



The Regulators Associated With N6-Methyladenosine in Lung Adenocarcinoma and Lung Squamous Cell Carcinoma Reveal New Clinical and Prognostic Markers

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N6-methyladenosine (m⁶A) methylation is of significant importance in the initiation and progression of tumors, but how specific genes take effect in different lung cancers still needs to be explored. The aim of this study is to analyze the correlation between the m⁶A RNA methylation regulators and the occurrence and development of lung cancer. The data of lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) were obtained through the TCGA database. We systematically analyzed the related pathological characteristics and prognostic factors by applying univariate and multivariate Cox regression, as well as LASSO Cox regression. Some of 23 m⁶A regulators are identified as having high expression in lung cancer. In addition, risk score has been shown to be an independent prognostic factor in lung cancer. Our research not only fully reveals that m⁶A regulators and clinical pathological characteristics are potentially useful with respect to survival and prognosis in different lung tumors but also can lay a theoretical root for the treatment for lung cancer—notably, to point out a new direction for the development of treatment.

Keywords: lung cancer, N6-methyladenosine, prognosis, regulators, risk scores

INTRODUCTION

M⁶A methylation, as a dynamic reversible process, is regulated by three enzymes: demethylase (erasers), function manager (readers), and methyltransferase complex (writers) (Yang et al., 2018; Zhou et al., 2020). Erasers include FTO and ALKBH5 (Li et al., 2017); readers include YTHDC1, YTHDC2, YTHDF1, YTHDF2, and HNRNPC (Zhang et al., 2019; He et al., 2020); and writers include MTETL3, METTL14, WTAP, KIAA1429, RBM15, and ZC3H13 (Tuncel and Kalkan, 2019; Asada et al., 2020). It is these m⁶A methylation regulators that are closely correlated with different human diseases, especially with cancer (Wang et al., 2018). A growing majority of evidence

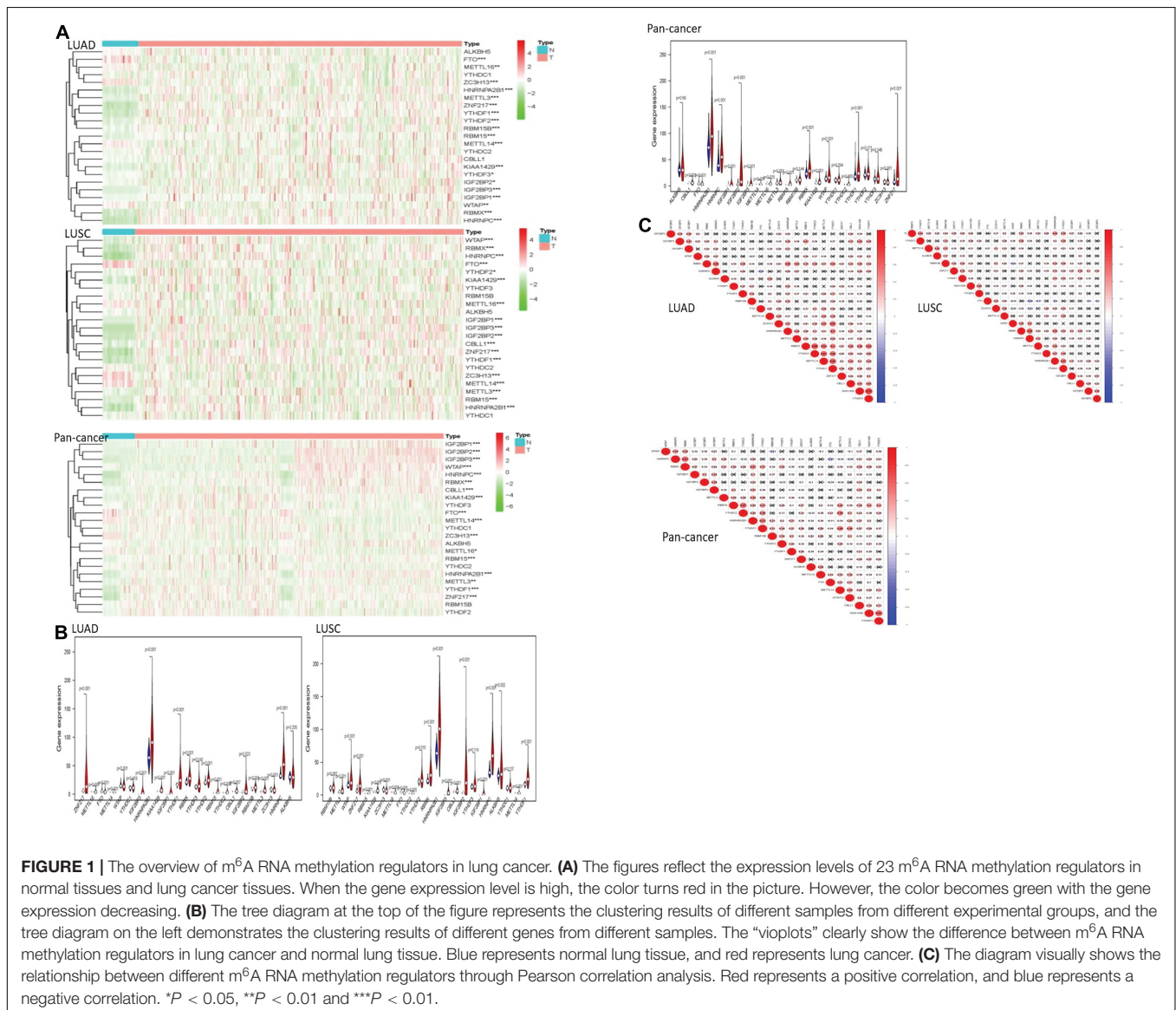
indicates that m⁶A has the capacity to exert a double effect in tumors. On the one hand, m⁶A regulates the expression of oncogenes and tumor suppressor genes, which act as promoting and suppressing cancer, respectively. On the other hand, the expression level of m⁶A and the expression and activity of m⁶A enzyme can be regulated to affect the function of m⁶A. Ultimately, they achieve the purpose of adjusting the differentiation of cancer stem cells and regulating T cell differentiation and immune homeostasis.

In the LUAD, the increased expression of METTL3 can enhance the translation of oncogene BRD4 by forming an mRNA ring with EIF3. With that, METTL3 accelerates the translation of oncogene by promoting ribosome recycling, thereby providing an easier way for the invasion and metastasis of lung cancer cells (Choe et al., 2018; Ni et al., 2021). The expression of FTO is elevated in the LUSC, and FTO inhibits cancer formation by inhibiting m⁶A methylation, which inhibits expression of

the oncogene MZF1 eventually. Both m⁶A with high expression and MZF1 with deficient expression inhibit tumor development (Liu et al., 2018).

Generally speaking, patients with early treatment of lung cancer have a good prognosis, but most patients are diagnosed to be in the advanced stage of lung cancer, whose prognosis is poor (Wozniak, 2012; Zhu et al., 2019). Nowadays, TNM staging is still the dominant method to analyze the prognosis of lung cancer, although there is a certain degree of error and lack of individuality (Wozniak and Schwartz, 2018). Our preoccupation is searching for further accurate prognostic factors from the point of view of gene and molecule, for the sake of making accurate prognostic analysis and the individualized treatment selection.

In this article, we acquired the information of patients with lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC), respectively. Pan-cancer data was the total of LUAD and LUSC data. The expression of m⁶A methylation regulators



was analyzed to shed light on if it was related to LUAD, LUSC, and pan-cancer. Especially significant, based on these data, we try to find out the clinical correlation between m⁶A methylation regulators and lung cancer (Li et al., 2021).

RESULTS

The Overview of m⁶A RNA Methylation Regulators in Lung Cancer

We drew a heatmap of LUAD, LUSC, and pan-cancer data after diff analysis firstly. Various gene expression levels were displayed as follows (Figure 1).

Most of the m⁶A RNA methylation regulators showed a weak to moderate positive correlation. KIAA1429 and YTHDF3 were the most correlated in LUAD. In LUSC, METTL3 and RBMX had the most obvious positive correlation. The negative correlation between HNRNPC gene and FTO gene was the most recognizable. In pan-cancer, KIAA1429 and YTHDF3 were

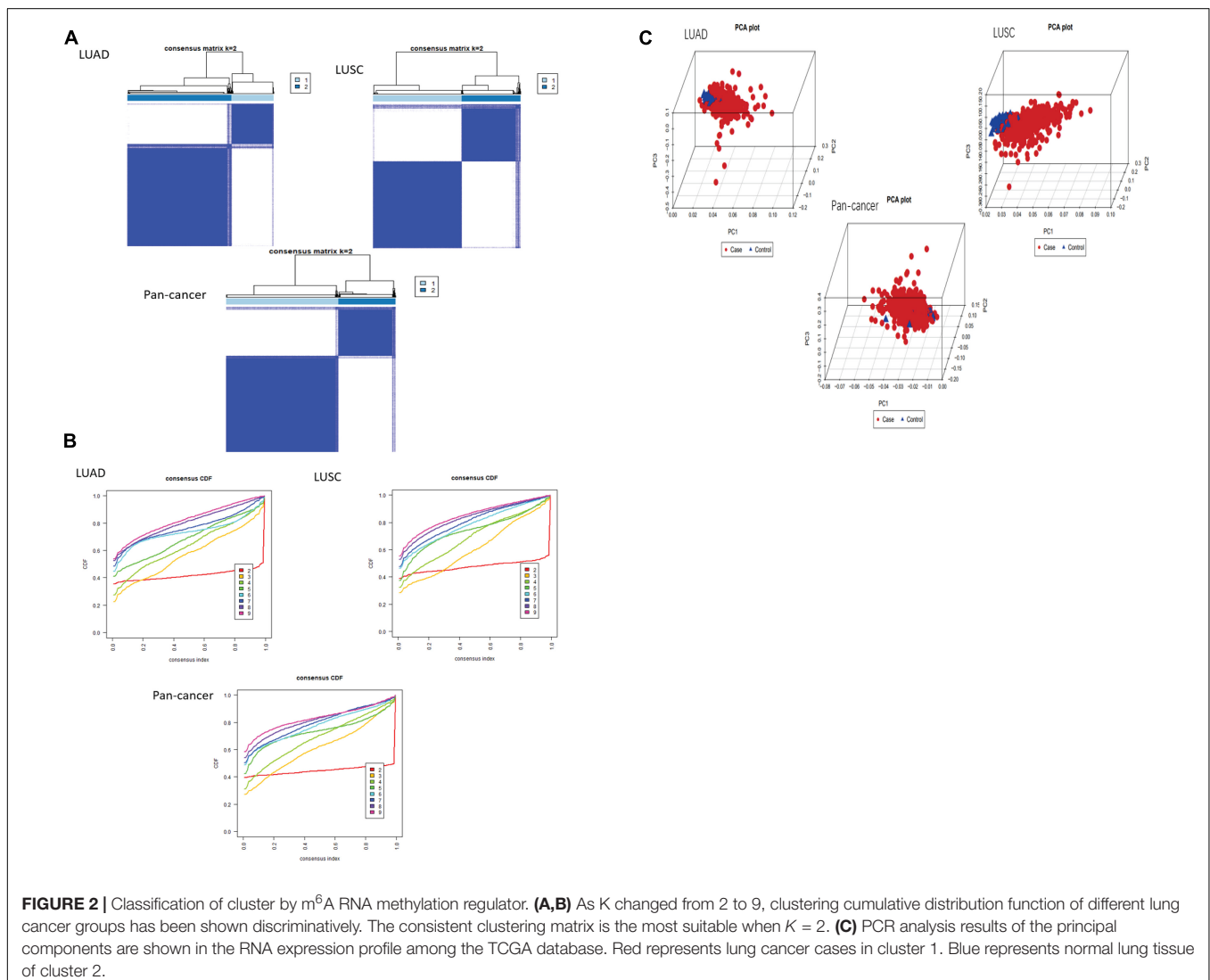
the most relevant. HNRNPC and FTO were the most obvious negative correlation.

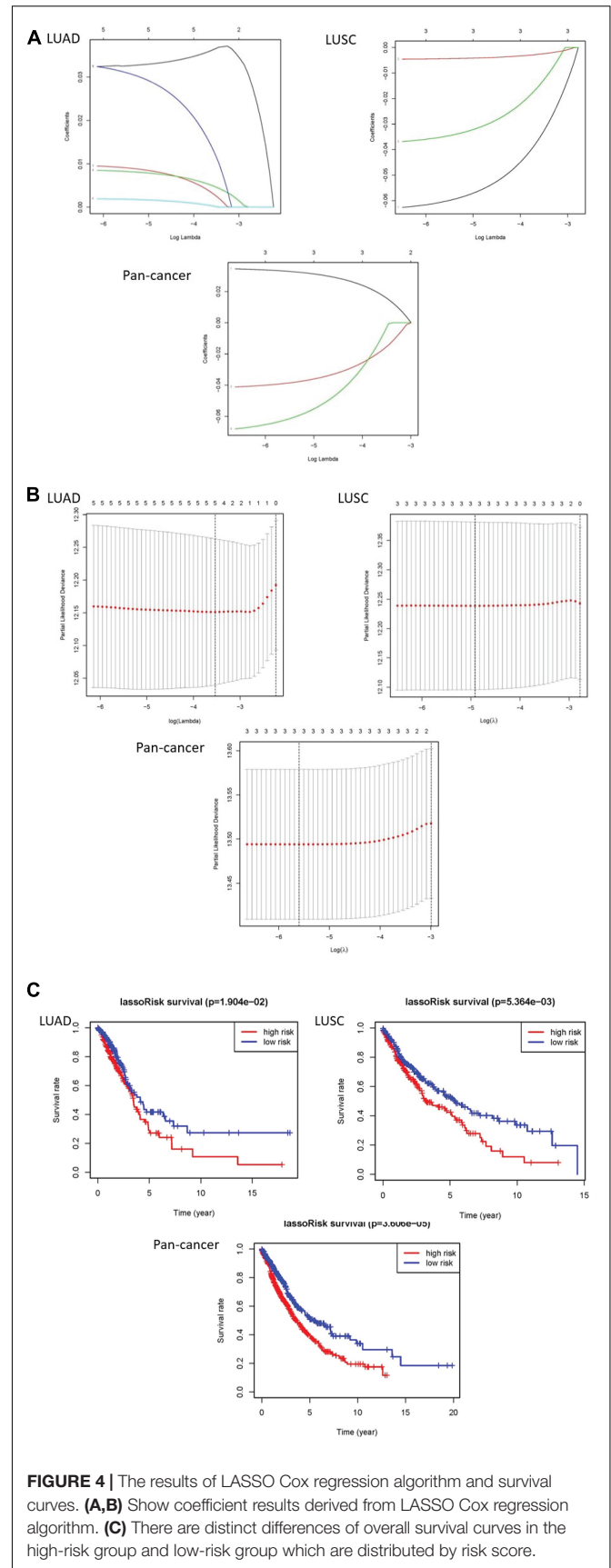
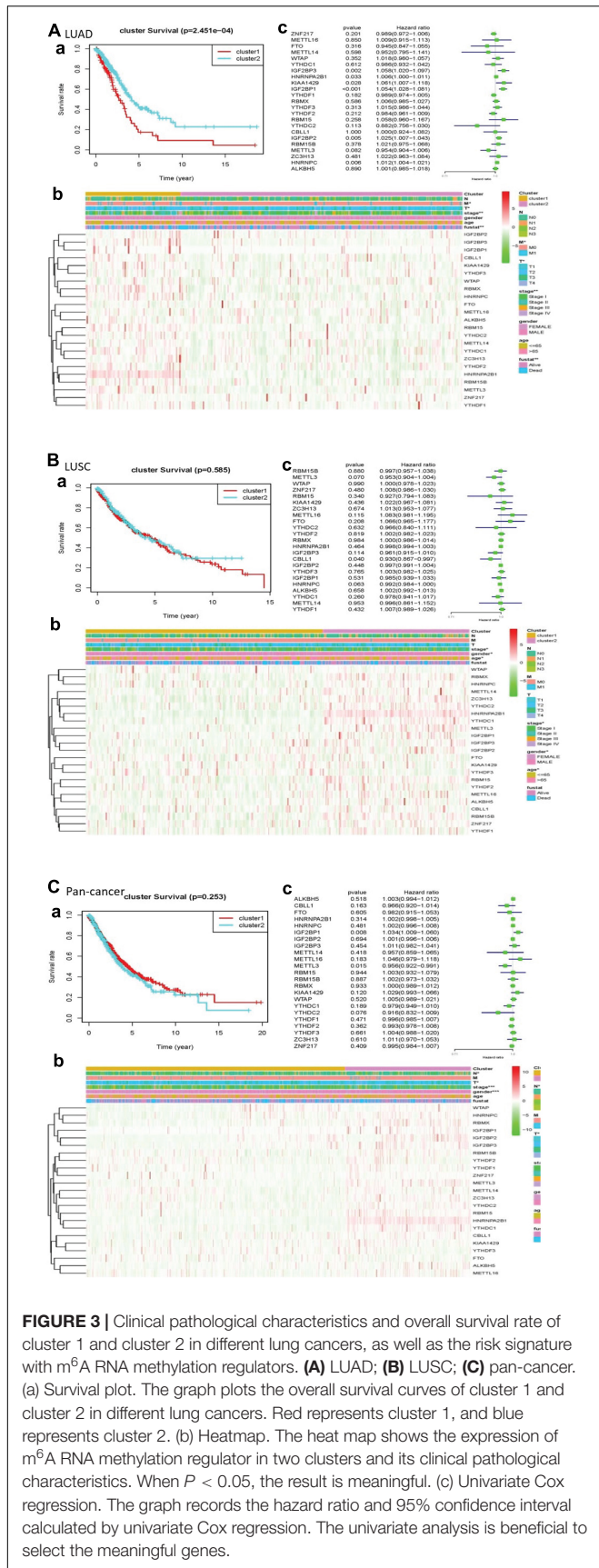
Consensus Clustering of m⁶A RNA Methylation Regulators Identified Two Clusters of Lung Cancer

After using “ConsensusClusterPlus” package to group LUAD, LUSC, and pan-cancer, the CDF value was small and the lines were smooth when $k = 2$. Next, PCA was used to verify whether the classification was correct. It was proved that the classification of cluster 1 and cluster 2 meets the requirements (Figures 2A–C).

The Categories Determined by Consensus Clustering Are Closely Related to Clinical Results

In the cleaned LUAD data, the survival probability of cluster 2 was greater than that of cluster 1. In assessing the relationship between clustering and clinical pathological features, the number





of cluster 1 was significantly less than that of cluster 2. The result revealed that cluster 1 and cluster 2 had significant differences in M stage, T stage, total stage, and survival status. In LUSCs after washing, the difference in overall survival between the two clusters was not apparent. In the heatmap, the length of cluster 1 was slightly longer than that of cluster 2. There were differences in total stage, gender, and age between the two clusters. Of the cleaned pan-cancer, cluster 1 had a higher survival probability than cluster 2 when the patients had the same survival time. In the heatmap, the length of cluster 1 was also slightly longer than that of cluster 2, and there were differences in N stage, T stage, total stage, and gender (Figure 3).

Prognostic Values of Risk Signature and m⁶A RNA Methylation Regulators

We used univariate Cox regression to get that, with these dangerous genes named IGF2BP3, HNRNPA2B1, KIAA1429, IGF2BP1, IGF2BP2, and HNRNPC, patients hardly survived. In LUSC, CBL1 is a risk gene. In pan-cancer, IGF2BP1 was a risk gene, while METTL3 was a protective gene (Figure 3).

The least absolute shrinkage and selection operator (LASSO) Cox regression algorithm was implemented to analyze 23 genes. According to the minimum standard, 5 genes were selected for LUAD, 3 for LUSC, and 3 for pan-cancer. Five genes in LUAD are IGF2BP1, IGF2BP2, HNRNPC, KIAA1429, and HNRNPA2B1. Three genes are CBL1, HNRNPC, and METTL3 in LUSC. In pan-cancer, three genes are IGF2BP1, METTL3, and YTHDC2. Then, risk characteristics were established, respectively. The coefficients were used to calculate the risk scores of each group. Among LUAD, LUSC, and pan-cancer, the survival rate of patients in the high-risk group is both lower than that in the low-risk group (Figures 4A–C).

Prognostic Risk Scores Are Related to the Characteristics of Lung Cancer Clinical Cases

We made a ROC curve to get the results that the risk score can be served as an independent prognostic factor. Next, a heatmap showed that there is a significant difference in survival status in LUAD. Similarly, the pan-cancer data have obvious differences in N stage, T stage, total stage, and survival status. However, there are no significant differences in any pathological features of LUSC (Figures 5A–C).

Finally, we performed univariate and multivariate Cox regression analysis. In univariate analysis, total stage, T stage, N stage, and risk scores are all related to overall survival in LUAD. Age, total stage, and risk scores are connected with overall survival in LUSC. Total staging, T staging, M staging, N staging, and risk scores have something to do with overall survival in pan-cancer. When all factors were incorporated into the multivariate analysis, the total stage and risk scores were still significantly correlated with the overall survival rate in LUAD. Age and risk scores are related to overall survival in LUSC. T staging and risk scores were in connection with overall survival in pan-cancer (Figure 6).

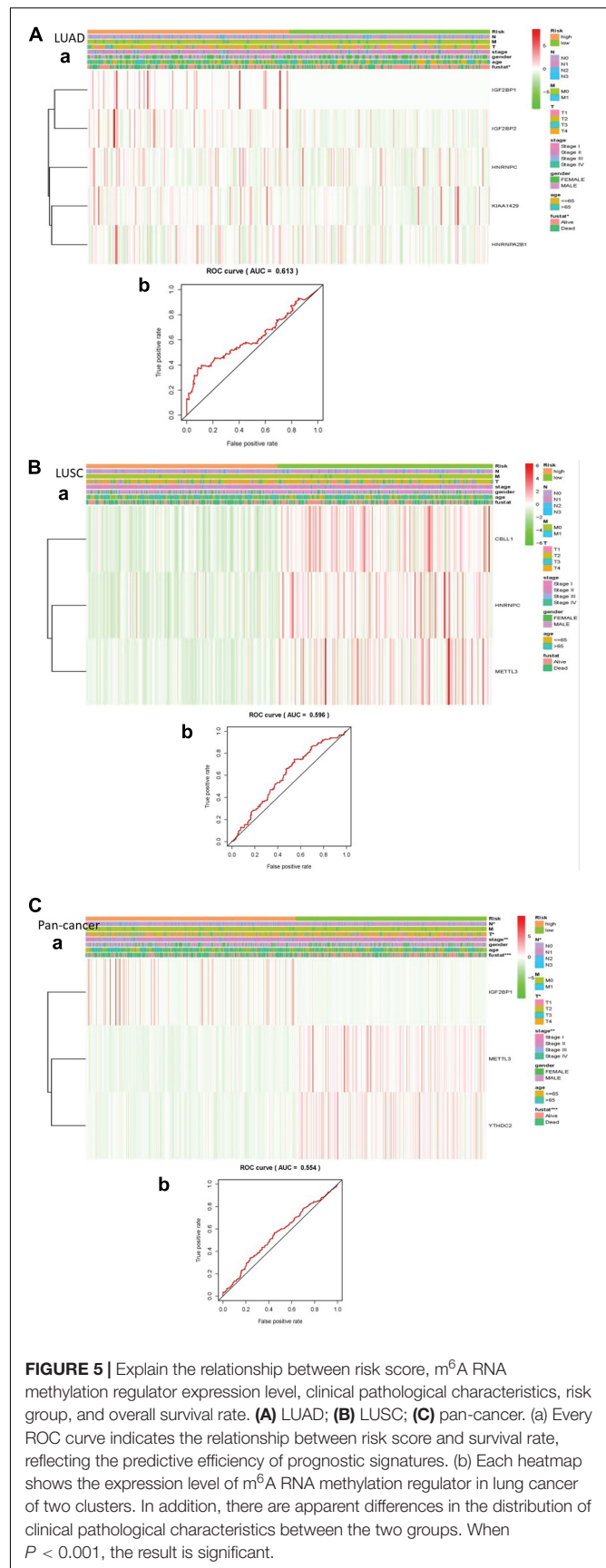
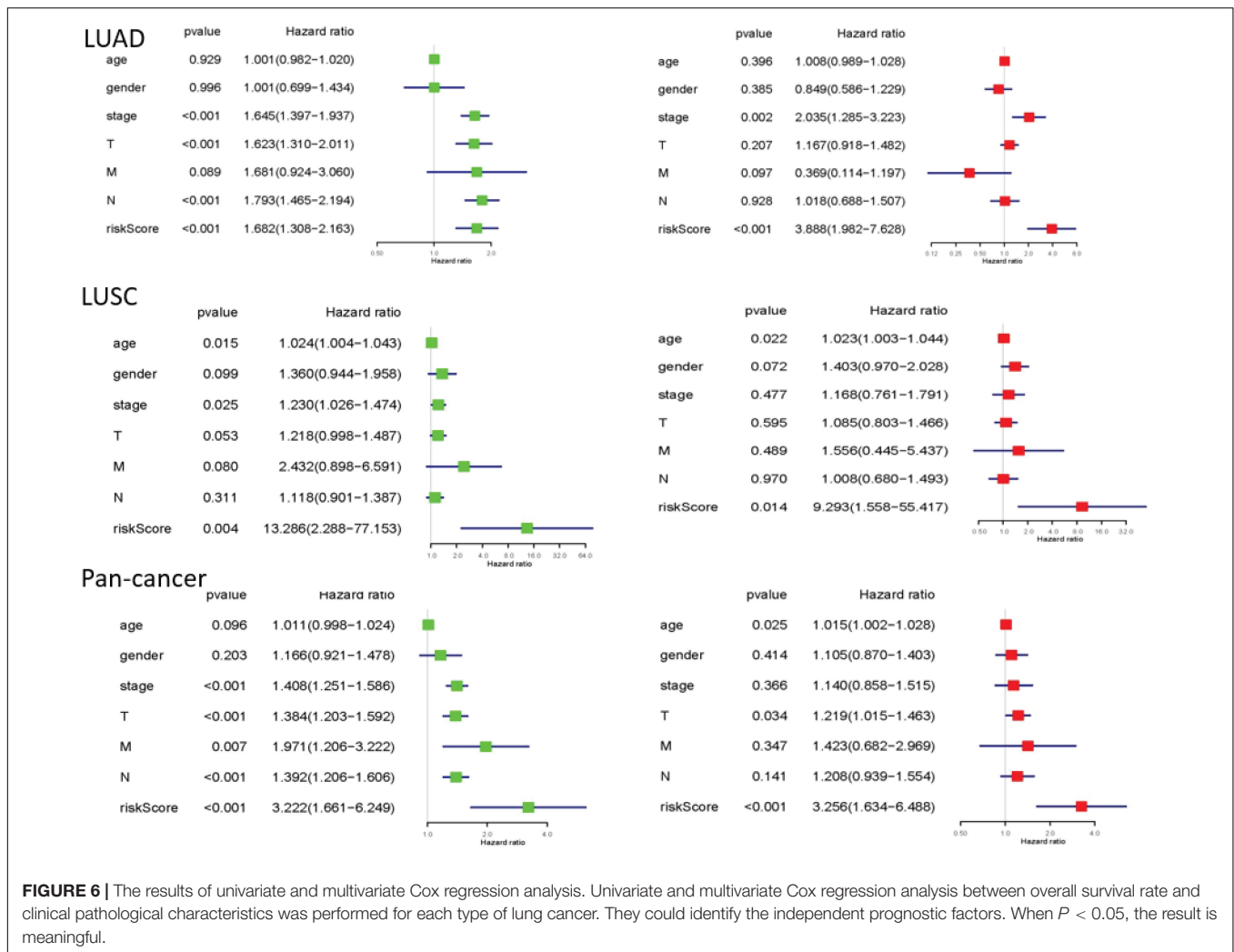


FIGURE 5 | Explain the relationship between risk score, m⁶A RNA methylation regulator expression level, clinical pathological characteristics, risk group, and overall survival rate. **(A)** LUAD; **(B)** LUSC; **(C)** pan-cancer. (a) Every ROC curve indicates the relationship between risk score and survival rate, reflecting the predictive efficiency of prognostic signatures. (b) Each heatmap shows the expression level of m⁶A RNA methylation regulator in lung cancer of two clusters. In addition, there are apparent differences in the distribution of clinical pathological characteristics between the two groups. When $P < 0.001$, the result is significant.



DISCUSSION

With the increasing incidence and mortality of lung cancer today, there are quite in-depth discoveries in DNA and protein modification, so the treatment of lung cancer is also updated at an alarming rate (Karlsson et al., 2019; Siegel et al., 2020). This study explored the clinical prognostic effect of m⁶A in LUAD, LUSC, and pan-cancer for the purpose of laying the foundation for treatment strategies based on RNA methylation.

In this study, we found that most of the 23 m⁶A RNA methylation regulators were abnormally expressed in LUAD, LUSC, and pan-cancer. Among them, KIAA1429 and YTHDF3 showed a significant positive correlation. YTHDF3 is a direct m⁶A reader, which can promote the translation of target mRNAs and improve their stability. The proliferation, migration, and invasion of cancer cells were enhanced with the upregulated expression of METTL3 (Cheng et al., 2019). When the expression level of METTL3 is upregulated, it enhances the proliferation, migration, and invasion of cancer cells *in vitro* and the growth of tumors *in vivo*. Meanwhile, YTHDF3 collaborates with YTHDF1 to promote protein synthesis and influence methylated mRNA

decay mediated by YTHDF2. Together with YTHDF1 and YTHDF2, YTHDF3 plays a key role in accelerating metabolism of mRNAs modified by m⁶A in the cytoplasm (Wang X. et al., 2014; Li et al., 2017; Shi et al., 2017).

In the LUAD, LUSC, and pan-cancer, there are significant differences in the overall survival of the two different clusters. They also have significant correlation in different clinical pathological features, indicating that the level of m⁶A RNA methylation regulator is distinctly related to the poor prognosis of lung cancer.

In the LUAD, KIAA1429 is associated with the formation of m⁶A methyltransferase complex (MTC), which promotes the formation of m⁶A (Bokar et al., 1997; Ping et al., 2014; Wang Y. et al., 2014). HNRNPC is considered as the promoter of tumorigenesis, and overexpression of HNRNPC can be found in the main regulators of various cancer progression-related genes, which is closely connected to the poor clinical prognosis of cancer (Huang et al., 2016; Fischl et al., 2019). The risk gene for LUSC is CBLL1. In addition, pan-cancer's risk gene is METTL3 and the protective gene is IGF2BP1. METTL3 can participate in the composition of MTC as well. Cytoplasmic

METTL3 is served as a m⁶A reader to promote the translation of target mRNA (Liu et al., 2014; Cheng et al., 2019; Ru et al., 2020; Shi et al., 2020). This result is used as a basis for predicting the survival rate of lung cancer according to the genetic differences of patients in the future. Even the known gene variation could be used to fundamentally treat lung cancer from the genetic perspective, for example, some medicine will target m⁶A methylation (Deng et al., 2018).

We selected the appropriate gene according to the minimum standard and calculated their respective risk scores in the wake of dividing lung cancer into high-risk and low-risk categories by the LASSO algorithm. Then, the ROC curve and heatmap are used to verify that the risk scores can effectively predict the survival of lung cancer patients. Above all, risk scores provide an important basis for clinical evaluation of lung cancer prognosis and provide personalized treatment options.

In summary, this study systematically described the differences in the expression levels of m⁶A RNA methylation regulators between LUAD, LUSC, and pan-cancer data, revealing

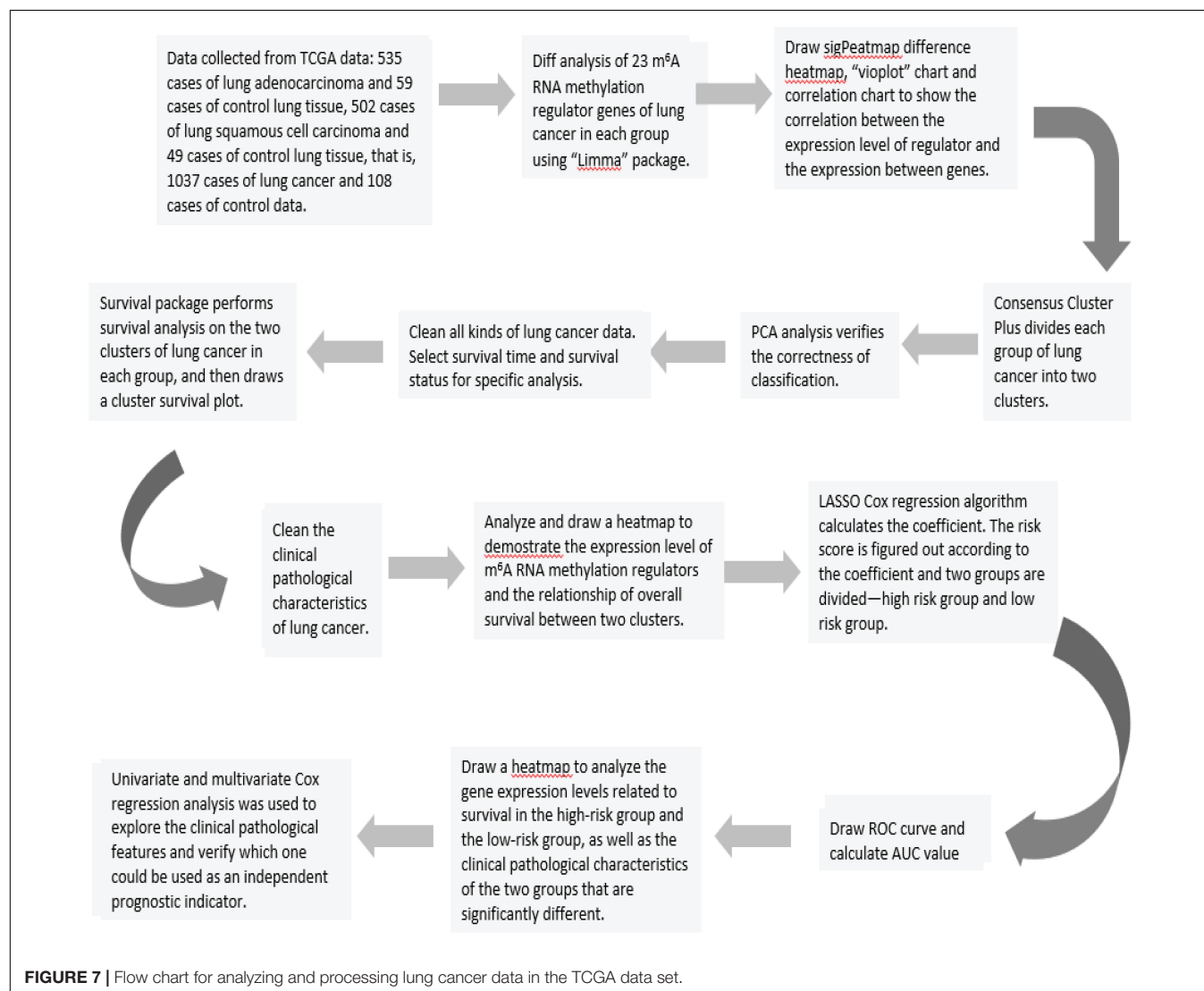
the prognostic role of their expressions in relation to the clinical pathological characteristics of lung cancer. M⁶A RNA methylation regulator obviously not only plays a vital role in the occurrence and development of lung cancer but also affects the clinical manifestations and prognostic development of lung cancer (Wang et al., 2021). Besides, risk scores can become a far-reaching factor in predicting the prognosis of lung cancer, providing essentially for clinical treatment guidance.

MATERIALS AND METHODS

Data Acquisition

A total of 1037 cases of lung cancer were obtained from the TCGA database,¹ including 535 cases of LUAD and 502 cases of LUSC. In addition, there were 108 cases in the control group, containing 59 cases of LUAD and 49 cases of LUSC.

¹<https://cancergenome.nih.gov/>



Selection of m⁶A RNA Methylation Regulators

Depending upon the literature (Shi et al., 2018; Li et al., 2019, 2020; Sun et al., 2019; Liu et al., 2020; Meng et al., 2020; Muthusamy, 2020; Zhou et al., 2020; Zhang et al., 2021; Zou et al., 2021), 23 tumor-related m⁶A RNA methylated modulators were selected. These 23 genes were extracted and systematically analyzed with the data of LUAD, LUSC, and pan-cancer in TCGA database. Through these procedures, we could search the relationship between the expression of 23 m⁶A-related genes and the clinical prognosis of lung cancer patients.

Bioinformatic Analysis

In order to examine the role of m⁶A RNA methylation regulator in lung cancer, we first used “limma” package to analyze 23 m⁶A RNA methylation regulator related genes in each lung cancer group. In the next step, expression levels of 23 m⁶A RNA methylation regulators had been distinctly demonstrated by “diff” analysis, “pheatmap” package, and “vioplot” package. Correlation figures were drawn to illustrate the relationship between different m⁶A RNA methylation regulators. Besides, we utilized the “ConsensusClusterPlus” package to delete the normal lung tissue data in each lung cancer group and divide the remaining lung cancer data into two groups. PCA could verify whether the classification was correct. The survival software package had the capability to study the survival of grouped lung cancers and draw a clinical heatmap of the correlation between different clusters and the relationship between survival and clinical data.

More than anything, for the purpose of finding the relationship between the gene and survival and its effect on the prognosis, the m⁶A RNA methylation Regulator genes were conducted by univariate Cox regression analysis and LASSO Cox regression algorithm (Bovelstad et al., 2007; Sauerbrei et al., 2007).

In the end, we could construct the survival-related linear risk assessment model—risk scores. Calculation formula: Risk score = $\sum_{i=1}^N \text{Exp}_i \times w_i$. Exp in the formula represents the expression level of the gene, and w represents the coefficient of the gene in the univariate or multivariate Cox regression analysis. ROC curve and heatmap were assessed if risk score could be a prognostic factor in lung cancer (Figure 7).

Statistical Analyses

“Diff” package was used to analyze the expression levels of 23 m⁶ARNA methylation modulator related genes in LUAD, LUSC, and pan-cancer. Then, the “ConsensusClusterPlus” software package was used to treat LUSC, LUSC, and total lung cancer. Also, each group of lung cancer was divided into two

clusters. With the help of univariate Cox regression analysis, the relationship between different clusters and clinical data was clarified. At the same time, the prognostic effect of related genes was analyzed in combination with multivariate regression analysis. Finally, LASSO Cox regression algorithm was performed to analyze related genes and divide each group of lung cancer into high-risk group and low-risk group to find independent prognostic factors. Overall survival referred to the time from the date of diagnosis to death for any reason. This study used Practical Extraction and Report Language (Perl) and R version 3.5.3 for statistical analysis. The results were statistically significant when $P < 0.05$.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Guangdong Medical University Ethics committee. The ethics committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

XZ and GP: conceptualization, methodology, supervision, funding, and software. ST: data curation and writing—original draft preparation. ST, KL, GL, ZT, ZZ, and XZ: visualization and investigation. ST, ZL, YL, JK, WC, ML, and XZ: writing—reviewing and editing. XZ: checked the statistical accuracy as an expert in statistics and bioinformatics. All authors read and approved the final version of the manuscript.

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REFERENCES

- Asada, K., Bolatkan, A., Takasawa, K., Komatsu, M., Kaneko, S., and Hamamoto, R. (2020). Critical Roles of N(6)-methyladenosine (m(6)A) in cancer and virus infection. *Biomolecules* 10:1071. doi: 10.3390/biom10071071
- Bokar, J. A., Shambaugh, M. E., Polayes, D., Matera, A. G., and Rottman, F. M. (1997). Purification and cDNA cloning of the AdoMet-binding subunit of the human mRNA (N6-adenosine)-methyltransferase. *RNA* 3, 1233–1247.
- Bovelstad, H. M., Nygard, S., Storvold, H. L., Aldrin, M., Borgan, O., Frigessi, A., et al. (2007). Predicting survival from microarray data—a comparative

- study. *Bioinformatics* 23, 2080–2087. doi: 10.1093/bioinformatics/btm305
- Cheng, M., Sheng, L., Gao, Q., Xiong, Q., Zhang, H., Wu, M., et al. (2019). The m(6)A methyltransferase METTL3 promotes bladder cancer progression via AFF4/NF- κ B/MYC signaling network. *Oncogene* 38, 3667–3680. doi: 10.1038/s41388-019-0683-z
- Choe, J., Lin, S., Zhang, W., Liu, Q., Wang, L., Ramirez-Moya, J., et al. (2018). mRNA circularization by METTL3-eIF3h enhances translation and promotes oncogenesis. *Nature* 561, 556–560. doi: 10.1038/s41586-018-0538-8
- Deng, X., Su, R., Weng, H., Huang, H., Li, Z., and Chen, J. (2018). RNA N(6)-methyladenosine modification in cancers, current status and perspectives. *Cell Res.* 28, 507–517. doi: 10.1038/s41422-018-0034-6
- Fischl, H., Neve, J., Wang, Z., Patel, R., Louey, A., Tian, B., et al. (2019). hnRNPc regulates cancer-specific alternative cleavage and polyadenylation profiles. *Nucleic Acids Res.* 47, 7580–7591. doi: 10.1093/nar/gkz461
- He, J. J., Li, Z., Rong, Z. X., Gao, J., Mu, Y., Guan, Y. D., et al. (2020). m(6)A reader YTHDC2 promotes radiotherapy resistance of nasopharyngeal carcinoma via activating IGF1R/AKT/S6 signaling axis. *Front. Oncol.* 10:1166.
- Huang, H., Han, Y., Zhang, C., Wu, J., Feng, J., Qu, L., et al. (2016). HNRNPC as a candidate biomarker for chemoresistance in gastric cancer. *Tumour Biol.* 37, 3527–3534. doi: 10.1007/s13277-015-4144-1
- Karlsson, A., Cirenajwis, H., Ericson-Lindquist, K., Brunnstrom, H., Reutersward, C., Jonsson, M., et al. (2019). A combined gene expression tool for parallel histological prediction and gene fusion detection in non-small cell lung cancer. *Sci. Rep.* 9:5207.
- Li, A., Chen, Y. S., Ping, X. L., Yang, X., Xiao, W., Yang, Y., et al. (2017). Cytoplasmic m(6)A reader YTHDF3 promotes mRNA translation. *Cell Res.* 27, 444–447. doi: 10.1038/cr.2017.10
- Li, K., Luo, H., Luo, H., and Zhu, X. (2020). Clinical and prognostic pan-cancer analysis of m6A RNA methylation regulators in four types of endocrine system tumors. *Aging (Albany NY)* 12, 23931–23944. doi: 10.18632/aging.104064
- Li, X., Feng, Z., Wang, R., Hu, J., He, X., and Shen, Z. (2021). Expression status and prognostic value of m(6)A RNA methylation regulators in lung adenocarcinoma. *Life (Basel)* 11:619. doi: 10.3390/life11070619
- Li, Y., Xiao, J., Bai, J., Tian, Y., Qu, Y., Chen, X., et al. (2019). Molecular characterization and clinical relevance of m(6)A regulators across 33 cancer types. *Mol. Cancer* 18:137.
- Liu, J., Ren, D., Du, Z., Wang, H., Zhang, H., and Jin, Y. (2018). m(6)A demethylase FTO facilitates tumor progression in lung squamous cell carcinoma by regulating MZF1 expression. *Biochem. Biophys. Res. Commun.* 502, 456–464. doi: 10.1016/j.bbrc.2018.05.175
- Liu, J., Yue, Y., Han, D., Wang, X., Fu, Y., Zhang, L., et al. (2014). A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. *Nat. Chem. Biol.* 10, 93–95. doi: 10.1038/nchembio.1432
- Liu, S., Li, Q., Chen, K., Zhang, Q., Li, G., Zhuo, L., et al. (2020). The emerging molecular mechanism of m(6)A modulators in tumorigenesis and cancer progression. *Biomed. Pharmacother.* 127:110098. doi: 10.1016/j.biopha.2020.110098
- Meng, Y., Li, S., Gu, D., Xu, K., Du, M., Zhu, L., et al. (2020). Genetic variants in m6A modification genes are associated with colorectal cancer risk. *Carcinogenesis* 41, 8–17.
- Muthusamy, S. (2020). m(6)A mRNA methylation, A pleiotropic regulator of cancer. *Gene* 736:144415. doi: 10.1016/j.gene.2020.144415
- Ni, X. F., Xie, Q. Q., Zhao, J. M., Xu, Y. J., Ji, M., Hu, W. W., et al. (2021). The hepatic microenvironment promotes lung adenocarcinoma cell proliferation, metastasis, and epithelial-mesenchymal transition via METTL3-mediated N6-methyladenosine modification of YAP1. *Aging (Albany NY)* 13, 4357–4369. doi: 10.18632/aging.202397
- Ping, X. L., Sun, B. F., Wang, L., Xiao, W., Yang, X., Wang, W. J., et al. (2014). Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase. *Cell Res.* 24, 177–189. doi: 10.1038/cr.2014.3
- Ru, W., Zhang, X., Yue, B., Qi, A., Shen, X., Huang, Y., et al. (2020). Insight into m(6)A methylation from occurrence to functions. *Open Biol.* 10:200091. doi: 10.1098/rsob.200091
- Sauerbrei, W., Royston, P., and Binder, H. (2007). Selection of important variables and determination of functional form for continuous predictors in multivariable model building. *Stat. Med.* 26, 5512–5528. doi: 10.1002/sim.3148
- Shi, H., Wang, X., Lu, Z., Zhao, B. S., Ma, H., Hsu, P. J., et al. (2017). YTHDF3 facilitates translation and decay of N(6)-methyladenosine-modified RNA. *Cell Res.* 27, 315–328. doi: 10.1038/cr.2017.15
- Shi, H., Zhang, X., Weng, Y. L., Lu, Z., Liu, Y., Lu, Z., et al. (2018). m(6)A facilitates hippocampus-dependent learning and memory through YTHDF1. *Nature* 563, 249–253. doi: 10.1038/s41586-018-0666-1
- Shi, Y., Zheng, C., Jin, Y., Bao, B., Wang, D., Hou, K., et al. (2020). Reduced expression of METTL3 promotes metastasis of triple-negative breast cancer by m6A Methylation-Mediated COL3A1 Up-regulation. *Front. Oncol.* 10:1126.
- Siegel, R. L., Miller, K. D., and Jemal, A. (2020). Cancer statistics, 2020. *CA Cancer J. Clin.* 70, 7–30.
- Sun, T., Wu, R., and Ming, L. (2019). The role of m6A RNA methylation in cancer. *Biomed. Pharmacother.* 112:108613. doi: 10.1016/j.biopha.2019.108613
- Tuncel, G., and Kalkan, R. (2019). Importance of m N(6)-methyladenosine (m(6)A) RNA modification in cancer. *Med. Oncol.* 36:36.
- Wang, H., Zhao, X., and Lu, Z. (2021). m(6)A RNA methylation regulators act as potential prognostic biomarkers in lung adenocarcinoma. *Front. Genet.* 12:622233.
- Wang, S., Chai, P., Jia, R., and Jia, R. (2018). Novel insights on m(6)A RNA methylation in tumorigenesis, a double-edged sword. *Mol. Cancer* 17:101.
- Wang, X., Lu, Z., Gomez, A., Hon, G. C., Yue, Y., Han, D., et al. (2014). N6-methyladenosine-dependent regulation of messenger RNA stability. *Nature* 505, 117–120. doi: 10.1038/nature12730
- Wang, Y., Li, Y., Toth, J. I., Petroski, M. D., Zhang, Z., and Zhao, J. C. (2014). N6-methyladenosine modification destabilizes developmental regulators in embryonic stem cells. *Nat. Cell Biol.* 16, 191–198. doi: 10.1038/ncb2902
- Wozniak, A. (2012). Challenges in the current antiangiogenic treatment paradigm for patients with non-small cell lung cancer. *Crit. Rev. Oncol. Hematol.* 82, 200–212. doi: 10.1016/j.critrevonc.2011.05.003
- Wozniak, A. J., and Schwartz, A. G. (2018). Risk of second lung cancer in patients with previously treated lung cancer, analysis of surveillance, epidemiology, and end results data. *J. Thorac. Oncol.* 13, e106–e107.
- Yang, Y., Hsu, P. J., Chen, Y. S., and Yang, Y. G. (2018). Dynamic transcriptomic m(6)A decoration: writers, erasers, readers and functions in RNA metabolism. *Cell Res.* 28, 616–624. doi: 10.1038/s41422-018-0040-8
- Zhang, C., Fu, J., and Zhou, Y. (2019). A review in research progress concerning m6A methylation and immunoregulation. *Front. Immunol.* 10:922.
- Zhang, X., Zhong, L., Zou, Z., Liang, G., Tang, Z., Li, K., et al. (2021). Clinical and prognostic pan-cancer analysis of N6-methyladenosine regulators in two types of hematological Malignancies, A Retrospective Study Based on TCGA and GTEx Databases. *Front. Oncol.* 11:623170.
- Zhou, Y., Kong, Y., Fan, W., Tao, T., Xiao, Q., Li, N., et al. (2020). Principles of RNA methylation and their implications for biology and medicine. *Biomed. Pharmacother.* 131:110731. doi: 10.1016/j.biopha.2020.110731
- Zhu, X., Luo, H., and Xu, Y. (2019). Transcriptome analysis reveals an important candidate gene involved in both nodal metastasis and prognosis in lung adenocarcinoma. *Cell Biosci.* 9:92.
- Zou, Z., Zhou, S., Liang, G., Tang, Z., Li, K., Tan, S., et al. (2021). The pan-cancer analysis of the two types of uterine cancer uncovered clinical and prognostic associations with m6A RNA methylation regulators. *Mol. Omics* 17:641. doi: 10.1039/d1mo90014e

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