NCOA4 is a Prognostic Biomarker Associated with Immune Infiltrates in Glioblastoma

Lingyu Liu
Tangdu Hospital, Fourth Military Medical University
Yufei Liu (yufei940830@sina.com)
Tangdu Hospital, Fourth Military Medical University

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Abstract

Background Glioblastoma (GBM) is a malignant primary craniocerebral tumor with median survival of less than 15 months. Nuclear receptor coactivator 4 (NCOA4) can regulate the growth of various malignant tumors by participating in various biological processes. However, in glioblastoma, the specific regulatory role and mechanism of NCOA4 have not been clearly explained.

Methods We collected GBM patient information from the Cancer Genome Atlas (TCGA). The expression of NCOA4 in GBM and normal tissues and the relationship between NCOA4 and various clinicopathological features, further the enrichment pathway of NCOA4 as well as the relationship between NCOA4 and prognosis of patients and the NCOA4-related nomogram for patient survival were analyzed by various bioinformatic tools including Wilcoxon rank sum test, Logistic regression analysis, Gene ontology term analysis (GO), Gene set enrichment analysis (GSEA), single-sample Gene Set Enrichment Analysis (ssGSEA), Kaplan-Meier analysis and Cox regression comprehensively.

Results Our study showed that NCOA4 was significantly overexpressed in GBM, and the higher the expression of NCOA4, the longer the survival. In order to further explore the potential role of NCOA4, we conducted enrichment analysis on NCOA4, established a NCOA4-related PPI network. We found that the expression of NCOA4 was negatively interrelated with the infiltration degree of NK CD56bright cells, and positively interrelated with the infiltration degree of mast cells, B cells, T cells, T helper cells. We created a Nomogram in order to predict the prognosis of patients with GBM.

Conclusion High expression of NCOA4 is associated with better prognosis and is associated with immune cell infiltration in GBM.

Background

Glioblastoma (GBM) is a malignant primary craniocerebral tumor with median survival of less than 15 months[1]. Despite recent advances in comprehensive treatment strategies for GBM, GBM patients still have a poor prognosis [1]. Glioma is recognized to be a highly heterogeneous CNS malignancy, whose diverse cellular composition and cellular interactions have not been well characterized[2]. To date, only a few therapies are approved for the treatment of GBM with the main reasons being: 1) the apparent heterogeneity of the tumor leads to its specific drug resistance 2) GBM can induce immunosuppression 3) the location of the intracranial tumor makes treatment more difficult 4) there is no clear biomarker for GBM to effectively diagnose and evaluate its prognosis[3]. Therefore, there is an urgent necessity for the elucidation of the identification of novel biomarkers for carcinoma diagnosis and therapeutic targets in GBM.

Nuclear receptor coactivator 4 (NCOA4), a coactivator of multiple nuclear hormone receptors has been reported can mediate the autophagy degradation of ferritin[4; 5]. It has been shown that NCOA4 can induce and mediate ferritin autophagy in neurodegenerative diseases and also cause ferroptosis[6; 7; 8].

Similarly, abnormal expression of NCOA4 is found in colorectal cancer and clear cell renal cell carcinoma[6]. However, the expression and role of NCOA4 in GBM have not been clearly described.

Tumor infiltrating immune cells is part of the tumor microenvironment and often has two sides to tumor growth[9]. At present, immunotherapy is gradually being tested and promoted in clinical practice [10]. NCOA4 is a selective cargo receptor that mediates the autophagy degradation of ferritin and thus participates in the regulation of ferroptosis[11]. Ferroptosis might contribute to tumor immune evasion by directly interfering with the function of various immune cells[12]. On the other hand, Ferroptosis can regulate the immune microenvironment and inhibit tumor growth. Therefore, ferroptosis is critically involved in the regulation of antitumor immunity and may provide potential strategies in immunotherapy.

In this study, we used various bioinformatics methods to analyze the role of NCOA4 in the progression of GBM, and produced nCOA4-related nomogram to predict patient survival outcomes

Methods

Data Source before Analysis

Gene expression data for analysis with clinical information about GBM projects (included 1157 normal and 168 GBM tissues were collected from TCGA. Overall survival less than 30 days were excluded and unavailable or unknown clinical features were missing values.

The Expression GBM and Paracancerous Tissues in the TCGA Database

The expression of NCOA4 in tumor and normal disease state was shown in boxplots and scatter plots. Receiver operating characteristic (ROC) curves were generated according to diagnostic performance of NCOA4. The level of expression of NCOA4 above the median value was defined as NCOA4-high and NCOA4-low from statistical ranking. We use "Xiantao" tool (https://www.xiantao.love/) expression differences" module to compare NCOA4 in GBM expression, tissue adjacent to carcinoma and normal tissue. We compare the NCOA4 in tissue and normal tissue expression of GBM Through the Human Protein Atlas database (https://www.proteinatlas.org/). The ROC curve of NCOA4 expression was drawn by ROC module for reference.

We through the "difference analysis" module of "Xiantao" tool, input "NCOA4" for analysis. And we input all DEGs into the "volcano plot" module of the "Xiantao" tool. The adjusted P value <0.05 and the absolute FC larger than 2.0 were considered to be statistically significant. All the DEGs identified were presented in volcano plots.

Functional Enrichment and Protein-Protein Interaction (PPI) Network of NCOA4

The expression difference between differences in NCOA4 signaling pathways between the high and low NCOA4 groups obtained by Xiantao tools (https://www.xiantao.love/), section GSEA predicting NCOA4-related phenotypes and signaling pathways. Search Tool for Interaction Gene/Proteins (STRING) website

(https://string-db.org/) analyzed protein-protein networks of NOCA4[13]. We explored NCOA4 internetworks by using the query of a single protein name (“NCOA4”) and organism (“Homo sapiens”). We set the following main parameters in “setting” module: minimum required interaction score [“Low confidence (0.150)”], meaning of network edges (“evidence”), max number of interactors to show (“no more than 50 interactors” in 1st shell) and active interaction sources (“experiments”). Finally, we get the available visualized NCOA4-binding proteins.

Analysis of the Interrelation Between NCOA4 and Immune Infiltration

ssGSEA was used to quantify the relative tumor infiltration levels of 24 immune cell types expression levels of genes[14]. In the module of "Xiantao tool" immune infiltration, Wilcoxon rank sum test and Xiantao tool Spearman correlation were used to analyze the relationship between NCOA4 and immune cell infiltration and the correlation between immune cell infiltration and different NCOA4 expression groups.

Clinical Statistical Analysis on Prognosis, Model Construction, and Evaluation

In the clinical meaning module of the Xiantao tool, prognosis features including overall survival (OS), progression-free interval (PFI), and disease-specific survival (DSS) in TCGA patients were analyzed by Cox regression and Kaplan-Meier methods. The truncation value of high and low NCOA4 expression was identified according to the median number. Wilcoxon signed rank sum test and Logistic regression were used to analyze the relationship between clinicopathological features and NCOA4. Multivariate Cox analysis calculated the effect of NCOA4 expression on survival rate and other clinical features. $P<0.05$ was considered significant. Based on Cox regression model, the independent prognostic factors obtained from multivariate analysis were continued, and we predicted the survival probabilities of 1, 3 and 5 years respectively. Compared the predicted probabilities with observed events by Calibration curves, with the 45° line representing the best predicted value. C-index, a conformance index was a useful tool to determine the differentiation of the nomogram, also can compare the prediction accuracy of the nomogram with independent prognostic factors.

Result

Differentially Expression of NCOA4 and NCOA4-related Differential Genes in GBM

The pan-cancer analyses were performed to compare the expression of NCOA4 in the tumor samples of GTEx combined with TCGA and the corresponding normal samples of TCGA by Wilcoxon rank sum test. NCOA4 was significant differential expressed in adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), breast infiltrating carcinoma (BRCA), cervical squamous cell carcinoma and adenocarcinoma (CESC), cholangiocarcinoma (CHOL), Colon adenocarcinoma (COAD), diffuse large B cell lymphoma (DLBC), Esophageal carcinoma (ESCA), pleomorphic glioma (GBM), head and neck squamous cell carcinoma (HNSC), renal chromophobe cell carcinoma (KICH), renal clear cell carcinoma (KIRC), renal papillary cell carcinoma (KIRP), acute myeloid leukemia (LAML), brain low grade glioma

(LGG), Liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), mesothelioma (MESO), ovarian serous cystadenocarcinoma (OV),pancreatic cancer (PAAD), pheochromocytoma and paraganglioma (PCPG), prostate cancer (PRAD), rectum adenocarcinoma (READ), sarcoma(SARC), skin melanoma (SKCM), gastric cancer (STAD), Testicular Germ Cell Tumors (TGCT), thyroid cancer (THCA), thymic cancer (THYM), endometrial cancer (UCEC), uterine sarcoma (UCS), uveal melanoma (UVM) ($P < 0.05$) (Figure 1A). Secondly, we compared the expression of NCOA4 in 1157 paracancerous samples and 168 GBM samples in TCGA GBM dataset. The expression of NCOA4 was significantly high in GBM samples ($P < 0.001$) (Figure 1B). ROC was used to analyze the distinguishing efficacy of NCOA4 between GBM tissues and normal paracancerous tissue. The area under the curve (AUC) of NCOA4 is 0.925, suggesting that NCOA4 may be a potentially identification molecule for GBM tissues (Figure 1C). Moreover, in the Human Protein Atlas, NCOA4 protein expression was increased in GBM tissues compared with normal tissues. We divided them into 84 NCOA4 high expression and 84 and low expression using median as the boundary. A total of 37 DEGs, covering 36 upregulated GENEs and 1 downregulated GENEs, were identified to be statistically significant between the two cohorts (adjusted p-value < 0.05, |Log2-fold change| > 2.0) (Figure 1D).

Functional Enrichment and Signaling Pathways of NCOA4 Related Genes in GBM

To search for functional enrichment of NCOA4-related genes, our GO enrichment analysis suggested that nCOA4-related genes were mainly enriched in focal adhesion, collagen-containing extracellular matrix, cell-substrate adherens junction, cell-substrate junction, cell-cell junction. (Figure 2A-B). The high and low expression of NCOA4 in GBM were analyzed by GSEA to search for NCOA4 enrichment signaling pathway. Finally, these signaling pathways include neutrophil Degranulation Pathway, signaling by Interleukins Pathway, r-RNA processing Pathway and translation Pathway (Figures 2C–D). We found from The Human Protein Atlas database that the expression of NCOA4 in GBM tissues was higher than that in normal tissues (Figure 1E-F).

Protein-Protein Interaction (PPI) Network Analysis in the differentially expressed genes

In order to acquire the interactions between the DEGs in the GBM group, a PPI network was constructed using the STRING database. In our study, the top 10 genes included AR, FTL, FTH1, CCDC6, RET, GFRA1, CTNNB1, HSP90AA1, FOXA1, PELP1(Figure.3).

The interrelation Between NCOA4 and Immune Infiltration

The interrelation between the expression level of NCOA4 and immune cell infiltration level quantified by ssGSEA was analyzed. The expression of NCOA4 was negatively interrelated with the infiltration degree of NK CD56bright cells, and positively interrelated with the infiltration degree of mast cells, B cells, T cells, T helper cells. (Figures 4A–K, $P < 0.001$).

Correlation between NCOA4 and clinical features

The clinical characteristics and expression of NCOA4 in 168 GBM patients in TCGA were analyzed. We analyzed 109 men and 59 women, as shown in Figures 5A-F and Table 1, overexpressed NCOA4 was significantly correlated with IDH status (wild type vs. mutational type, P = 0.027). Interestingly, Our Logistic regression analysis showed that NCOA4 overexpression was also related to IDH mutation (wild type vs. mutational type, P = 0.024). (Table 2).

The OS rate of patients with high NCOA4 expression was significantly higher than that of patients with low NCOA4 expression (P = 0.042; Figure 6A). However, there were no significant differences in DSS rate and PFI between NCOA4 group and NCOA4 group (P = 0.106; P = 0.383; Figures 6B, C). In our opinion, this may be due to the rapid disease progression of GBM patients, and it is difficult to show significant differences between DSS and PFI.

Construction of NCOA4 Related Nomogram

We constructed a Nomogram using NCOA4 and other clinical indicators to predict the prognosis of patients with GBM. (Figure 7A). We obtained the final Nomogram by multivariate COX regression analysis. In the Nomogram, each factor is assigned a score range, and the sum of all factor scores is the final score, corresponding to the 1-year, 2-year and 3-year survival probability of GBM patients. (Figure 7A).

To test the prediction accuracy of this Nomogram for prognosis, we calculate a C-index of 0.622(CI: 0.5900-0.653). And the calibration curve shows that our prediction and observation have a good consistency. (Figure7B).

Discussion

Since the expression of NCOA4 in glioblastoma and its effect on the prognosis of glioblastoma have not been clarified. Therefore, we systematically analyzed the expression of NCOA4 in GBM and the effect of NCOA4 on the prognosis of GBM patients, and briefly analyzed its possible mechanism of action. As a cargo carrier protein of ferritin autophagy, NCOA4 can promote ferroptosis[4; 15]. This process involves intracellular accumulation of iron, production of Reactive oxygen species (ROS), supply of fatty acids, and lipid peroxidation[16]. Moreover, when NCOA4 decreased, intracellular iron and oxidative stress decreased, while glutathione levels increased[8]. When these changes occur, the sensitivity of the cell to ferroptosis also changes[17; 18]. Ferroptosis interacts with tumor immunity. However, tumor immune invasion can not only inhibit tumor growth, but also promote tumorigenesis under certain conditions. Similarly, ferroptosis also has two sides to tumor progression, so we analyzed the relationship between NCOA4 and GBM in the two aspects.

NCOA4 can promote the development of certain tumors. Studies have shown that NCOA4 is highly expressed in breast cancer, and can induce the transformation of normal cells into cancer cells and promote tumor formation[19]. In addition, NCOA4-RET fusion can occur in colorectal cancer and promote the progression of colon cancer[20]. Interestingly, NCOA4-RET is also frequently found in papillary thyroid

carcinoma [21] In hepatocellular carcinoma, when NCOA4 is highly expressed, it can induce iron death in hepatocellular carcinoma cells, thus improving the survival rate of patients[22; 23]. These studies suggest that NCOA4 may play a dual role in tumorigenesis, with NCOA4α acting as a positive regulator of tumor development in ovarian cancer and NCOA4β acting as a tumor suppressor in ovarian cancer. But these effects may be reversed in prostate cancer and breast cancer[24; 25]. In our study, NCOA4 was significantly overexpressed in patients with GMB. And when NCOA4 is highly expressed, patients have a better prognosis.

In order to study the function of NCOA4 in GBM, we performed GO, GESA and ssGSEA analyses using TCGA data. In our result, differential enrichment of neutrophil degranulation pathway, signaling by interleukins pathway, r-RNA processing pathway and translation pathway. The expression of NCOA4 was negatively interrelated with the infiltration degree of NK CD56bright cells, and positively interrelated with the infiltration degree of mast cells, B cells, T cells, T helper cells.

Studies have shown that neutrophil degranulation is associated with immune evasion of GBM[26]. In addition, various interleukins can enhance the collective immunity against GBM[27]. This may suggest that NCOA4 is correlated with the immune microenvironment of GBM.

Glioblastoma (GBM) is one of the most common malignant brain tumors with poor prognosis. Infiltrating immune cells in the tumor microenvironment (TME) can promote or inhibit tumor progression[28; 29]. In our study, GBM with high NCOA4 expression was accompanied by more B cells, mast cells, T cells and T helper cells and fewer NKCD56 bright cells, Of course, when NCOA4 is low expression, the results are reversed. It has been shown that activated CD8 + T cells can induce ferroptosis, which also proves the strong correlation between ferroptosis and immunotherapy[4]. T cells are the key agents of tumor killing[30], and B cells in the process of formation of antitumor immune response plays an important role, can interact with T cells and synergistic selection, and present cognate tumor-derived antigens to T cells[31]. Meanwhile, TH cells can produce antigen-specific memory B cells in the immune process of B cells[32]. Recent research shows that mast cells can transform the tumor microenvironment into anti-tumor immunity when adequately triggered[33]. In our study, the infiltration of T cells, mast cells, T helper cells and B cells was positively correlated with the expression of NCOA4, indicating that the expression of NCOA4 may promote the GBM immune microenvironment to show a characteristic of inhibiting tumor growth. NCOA4 can mediate ferritin autophagy and induce ferroptosis, which is related to the immune regulation of GBM. Therefore, NCOA4 may be a common regulator of GBM immune infiltration and ferroptosis, which has predictive value for the prognosis of patients with GBM.

In our study, high expression of NCOA4 was associated with a better prognosis, and we evaluated NCOA4 in combination with other important clinical indicators. Since our model is based on a complementary view of each tumor, it is able to provide personalized scores for individual patients. Based on the calibration plot, there was a good agreement between the predicted value and the actual value of the survival status of GBM patients at 1, 2 and 3 years.

However, our study is based on publicly available online databases and has not been extensively validated. Although our Nomogram is with a high prediction accuracy, it still needs a large number of experiments to verify our views. We will further verify our results in the future.

Conclusion

In our study, we analyzed the expression of NCOA4 in GBM and demonstrated that high expression is associated with long survival in GBM patients. The high expression of NCOA4 was positively correlated with the infiltration of mast cells, B cells, T cells and T helper cells, and it was speculated that NCOA4 inhibited GBM growth by inducing ferroptosis and regulating immune infiltration. We constructed a Nomogram using NCOA4 and other clinical indicators to predict the prognosis of patients with GBM. Our Nomogram may be a valuable new prognostic method for clinicians in the future.

Abbreviations

nuclear receptor coactivator 4 (NCOA4); glioblastoma (GBM); the cancer genome atlas (TCGA); gene ontology (GO); gene set enrichment analysis (GSEA); single-sample gene set enrichment analysis (ssGSEA); search tool for interaction gene/proteins (STRING); overall survival (OS); progression-free interval (PFI); disease-specific survival (DSS); adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), breast infiltrating carcinoma (BRCA), cervical squamous cell carcinoma and adenocarcinoma (CESC), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), diffuse large B cell lymphoma (DLBC), esophageal carcinoma (ESCA), pleomorphic glioma (GBM), head and neck squamous cell carcinoma (HNSC), renal chromophobe cell carcinoma (KICH), renal clear cell carcinoma (KIRC), renal papillary cell carcinoma (KIRP), acute myeloid leukemia (LAML), brain low grade glioma (LGG), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), mesothelioma (MESO), ovarian serous cystadenocarcinoma (OV),pancreatic cancer (PAAD), pheochromocytoma and paraganglioma (PCPG), prostate cancer (PRAD), rectum adenocarcinoma (READ), sarcoma(SARC), skin melanoma (SKCM), gastric cancer (STAD), testicular Germ Cell Tumors (TGCT), thyroid cancer (THCA), thymic cancer (THYM), endometrial cancer (UCEC), uterine sarcoma (UCS), uveal melanoma (UVM); area under the curve (AUC); receiver operating characteristic curve (ROC); isocitrate dehydrogenase (IDH); reactive oxygen species (ROS); tumor microenvironment (TME).

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Availability of data and material

The datasets analyzed during the current study are available in the "Xiantao" tool (https://www.xiantao.love/), String (https://string-db.org/), the Human Protein Atlas database (https://www.proteinatlas.org/) and The Cancer Genome Atlas (TCGA) (https://www.cancer.gov/)database.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

L.Y.L. and Y.F.L. designed and performed experiments, analyzed data and

interpreted the results. Y.F.L. assisted with experiments and participated in discussion and manuscript writing. The author(s) read and approved the manuscript.

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References

1. W. Alafate, D. Xu, W. Wu, J. Xiang, X. Ma, W. Xie, X. Bai, M. Wang, and J. Wang, Loss of PLK2 induces acquired resistance to temozolomide in GBM via activation of notch signaling. J Exp Clin Cancer Res 39 (2020) 239.
2. M.E. Berens, A. Sood, J.S. Barnholtz-Sloan, J.F. Graf, S. Cho, S. Kim, J. Kiefer, S.A. Byron, R.F. Halperin, S. Nasser, J. Adkins, L. Cuyugan, K. Devine, Q. Ostrom, M. Couce, L. Wolansky, E. McDonough, S. Schyberg, S. Dinn, A.E. Sloan, M. Prados, J.J. Phillips, S.J. Nelson, W.S. Liang, Y. Al-Kofahi, M. Rusu, M.I. Zavodszky, and F. Ginty, Multiscale, multimodal analysis of tumor heterogeneity in IDH1 mutant vs wild-type diffuse gliomas. PLoS One 14 (2019) e0219724.
3. T.I. Janjua, P. Rewatkar, A. Ahmed-Cox, I. Saeed, F.M. Mansfeld, R. Kulshreshtha, T. Kumeria, D.S. Ziegler, M. Kavallaris, R. Mazzieri, and A. Popat, Frontiers in the treatment of glioblastoma: Past, present and emerging. Adv Drug Deliv Rev 171 (2021) 108-138.
4. Y. Mou, J. Wu, Y. Zhang, O. Abdihamid, C. Duan, and B. Li, Low expression of ferritinophagy-related NCOA4 gene in relation to unfavorable outcome and defective immune cells infiltration in clear cell renal carcinoma. BMC Cancer 21 (2021) 18.

5. X. Li, L. Lozovatsky, A. Sukumaran, L. Gonzalez, A. Jain, D. Liu, N. Ayala-Lopez, and K.E. Finberg, NCOA4 is regulated by HIF and mediates mobilization of murine hepatic iron stores after blood loss. Blood 136 (2020) 2691-2702.
6. M. Quiles Del Rey, and J.D. Mancias, NCOA4-Mediated Ferritinophagy: A Potential Link to Neurodegeneration. Front Neurosci 13 (2019) 238.
7. N. Santana-Codina, and J.D. Mancias, The Role of NCOA4-Mediated Ferritinophagy in Health and Disease. Pharmaceuticals (Basel) 11 (2018).
8. J.D. Mancias, X. Wang, S.P. Gygi, J.W. Harper, and A.C. Kimmelman, Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy. Nature 509 (2014) 105-9.
9. P. Domingues, M. Gonzalez-Tablas, A. Otero, D. Pascual, D. Miranda, L. Ruiz, P. Sousa, J. Ciudad, J.M. Goncalves, M.C. Lopes, A. Orfao, and M.D. Tabernero, Tumor infiltrating immune cells in gliomas and meningiomas. Brain Behav Immun 53 (2016) 1-15.
10. O. Cohen-Inbar, and M. Zaaroor, Immunological Aspects of Malignant Gliomas. Can J Neurol Sci 43 (2016) 494-502.
11. N. Santana-Codina, A. Gikandi, and J.D. Mancias, The Role of NCOA4-Mediated Ferritinophagy in Ferroptosis. Adv Exp Med Biol 1301 (2021) 41-57.
12. L. Shi, Y. Liu, M. Li, and Z. Luo, Emerging roles of ferroptosis in the tumor immune landscape: from danger signals to anti-tumor immunity. FEBS J (2021).
13. D. Szklarczyk, A.L. Gable, K.C. Nastou, D. Lyon, R. Kirsch, S. Pyysalo, N.T. Doncheva, M. Legeay, T. Fang, P. Bork, L.J. Jensen, and C. von Mering, The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res 49 (2021) D605-D612.
14. G. Bindea, B. Mlecnik, M. Tosolini, A. Kirilovsky, M. Waldner, A.C. Obenauf, H. Angell, T. Fredriksen, L. Lafontaine, A. Berger, P. Bruneval, W.H. Fridman, C. Becker, F. Pages, M.R. Speicher, Z. Trajanoski, and J. Galon, Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity 39 (2013) 782-95.
15. N. Santana-Codina, S. Gableske, M. Quiles del Rey, B. Malachowska, M.P. Jedrychowski, D.E. Biancur, P.J. Schmidt, M.D. Fleming, W. Fendler, J.W. Harper, A.C. Kimmelman, and J.D. Mancias, NCOA4 maintains murine erythropoiesis via cell autonomous and non-autonomous mechanisms. Haematologica 104 (2019) 1342-1354.
16. X. Chen, R. Kang, G. Kroemer, and D. Tang, Broadening horizons: the role of ferroptosis in cancer. Nat Rev Clin Oncol 18 (2021) 280-296.
17. M. Gao, P. Monian, Q. Pan, W. Zhang, J. Xiang, and X. Jiang, Ferroptosis is an autophagic cell death process. Cell Res 26 (2016) 1021-32.
18. Y. Wang, Z. Wei, K. Pan, J. Li, and Q. Chen, The function and mechanism of ferroptosis in cancer. Apoptosis 25 (2020) 786-798.
19. B.S. Paratala, J.H. Chung, C.B. Williams, B. Yilmazel, W. Petrosky, K. Williams, A.B. Schrock, L.M. Gay, E. Lee, S.C. Dolfi, K. Pham, S. Lin, M. Yao, A. Kulkarni, F. DiClemente, C. Liu, L. Rodriguez-Rodriguez, S.

Ganesan, J.S. Ross, S.M. Ali, B. Leyland-Jones, and K.M. Hirshfield, RET rearrangements are actionable alterations in breast cancer. Nat Commun 9 (2018) 4821.

20. S.Y. Kim, S.O. Oh, K. Kim, J. Lee, S. Kang, K.M. Kim, W. Lee, S.T. Kim, and D.N. Nam, NCOA4-RET fusion in colorectal cancer: Therapeutic challenge using patient-derived tumor cell lines. J Cancer 9 (2018) 3032-3037.
21. R. Roskoski, Jr., and A. Sadeghi-Nejad, Role of RET protein-tyrosine kinase inhibitors in the treatment RET-driven thyroid and lung cancers. Pharmacol Res 128 (2018) 1-17.
22. Z. Zhang, Z. Yao, L. Wang, H. Ding, J. Shao, A. Chen, F. Zhang, and S. Zheng, Activation of ferritinophagy is required for the RNA-binding protein ELAVL1/HuR to regulate ferroptosis in hepatic stellate cells. Autophagy 14 (2018) 2083-2103.
23. Z. Zhang, M. Guo, Y. Li, M. Shen, D. Kong, J. Shao, H. Ding, S. Tan, A. Chen, F. Zhang, and S. Zheng, RNA-binding protein ZFP36/TTP protects against ferroptosis by regulating autophagy signaling pathway in hepatic stellate cells. Autophagy 16 (2020) 1482-1505.
24. Y. Peng, C.X. Li, F. Chen, Z. Wang, M. Ligr, J. Melamed, J. Wei, W. Gerald, M. Pagano, M.J. Garabedian, and P. Lee, Stimulation of prostate cancer cellular proliferation and invasion by the androgen receptor co-activator ARA70. Am J Pathol 172 (2008) 225-35.
25. X. Wu, F. Chen, A. Sahin, C. Albarracin, Z. Pei, X. Zou, B. Singh, R. Xu, G. Daniels, Y. Li, J. Wei, M. Blake, R.J. Schneider, P. Cowin, and P. Lee, Distinct function of androgen receptor coactivator ARA70alpha and ARA70beta in mammary gland development, and in breast cancer. Breast Cancer Res Treat 128 (2011) 391-400.
26. T.R. Sippel, J. White, K. Nag, V. Tsvankin, M. Klaassen, B.K. Kleinschmidt-DeMasters, and A. Waziri, Neutrophil degranulation and immunosuppression in patients with GBM: restoration of cellular immune function by targeting arginase I. Clin Cancer Res 17 (2011) 6992-7002.
27. A. Daga, C. Bottino, R. Castriconi, R. Gangemi, and S. Ferrini, New perspectives in glioma immunotherapy. Curr Pharm Des 17 (2011) 2439-67.
28. S. Huang, Z. Song, T. Zhang, X. He, K. Huang, Q. Zhang, J. Shen, and J. Pan, Identification of Immune Cell Infiltration and Immune-Related Genes in the Tumor Microenvironment of Glioblastomas. Front Immunol 11 (2020) 585034.
29. M.K. Callahan, and J.D. Wolchok, Recruit or Reboot? How Does Anti-PD-1 Therapy Change Tumor-Infiltrating Lymphocytes? Cancer Cell 36 (2019) 215-217.
30. R.J. Kishton, M. Sukumar, and N.P. Restifo, Metabolic Regulation of T Cell Longevity and Function in Tumor Immunotherapy. Cell Metab 26 (2017) 94-109.
31. G.V. Sharonov, E.O. Serebrovskaya, D.V. Yuzhakova, O.V. Britanova, and D.M. Chudakov, B cells, plasma cells and antibody repertoires in the tumour microenvironment. Nat Rev Immunol 20 (2020) 294-307.
32. L.J. McHeyzer-Williams, and M.G. McHeyzer-Williams, Antigen-specific memory B cell development. Annu Rev Immunol 23 (2005) 487-513.

33. A. Dudeck, M. Koberle, O. Goldmann, N. Meyer, J. Dudeck, S. Lemmens, M. Rohde, N.G. Roldan, K. Dietze-Schwonberg, Z. Orinska, E. Medina, S. Hendrix, M. Metz, A.C. Zenclussen, E. von Stebut, and T. Biedermann, Mast cells as protectors of health. J Allergy Clin Immunol 144 (2019) S4-S18.

Tables

Table 1. Association with NCOA4 expression and clinicopathological variables.

Characteristic	Low expression of NCOA4	High expression of NCOA4	p
n	84	84	
Gender, n (%)			0.518
Female	32 (19%)	27 (16.1%)	
Male	52 (31%)	57 (33.9%)	
Race, n (%)			0.427
Asian	1 (0.6%)	4 (2.4%)	
Black or African American	5 (3%)	6 (3.6%)	
White	77 (46.4%)	73 (44%)	
Age, n (%)			0.354
<=60	40 (23.8%)	47 (28%)	
>60	44 (26.2%)	37 (22%)	
Karnofsky performance score, n (%)			1.000
<80	18 (14.1%)	18 (14.1%)	
>=80	45 (35.2%)	47 (36.7%)	
IDH status, n (%)			0.027
WT	81 (50.3%)	68 (42.2%)	
Mut	2 (1.2%)	10 (6.2%)	

Table 2. The univariate analysis with Logistic regression illuminated NCOA4 expression as a categorical

Characteristics	Total(N)	Odds Ratio(OR)	P value
Gender (Male vs. Female)	168	1.299 (0.689-2.463)	0.419
Race (White vs. Asian&Black or African American)	166	0.569 (0.185-1.611)	0.298
Age (>60 vs. <=60)	168	0.716 (0.388-1.312)	0.280
Karnofsky performance score (>=80 vs. <80)	128	1.044 (0.482-2.266)	0.912
IDH status (Mut vs. WT)	161	5.956 (1.505-39.614)	0.024

Figures

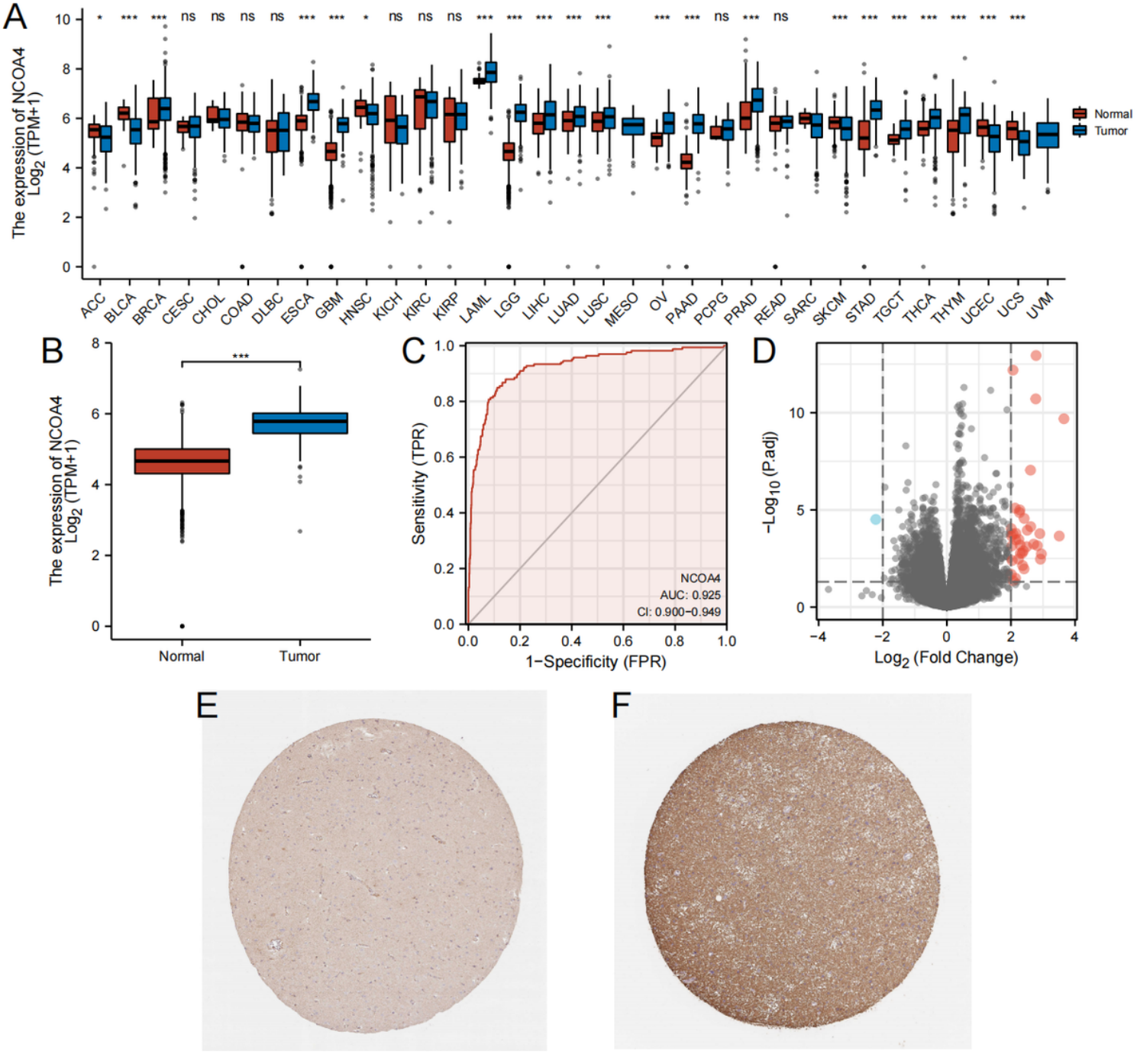

Figure 1

Expression of NCOA4 in pan cancer and GBM. (A) Overexpression of NCOA4 in pan cancer. (B) The expression of NCOA4 was significantly high in GBM samples. (C) The area under the curve (AUC) of NCOA4 is 0.925. (D) A total of 37 DEGs, covering 36 upregulated GENEs and 1 downregulated GENEs, were identified to be statistically significant. (E-F) The expression of NCOA4 in GBM tissues was higher than that in normal tissues in The Human Protein Atlas database. * p<0.05, ** p<0.01, *** p<0.001.

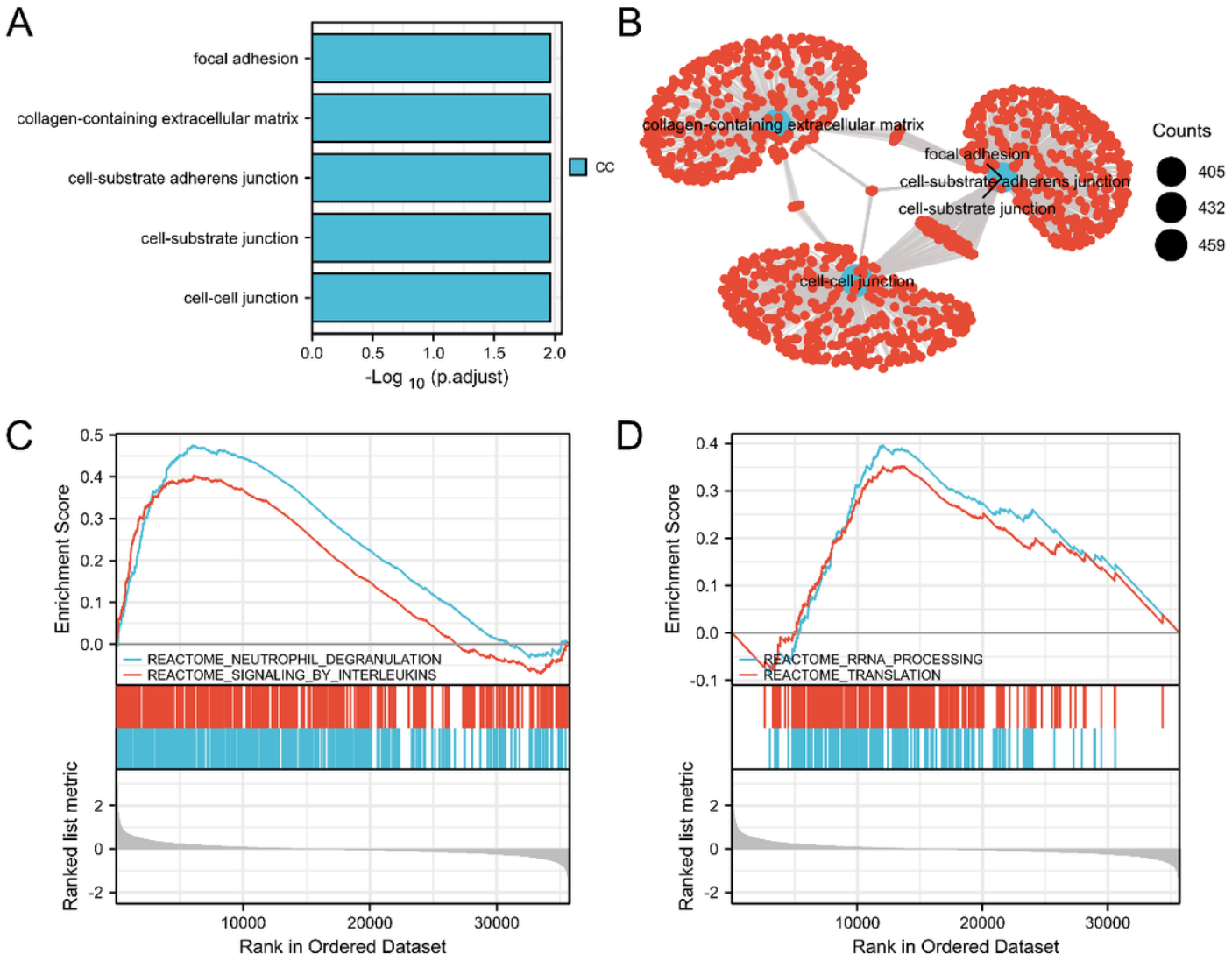

Figure 2

The functional enrichment information and signaling pathway of NCOA4 in GBM. (A-B) NCOA4-related genes were involved in focal adhesion, collagen-containing extracellular matrix, cell-substrate adherens junction, cell-substrate junction, cell-cell junction. (C-D) NCOA4 signaling pathways include neutrophil degranulation pathway, signaling by interleukins pathway, rRNA processing pathway and translation pathway.

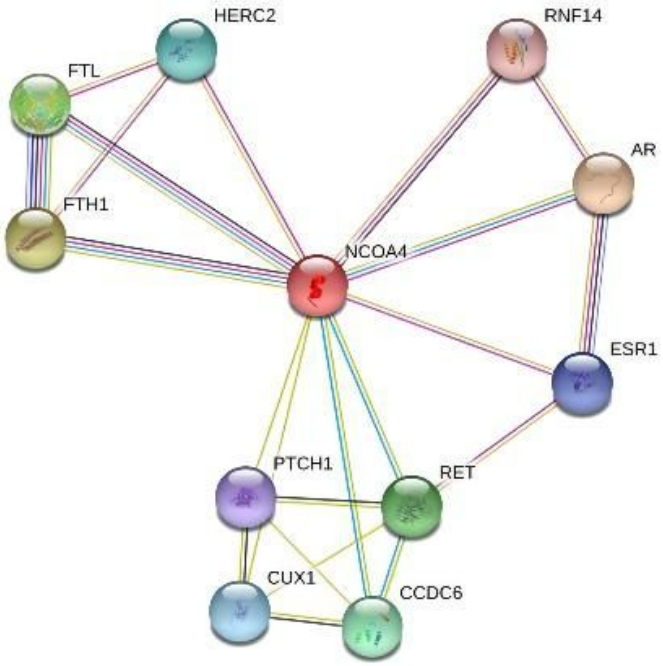

Figure 3

In Protein-Protein Interaction (PPI) Network, the top 10 hub genes included AR, FTL, FTH1, CCDC6, RET, GFRA1, CTNNB1, HSP90AA1, FOXA1, PELP1.

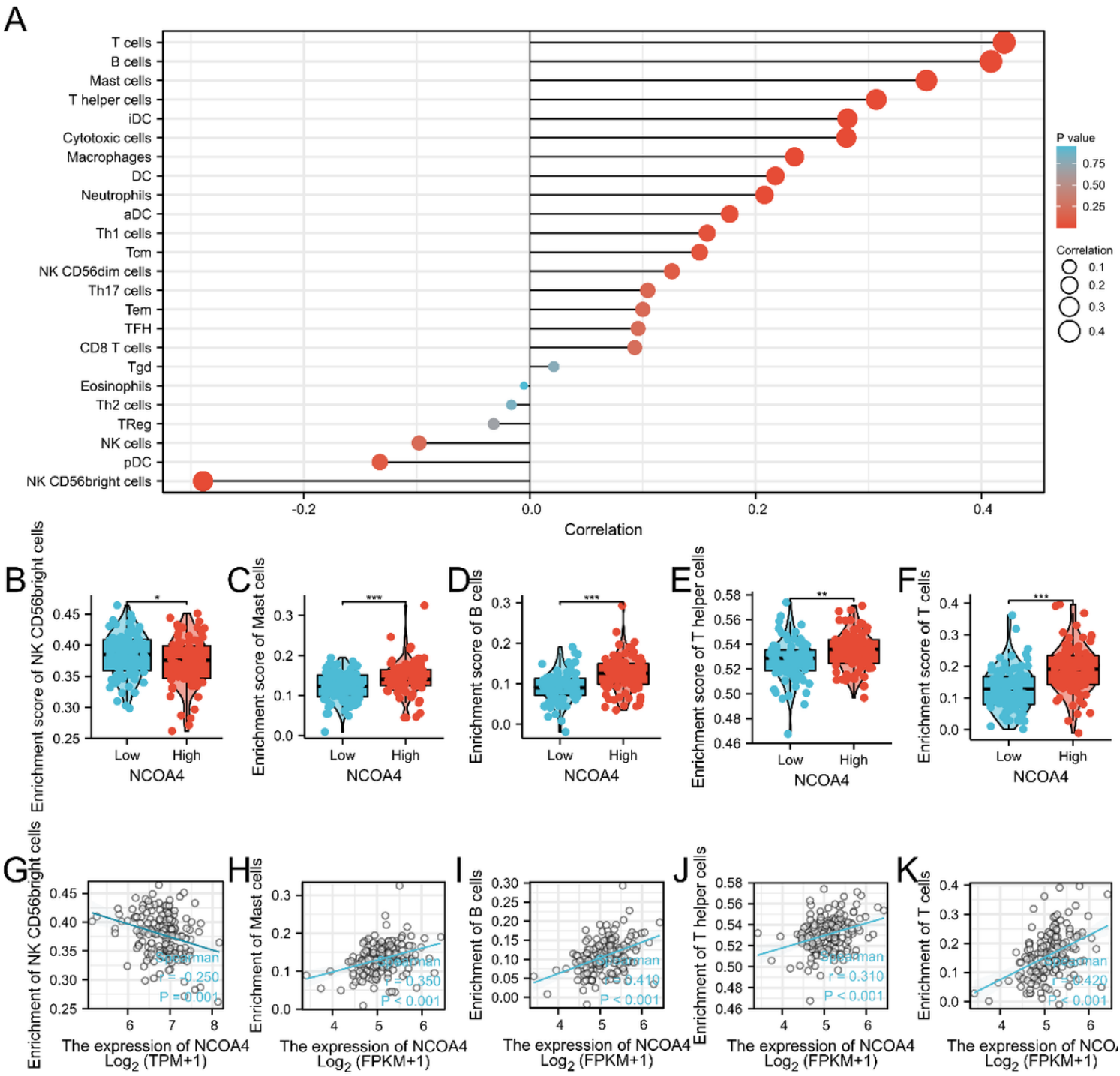

Figure 4

The correlation between NCOA4 expression and immune infiltration. (A) The correlation between the expression level (TPM) of NCOA4 and immune cell infiltration level quantified by ssGSEA was analyzed by spearman correlation. (B-K) The expression of NCOA4 was negatively correlated with the abundance of NK CD56 bright cells, and positively correlated with the abundance of mast cells, B cells, T cells, T helper cells.

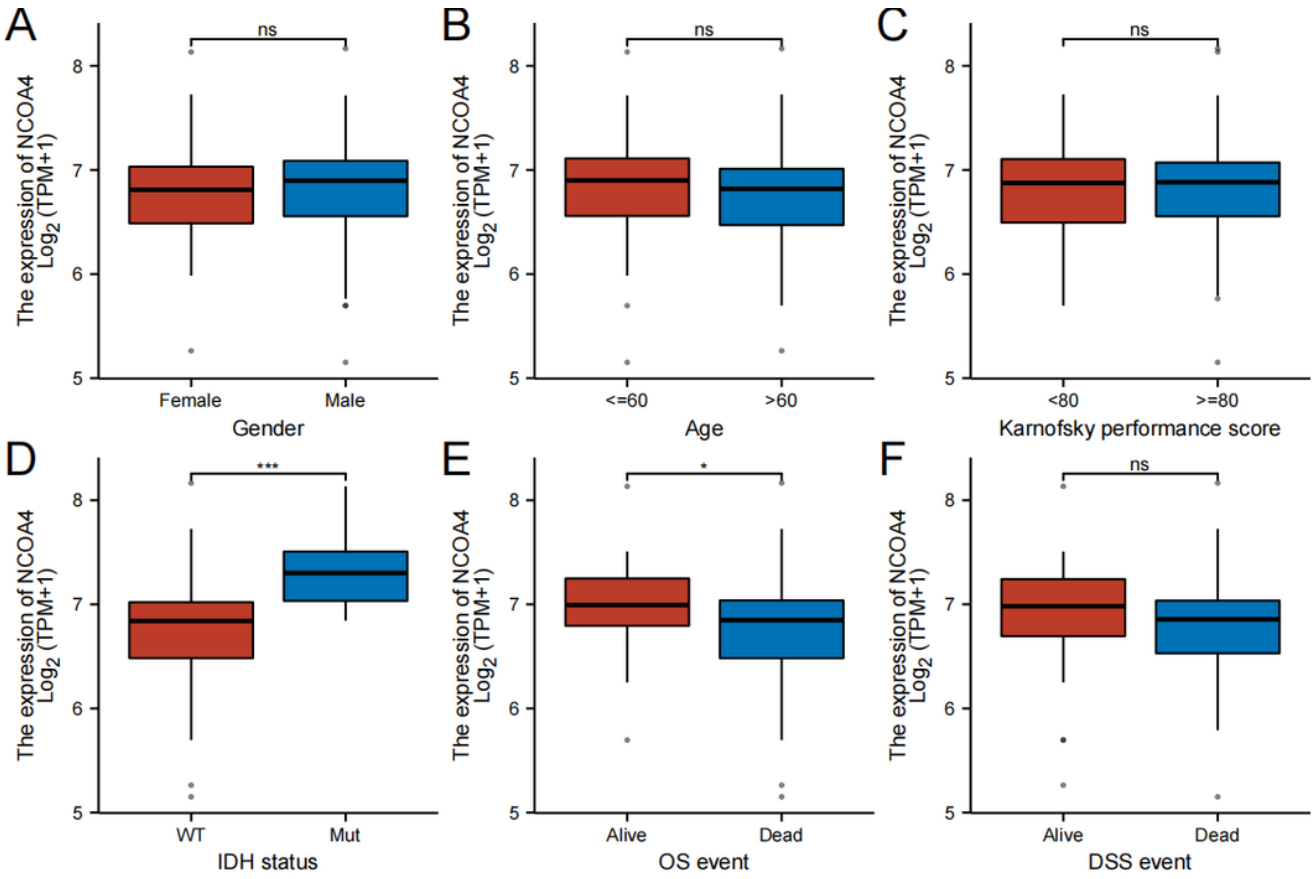

Figure 5

Association with NCOA4 expression and clinicopathological variables. (A-F) overexpressed NCOA4 was significantly correlated with IDH status and age. Others are not significant. ns p>0.05, * p<0.05, ** p<0.01, *** p<0.001.

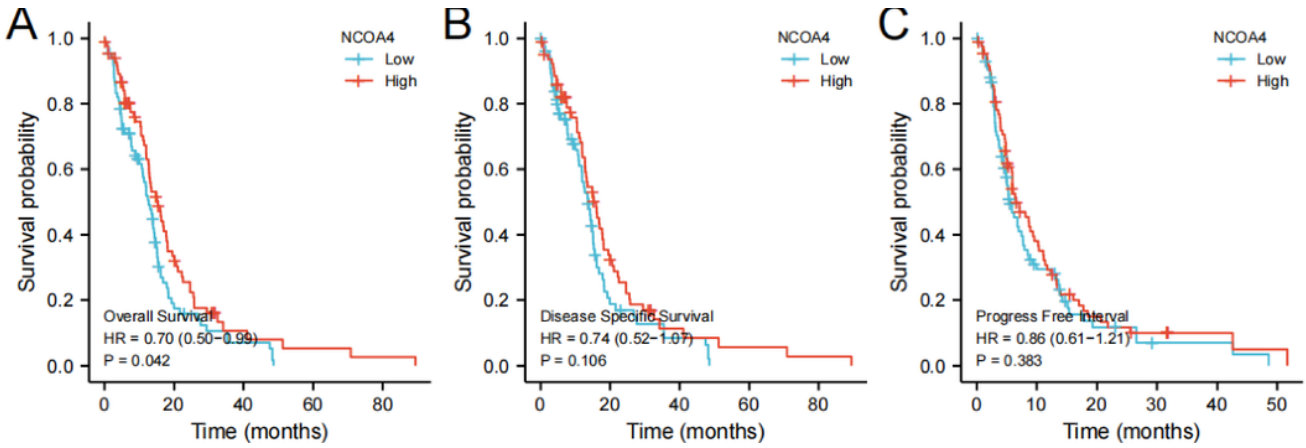

Figure 6

Low NCOA4 expression was closely associated with poor prognosis of patients with GBM. (A) The OS rates were significantly higher among patients with low NCOA4 expression than those with high NCOA4

expression. (B-C) The DSS rates and PFI in the NCOA4-high group were not significantly higher than those in the NCOA4-low group.

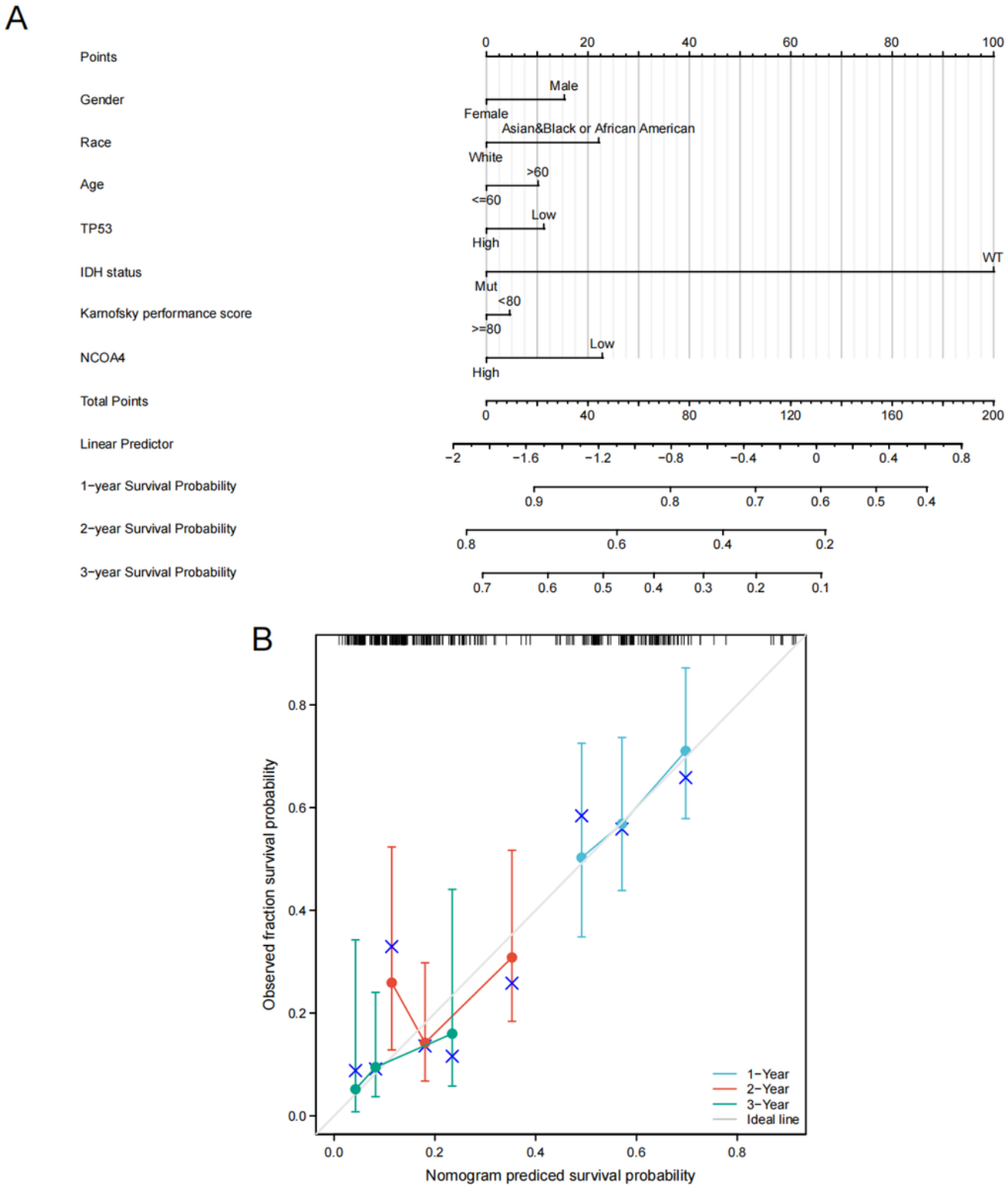

Figure 7

Construction and validation of a Nomogram based on the NCOA4. (A) NCOA4 and independent clinical risk factors were used to construct a nomogram. (B) The prediction efficiency of the nomogram, and the

C-index of the model was 0.622(CI: 0.0.590–0.653).