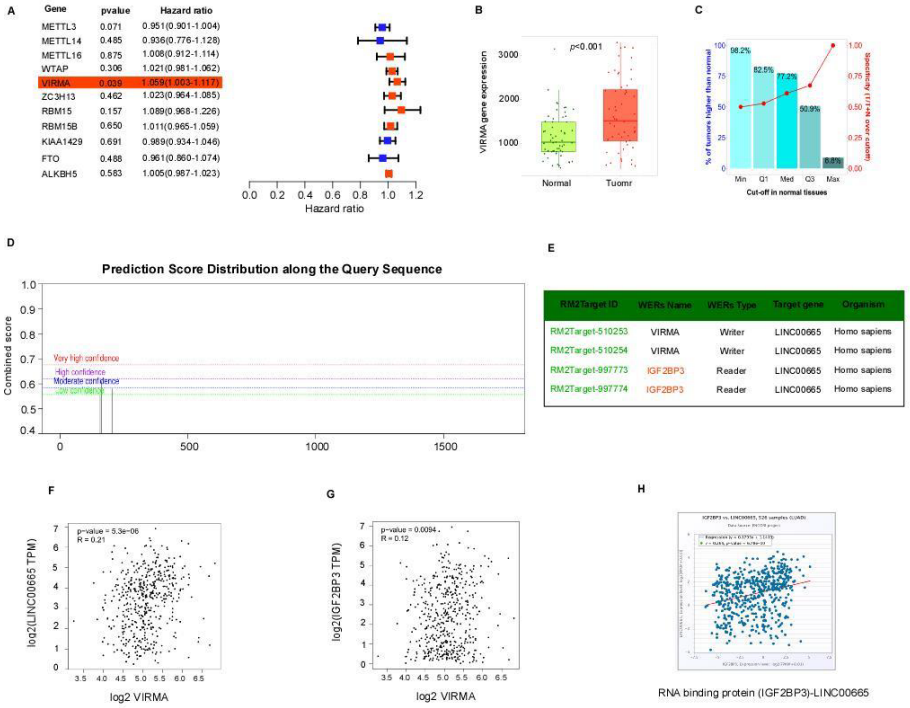
*(A, B, C, D) Correlation analysis between XIST, AL024498.1, LINC00655 and hsa-let-7c-5p. (A, E, F, G): Correlation analysis between XIST, AL024498.1, LINC00655 and IGF2BP3. *p value < 0.05; **p value < 0.01; ***p value < 0.001.*

Figure 6: Correlation analysis between lncRNA and miRNA or lncRNA and IGF2BP3 in LUAD

ISSN 2706-6819 Vol.6, Issue 5: 55-66, DOI: 10.25236/IJFM.2024.060509

3.5 Prediction and analysis of the regulatory factor upstream of LINC00655

The upstream regulatory factor of the lncRNA plays a key role in the m6A modification [27]. To determine the regulatory factor of lncRNAs, we used the SRAMP database to predict the modification points on LINC00655 and found 11 potential genes that might be the upstream "writer" or "eraser" of LINC00655. After the analysis of hazard ratio and expression, we found the VIRNA is statistically significant with the prognosis of LUAD, so we determined that VIRMA could be the most significant key regulatory factor upstream of LINC00633 (Figure 7 A-C). Next, we predicted the sequence distribution of LINC00655, and we were surprised to find that VIRMA was the "writer" of LINC00655 and IGF2BP3 was the "reader" of LINC00655 (Figure 7 D-E). Through correlation analysis, we found that VIRMA was significantly positively correlated with LINCOO655 and IGF2BP3 (Figure 7 F-G). IGF2BP3 was also significantly positively correlated with LINC00655 (Figure 7 H). Finally, based on these results, we determined that VIRMA might be the key "writer" of LINC00655, regulate m6A modification and increase the expression of LINC00655 in LUAD. IGF2BP3 can also increase the expression of LINC00655 in turn, forming positive feedback.



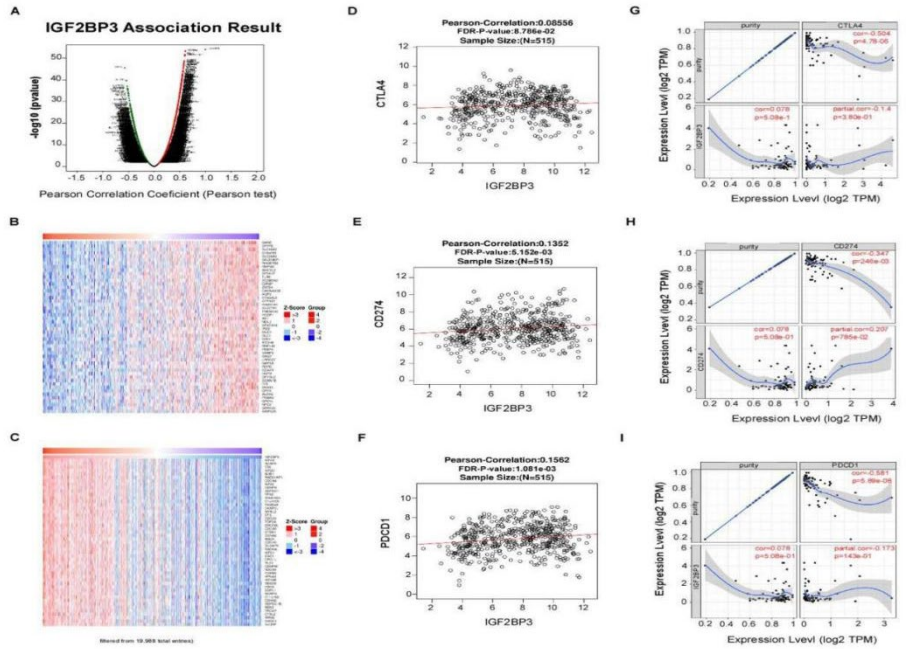
*(A): Hazard ratio analysis of potential upstream "writers" or "erasers" of. LINC00655. (B, C): Expression analysis of VIRMA in LUAD. (D, E): The prediction of the "reader" and "writer" of LINC00655. (F-H): Correlation analysis between VIRMA and LINCOO655, VIRMA and IGF2BP3, IGF2BP3 and LINC00655. *p value < 0.05; **p value < 0.01; ***p value < 0.001.*

Figure 7: Identification of VIRMA as the upstream regulatory factor of LINC00655 and identification of the "reader" and "writer" of m6A modification in LUAD.

3.6 Correlation analysis between IGF2BP3 and immune checkpoints in LUAD

The IGF2BP family not only participates in multiple metabolic processes through m6A modification but is also involved in many biological processes, including the immune response [28]. Many studies have shown that PD1, PD-L1 and CTLA4 are the key immune checkpoints, and they are responsible for tumor immune escape. To verify that IGF2BP3 is a potential oncogene in LUAD, we analyzed the correlation of IGF2BP3 with all 19988 immune checkpoints by linkedmics.org (Figure 8 A-C). We found that IGF2BP3 was most significantly positively correlated with PD1 (PDCD1) and PD-L1 (CD274) in LUAD. (Figure 8 D-F) Moreover, by using TIMER data analysis, as shown in Figure 8 G-I, we also found that PD1, PD-L1 and CTLA-4 had significantly positive correlations with IGF2BP3. The two results indicate that tumor immune escape might participate in VIRMA/LINC00655/ has-let-7c-5p/IGF2BP3 axis-related m6A modification-mediated carcinogenesis of LUAD.

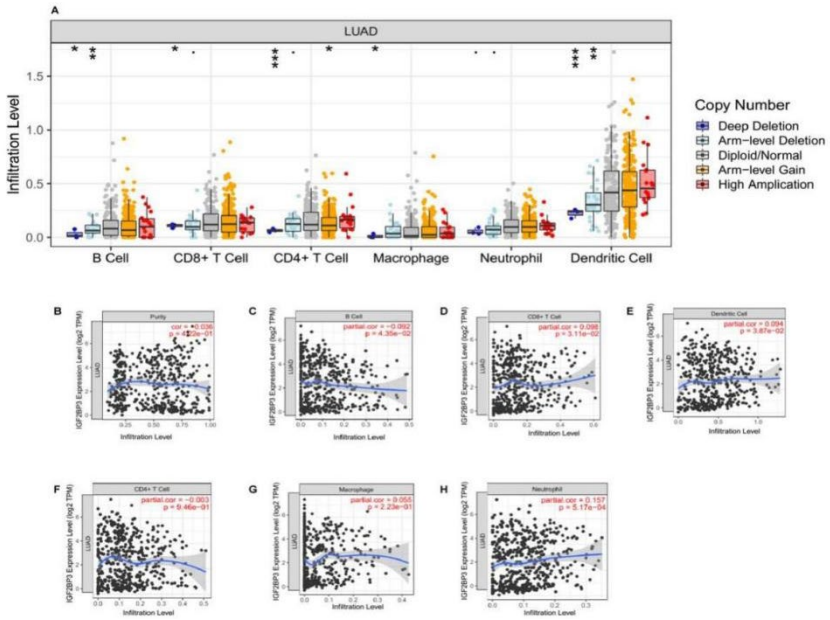
ISSN 2706-6819 Vol.6, Issue 5: 55-66, DOI: 10.25236/IJFM.2024.060509



*(A- C): Correlation analysis of 19988 immune checkpoints with IGF2BP3 form a database. (D): The expression correlation of IGF2BP3 with PD1 in LUAD determined by the GEPIA database. (E): The expression correlation of IGF2BP3 with PD-L1 in LUAD determined by the GEPIA database. (F): The expression correlation of IGF2BP3 with CTLA-4 in LUAD determined by the GEPIA database. (G): Spearman correlation of IGF2BP3 with the expression of PD-1 in LUAD adjusted by purity using TIMER. (H): Spearman correlation of IGF2BP3 with the expression of PD-L1 in LUAD adjusted by purity using TIMER. (I): Spearman correlation of IGF2BP3 with the expression of CTLA-4 in LUAD adjusted by purity using TIMER. *p value < 0.05; **p value < 0.01; ***p value < 0.001.*

Figure 8: Correlation of IGF2BP3 expression with PD-1, PD-L1 and CTLA-4 expression in LUAD.

3.7 IGF2BP3 positively correlates with immune cell infiltration in LUAD



*(A): The infiltration level of 6 immune cells under different copy numbers of IGF2BP3 in HCC. (B–H): The correlation of IGF2BP3 expression level with purity (B), B-cell (C), CD8+ T-cell (D), dendritic cell (E), CD4+ T-cell (F), macrophage (G), or neutrophil (H) infiltration level in LUAD. *p value < 0.05; **p value < 0.01; ***p value < 0.001.*

Figure 9: The relationship of immune cell infiltration with IGF2BP3 levels in LUAD.

ISSN 2706-6819 Vol.6, Issue 5: 55-66, DOI: 10.25236/IJFM.2024.060509

We also predicted that IGF2BP3 is closely correlated with immune cell infiltration in LUAD. As listed in Figure 7A, we found a significant change in some immune cell infiltration levels in LUAD under some copy number of IGF2BP3. For example, we found that under deep deletion of IGF2BP3 in B cells, CD8+ T cells, CD4+ T cells, macrophages and dendritic cells, the cell infiltration level was reduced significantly. In addition, cell infiltration under arm-level deletion of IGF2BP3 copy number was observed in B cells and dendritic cells. The infiltration of CD4+ T cells under arm-level gain-of-copy-number IGF2BP3 was also significantly reduced (Figure 9 A). Other key clues that can prove the close relationship between IGF2BP3 and immune cells appeared when we performed correlation analysis between them, as shown in Figure 9 B-H. By correlation analysis, we found that IGF2BP3 was significantly negatively associated with B cells and CD4+ T cells in LUAD, but it was significantly positively associated with CD8+ T cells, macrophages and neutrophils in LUAD.

4. Discussion

Today, LUAD is still notorious for its poor prognosis, and many patients lose treatment opportunities due to early metastasis. Exposing the molecular mechanism of LUAD carcinogenesis could be an important way to find new effective therapeutic targets. Increasing evidence has demonstrated that IGF2BP3 plays important roles in the initiation and progression of LUAD through m6A modification and can also impact tumor immunity. However, knowledge of IGF2BP3 in LUAD is still inadequate, and more studies are needed.

In this study, we first used The Cancer Genome Atlas (TCGA) and GEPIA databases to perform pancancer analysis of the m6A-related gene family of IGF2BP expression. Next, survival analysis was performed. Combining the results, we identified IGF2BP3 as the key gene in LUAD, and we also proved that IGF2BP3 was expressed at higher levels than in normal tissues and that high expression of IGF2BP3 can cause poor prognosis in patients with LUAD. According to another previous study [29], with our results, we determined that IGF2BP3 is the oncogene of LUAD. ncRNAs, including miRNAs and lncRNAs, participate in the regulation of gene expression through the ceRNA mechanism [21, 29, 30]. To identify the upstream regulatory miRNAs of IGF2BP3, we processed prediction programs, including RNA22, miRmap, microT, and others programs, to determine which miRNAs can potentially bind to IGF2BP3. Finally, we obtained 13 miRNAs, most of which act as tumor-suppressive miRNAs in LUAD. Through correlation analysis, expression analysis, and survival analysis, we selected hsa-let-7c-5p as the most likely upstream miRNA of IGF2BP3. Another study also agrees that hsa-let-7c-5p plays a key role in cancer [31]. According to the ceRNA hypothesis [32]. The potential lncRNAs hsa-let-7c-5p and IGF2BP3 should be oncogenic lncRNAs in LUAD. We predicted upstream lncRNAs of the hsa-let-7c-5p/IGF2BP3 axis. First, we found a series of possible lncRNAs, including XIST, LINC00665 and AL024498. Next, we performed expression analysis, correlation analysis, and survival analysis. Finally, combined with other reports [33, 34], we identified LINC00655 as an oncogene in LUAD. Finally, we identified LINC00655/hsa-let-7c-5p/IGF2BP3 as potential m6A-related regulatory pathways in LUAD.

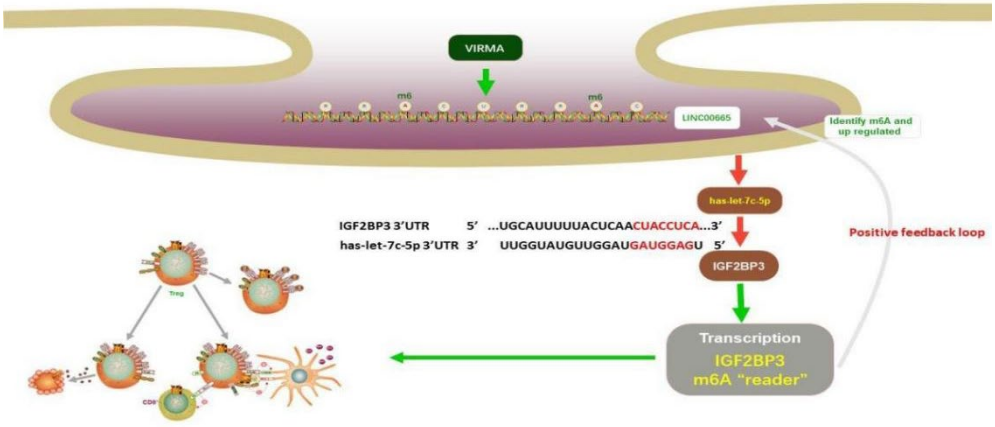


Figure 10: The model of the m6A modification pathway: VIRMA/LINC00655/has-let-7c-5p/IGF2BP3 axis in carcinogenesis in LUAD.

The upstream regulatory factor of lncRNAs might be a "writer" or "eraser" of the m6A modification[35]. We first analyzed the database and chose 11 potential upstream "writer" or "eraser" genes of the lncRNAs. Next, we performed expression analysis, correlation analysis and survival analysis.

ISSN 2706-6819 Vol.6, Issue 5: 55-66, DOI: 10.25236/IJFM.2024.060509

VIRMA was chosen as the most significant potential upstream writer gene of LINC00655, and it was positively related to LINC00655. Meanwhile, we were surprised to find IGF2BP3 as the "reader" of LINC00655. According to the study by Wen Ni[36], we know that the "reader" gene of lncRNAs can regulate the expression of lncRNAs. After the correlation analysis between IGF2BP3 and LINC00655, we found that IGF2BP3 was positively correlated with LINC00655, and the m6A modification regulatory pathway can form a positive feedback regulation mechanism. Finally, we determined the m6A modification mechanism of LUAD, which we named the VIRMA/LINC00655/has-let-7c-5p/IGF2BP3 axis, as shown in Figure 10.

Many studies have confirmed that the efficacy of immunotherapy depends on the sufficient expression of immune checkpoints [37]. Thus, we processed the relationship between IGF2BP3 and immune checkpoints. The results demonstrated that high expression of IGF2BP3 was closely related to PD1, PD-L1 and CTLA-4 in LUAD. Tumor immune cell infiltration could also influence the efficacies of chemotherapy and immunotherapy in patients [38-40]. Our study suggested that IGF2BP3 was significantly positively correlated with immune cells, including B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells, in LUAD. These findings indicated that the m6A modification pathway VIRMA/LINC00655/has-let-7c-5p/IGF2BP3 axis might impact tumor immunity and that targeting IGF2BP3 might increase the efficacy of immunotherapy in LUAD.

5. Conclusions

We found that IGF2BP3 was an m6A-related gene that was highly expressed in LUAD and positively correlated with poor prognosis in LUAD. We identified an upstream m6A modification regulatory mechanism of IGF2BP3 in LUAD, namely, the VIRMA/LINC00655/has-let-7c-5p/ IGF2BP3 axis. Furthermore, our findings also indicated that the m6A modification pathway might exert its action by increasing tumor immune cell infiltration and immune checkpoint expression. However, these results should be proven by more basic experiments and large clinical trials in the future.

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