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NCAPD3 is a prognostic biomarker and is correlated with immune infiltrates in glioma

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Summary. Non-SMC Condensin II Complex Subunit D3 (NCAPD3) has been linked with the genesis and progression of multiple human cancers. Nevertheless, the scientific value and molecular process of NCAPD3 in glioma remain unclear. We explored the level of NCAPD3 expression in pan-cancer by multiple online databases. And we focused on the level and prognostic value of NCAPD3 expression in glioma by immunohistochemistry (IHC) and survival analysis. Meanwhile, we verified the relationship between NCAPD3, biological function and immune infiltration in glioma by Linkedomics and SangerBox databases. The expression of NCAPD3 was increased in a variety of cancers, including glioma. Its high expression was strongly related to WHO grade (P=0.002) and programmed cell death ligand 1 (PD-L1) expression of glioma (P=0.001). Patients with a high level of NCAPD3 expression had a lower overall survival (OS) in glioma than patients with a low level of NCAPD3 expression. Multivariate statistical analyses showed NCAPD3 expression (P=0.040), WHO grade P<0.001), 1p/19q codeletion (P<0.001), recurrence (P<0.001), age (P=0.023), and chemotherapy status (P=0.001) were meaningful independent prognostic factors in patients with glioma. Furthermore, bioinformatics analysis proved that NCAPD3 has been linked to immune infiltration in glioma. High level of NCAPD3 expression may serve as a promising prognostic biomarker and correlate with dendritic cell infiltration, representing a potential immunotherapy target in glioma.

Key words: NCAPD3, Glioma, Prognosis, Immune infiltrates, Biomarker

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Introduction

Glioma is the brain tumor with the highest incidence and mortality in adults (Sung et al., 2021). As to the categorization established by the World Health Organization (WHO), glioma is categorized into various grades, namely grade 1, grade 2 (referred to as low-grade glioma or LGG), grade 3, and grade 4 (known as high-grade glioma or HGG) (Louis et al., 2016). LGG has a median survival of 11.6 years and a 10-year survival rate of 47% (Ohgaki and Kleihues, 2005). However, the median overall survival (OS) time of HGG patients has a poor median OS time, only 15 months (Bleeker et al., 2012). The poor prognosis of glioma is due to the diffuse invasive growth of glioma cells and the immunosuppressive microenvironment (Danen et al., 2005; Cuddapah et al., 2014; de Gooijer et al., 2018). Studies have shown that there are many ways in which glioma cells can exert local immunosuppressive effects (Huang et al., 2020). On the one side, Glioma cells have the capacity to release a diverse array of cytokines and chemokines that promote tumor growth. Consequently, this secretion can influence the polarization of macrophages, facilitate the recruitment of regulatory T cells (Tregs), impede the maturation of dendritic cells (DCs), and hinder the activity of natural killer (NK) cells. Conversely, immunosuppressive substances like programmed cell death ligand 1 (PD-L1) can be expressed by glioma cells and it is known to restrain T cell proliferation and activation (Nduom et al., 2015). Although immunotherapy has been a breakthrough in the treatment of glioma, there are still some limitations. Therefore, identifying new and effective immune-related biomarkers and therapeutic targets will be helpful in advancing glioma treatment.

Eukaryotic cells contain two different types of condensin complexes, known as condensin I and condensin II (Kschonsak et al., 2017). They have the same subunits termed (SMC) family proteins (SMC2, SMC4), but the NCAPD3, NCAPG2, and NCAPH2 subunits are only present in condensin II complex. Non-

SMC Condensin II Complex Subunit D3 (NCAPD3) is one of the three non-SMC subunits of condensin II complex (Ono et al., 2003), which plays an important role in condensing and segregating mitotic chromosomes (Ono et al., 2013). Baergen et al. (2019) found that the condensin related genes (SMC4, NCAPD2, NCAPD3, NCAPG, NCAPG2, NCAPH) are frequently deletions or amplifications in many cancer types, including glioblastoma. Study confirms that overexpression of NCAPD3 condensin family genes results in poor OS and disease-specific survival in LGG (Dong et al. 2023; Qin et al. 2023). SMC4 was shown to contribute to the promotion of glioma cell proliferation, migration/invasion and tumorigenicity and to activate the TGFβ/Smad signaling pathway by Jiang et al. (2022). Li et al. (2023) identified a novel circ RNA (circ NCAPG) derived from NCAPG, which was overexpressed in glioma, promoting glioma stem cell progression. NCAPG2 enhances the malignant properties of glioblastoma cells and promotes tumour growth in xenografts by activating HBO1 via phosphorylation (Wu et al., 2021). These discovered functions indicated that the condensin family genes play an indispensable role in the tumorigenesis of glioma. Anyway, the clinical role and biological functions of NCAPD3 have not been elucidated in glioma.

Using public databases and immunohistochemistry (IHC), in this study we tried to measure the different-level expression of NCAPD3 in normal tissues compared to glioma tissues. We investigated the clinicopathological features and prognostic value of NCAPD3 in patients with glioma. We combined LinkedOmics, bioinformatics related to The Cancer Genome Atlas (TCGA), Gene Ontology (GO) and gene set enrichment analysis (GSEA) to analyse the relationship between NCAPD3-related genes and biological functions. Finally, the relationship between NCAPD3 and immune cells was analysed using the Tumour Immune Estimation Resource (TIMER) database and Gene Expression Profiling Interactive Analysis 2 (GEPIA2). Our results indicate that NCAPD3 is closely related to the cell cycle and immune processes of glioma, and the expression level of NCAPD3 is significantly correlated with DCs. These findings imply that NCAPD3 may influence the immunological process of glioma via DCs. Our findings propose new opportunities for pinpointing molecular indicators for treating glioma and predicting its prognosis.

Materials and methods

Clinical specimens

The patients participating in this study have given their consent and agreed to the utilization of their tumor tissue samples for examination, in accordance with the principles outlined in the Helsinki Declaration. The glioma tissue microarrays, comprising 122 samples of glioblastoma tissue and 27 samples of normal tissue, were supplied by Shanghai Outdo Biological Co., Ltd. All patients' clinical, pathological, and laboratory data were comprehensive. The categorization and staging of clinicopathological conditions were determined using criteria established by the American Joint Committee on Cancer (AJCC).

Immunohistochemistry

The tissue sections underwent deparaffinization using xylene and subsequent rehydration in a graded alcohol bath. This process was carried out by initially placing the sections at room temperature (RT), followed by rewarming and conventional dewaxing in an oven set at 65°C for a duration of 1 hour. The citric acid buffer with a concentration of 1% was subjected to boiling temperature prior to immersing portions of it for the purpose of antigenic thermal repair. Following the administration of three PBS flushes, a 3% H_2O_2 solution was applied to the tissue in order to suppress the activity of endogenous peroxidase. The slides were subsequently treated with the NCAPD3 monoclonal antibody (1:200, Cat No. 10727-1-AP, Proteintech, USA). In order to assess the level of coloring using a microscope, a reaction boosting reagent, secondary antibody reagent, and DAB color solution were used. Subsequently, PBS buffer was introduced to halt the progression of color development. PBS buffer was utilized for hematoxylin and reverse blue staining. The sheet was affixed using a neutral adhesive, and the image was subjected to microscopic examination in order to obtain statistical data on stains.

Evaluation of IHC staining

Using a double-blind control procedure, two pathologists (Lin Z and Yang Y) evaluated and scored all tissue specimens. For immunohistochemical analysis, A semi-quantitative score was used in combination with the percentage of positive area and the intensity of the staining. NCAPD3 staining intensity score (negative=0, weak=1, moderate=2, strong=3) multiplied by percentage of stained cells (25%=1, 26-50%=2, 51-75%=3, >75%=4). The staining degree was determined (value 0-12). We defined NCAPD3 immunostaining values 0-3 as normal expression and 4 or above as overexpression.

The TIMER database: Tumor Immune Estimation Resource

TIMER: Tumor Immune Estimation Resource (https://cistrome.shinyapps.io/timer/), A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. The TIMER database provides a complete investigation of the expression patterns of NCAPD3 mRNA across both cancerous and non-cancerous tissues. The utilization of this information facilitates the investigation of the potential correlation between the

expression of NCAPD3 and the levels of immune infiltration in various cancer subtypes. Additionally, the database enables the generation of scatter plots to visually represent these relationships. The DiffExp module enables users to investigate the variation in gene expression between tumour and surrounding normal tissues in all TCGA tumors for any gene of interest. Gene expression levels are depicted through box plots, while the Wilcoxon test is employed to assess the statistical significance of differential expression. The "SCNA" module compared the levels of tumor infiltration in glioma with different copy number changes of NCAPD3 (Li et al., 2017).

The GEPIA2 database

The GEPIA2 database, available at http://gepia2.cancer-pku.cn/#analysis, is a web server that has been upgraded to provide massive profiling of expression and interactive analysis (Tang et al., 2019). The present work investigated the expression degree of NCAPD3 across a diverse range of malignancies by utilizing the GEPIA2 database. In GEPIA2, additional analysis was undertaken to assess NCAPD3 expression in both normal brain tissue and gliomas.

The SangerBox database

The SangerBox database was utilized to assess the expression of NCAPD3 across various types of tumors. This work involved the examination of NCAPD3 expression in normal tissues and glioma tissues utilizing the given database. The information about healthy tissue was acquired from the Genotype-Tissue Expression (GTEx) database. Furthermore, the data pertaining to the glioma cell lines were obtained from the Cancer Cell Line Encyclopedia (CCLE) database. A comparative analysis was undertaken to assess the differential expression levels of NCAPD3 in glioma tumors and normal tissues. This research involved the integration of data from the TCGA and GTEx databases.

The UALCAN database

The "TCGA" module of the UALCAN database was employed to do a comparative study of NCAPD3 mRNA expression levels of tumor and healthy tissue. In this study, we evaluated the expression of NCAPD3 in glioma, taking into consideration the TP53 mutant status.

The LinkedOmics Database

The LinkedOmics database, accessible at http://www.linkedomics.org/, encompasses a comprehensive collection of multiomics and clinical profiles derived from 32 distinct cancer types. Additionally, it includes a substantial dataset of 11,158 patient profiles obtained from the TCGA research. We investigated the biological processes of the NCAPD3 gene family using the Link Interpreter module's GSEA. The GSEA was conducted utilizing the subsequent criteria: The FDR value corresponds to the Rank Criteria obtained from the LinkFinder Result. The Minimum Number of Genes (Size) is set to 3, and the Simulations are conducted for a total of 500 iterations.

The CGGA database

The CGGA record (http://www.cgga.org.cn/) is a database that comprises figures of brain tumours from a Chinese group of over 2000 examples. The clinical data and mRNA-seq data of glioma patients were acquired from the CGGA database. It includes examining the entire exome, DNA methylation, mRNA sequencing, microRNA and mRNA microarrays, as well as relevant clinical information (Zhao et al., 2021). The clinical data encompassed several parameters, including the diagnosis, age, sex, cancer type, grade, IDH1 mutation status, administration of radiation therapy, vital status, and OS rates. This study utilizes mRNAseq data from 693 and 325 samples in the CGGA dataset. We also examined factors that affect the prognosis of glioma using COX regression models.

Statistical analysis

The data analysis was performed by SPSS 26.0 software and GraphPad Prism 9.5.1 software. Paired T-test for independent means was used for group comparisons. To compare various groups, one-way ANOVA was used, while the Chi-square test was utilized to evaluate the association between NCAPD3 expression and clinicopathological features. The survival curves were constructed using the Kaplan-Meier methodology. Univariate and multivariate ratios of the risk of study factors were analysed using the Cox proportional hazards regression method. The value of $P<0.05$ was considered statistically significant.

Results

NCAPD3 was abnormally expressed in human pan-cancer

The TIMER database revealed that NCAPD3 mRNA was found to be significantly elevated in most human cancers relative to the corresponding normal tissues, such as bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESE), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), pheochromocytoma and paraganglioma (PCPG), rectum

adenocarcinoma (READ), stomach adenocarcinoma (STAD), and uterine corpus endometrial carcinoma (UCEC) (P<0.05). In contrast, NCAPD3 expression in thyroid carcinoma (THCA) was lower than those in normal tissues (P<0.05) (Fig. 1A). Similar results were found in the GEPIA2 database (Fig. 1B). Further checking of NCAPD3 levels in glioma tissues and neighboring tissues, using TCGA and SangerBox databases, revealed that NCAPD3 expression was notably greater in cancerous tissues than in neighboring tissues. (Fig. 1C,D, all P<0.05). Interestingly, we discovered that grade 3 glioma has higher levels of NCAPD3 expression than grade 2 glioma (Fig. 1E,F). And we found that NCAPD3 was significantly

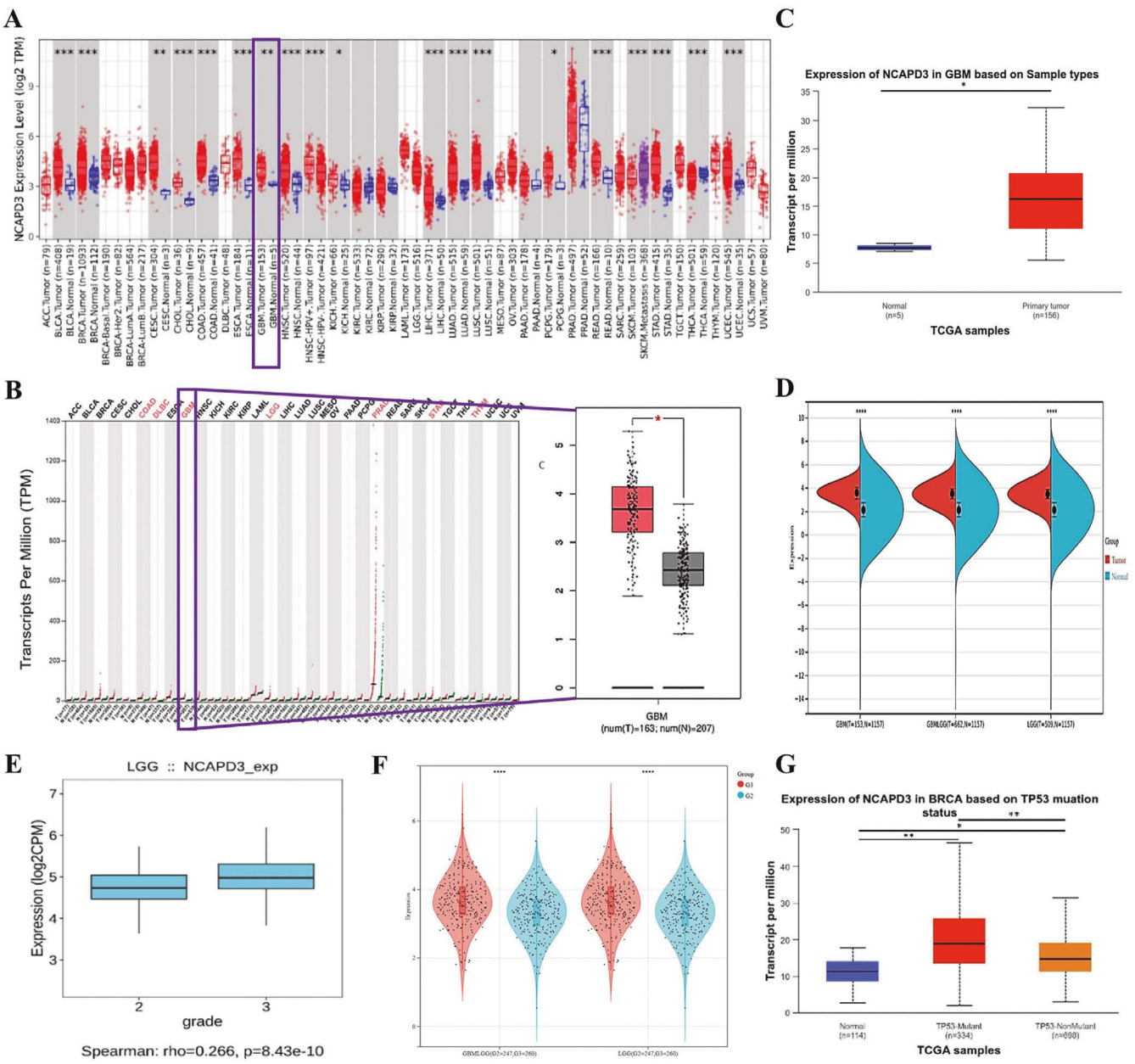

Fig. 1. High expression of NCAPD3 in glioma. **A.** Utilizing the TIMER database, the expression of NCAPD3 in numerous tumors was investigated. **B.** Using the GEPIA2 database, the differential expression of NCAPD3 across tumors was analyzed. **C, D.** The expression of NCAPD3 in cancer tissues was significantly higher than that in normal tissues (*P<0.05, ****P<0.0001). **E, F.** NCAPD3 expression was greater in grade 3 glioma compared to grade 2 glioma (****P<0.0001). **G.** NCAPD3 expression was significantly higher in TP53-mutant GBM than in normal tissues and non-mutant GBM (*P<0.05, **P<0.01).

differentially expressed in the TP53 mutation status of GBM. As shown, NCAPD3 expression levels were significantly different in normal tissues and TP53-mutant GBM (Fig. 1G, $P<0.01$); NCAPD3 expression levels were significantly different in normal tissues and non-TP53-mutant GBM (Fig. 1G, $P<0.05$); and NCAPD3 expression levels were significantly different in TP53-mutant and non-TP53-mutant GBM (Fig. 1G, $P<0.01$). These results indicate that NCAPD3 was extensively expressed in various types of cancer and the high expression level had a relation to the grade and the TP53 mutation status of glioma.

High level of NCAPD3 expression was closely associated with the malignancy degree of glioma

The GEO DataSet (GDS4515/201664_at) and TCGA revealed that the mRNA level of NCAPD3 expression was noticeably higher in GBM tissues compared to surrounding non-tumor tissues (Fig. 2A, $P<0.001$). IHC labeling for NCAPD3 was done on 122 GBM specimens and 27 neighboring nontumor samples to measure the degree of NCAPD3 expression in GBM. Cancer cells primarily had positive NCAPD3 staining in their cytoplasm. (Fig. 2B). The positive rate of NCAPD3 expression was dramatically higher in GBM (78.69%, 96/122) than in normal tissues (29.63%, 8/27) ($P<0.01$). In addition, the strongly positive rate of NCAPD3 was 59.84% (73/122) in GBM, which was also markedly higher than that in normal tissues (22.22%, 6/27) ($P<0.01$) (Fig. 2C). In addition, we looked at the correlation between glioma clinical pathological characteristics and NCAPD3 expression levels. It verified that NCAPD3 overexpression was correlated with WHO grade ($P=0.002$), and PD-L1 expression ($P=0.001$), but not correlated with gender, age, tumor size, or tumor location (Fig. 2D, Table 1). The results provide evidence for a favorable correlation between high levels of NCAPD3 expression and PD-L1 expression in gliomas.

High level of NCAPD3 expression indicates poor prognosis in patients with glioma

Kaplan-Meier analysis showed that glioma patients in the CGGA database who had high NCAPD3

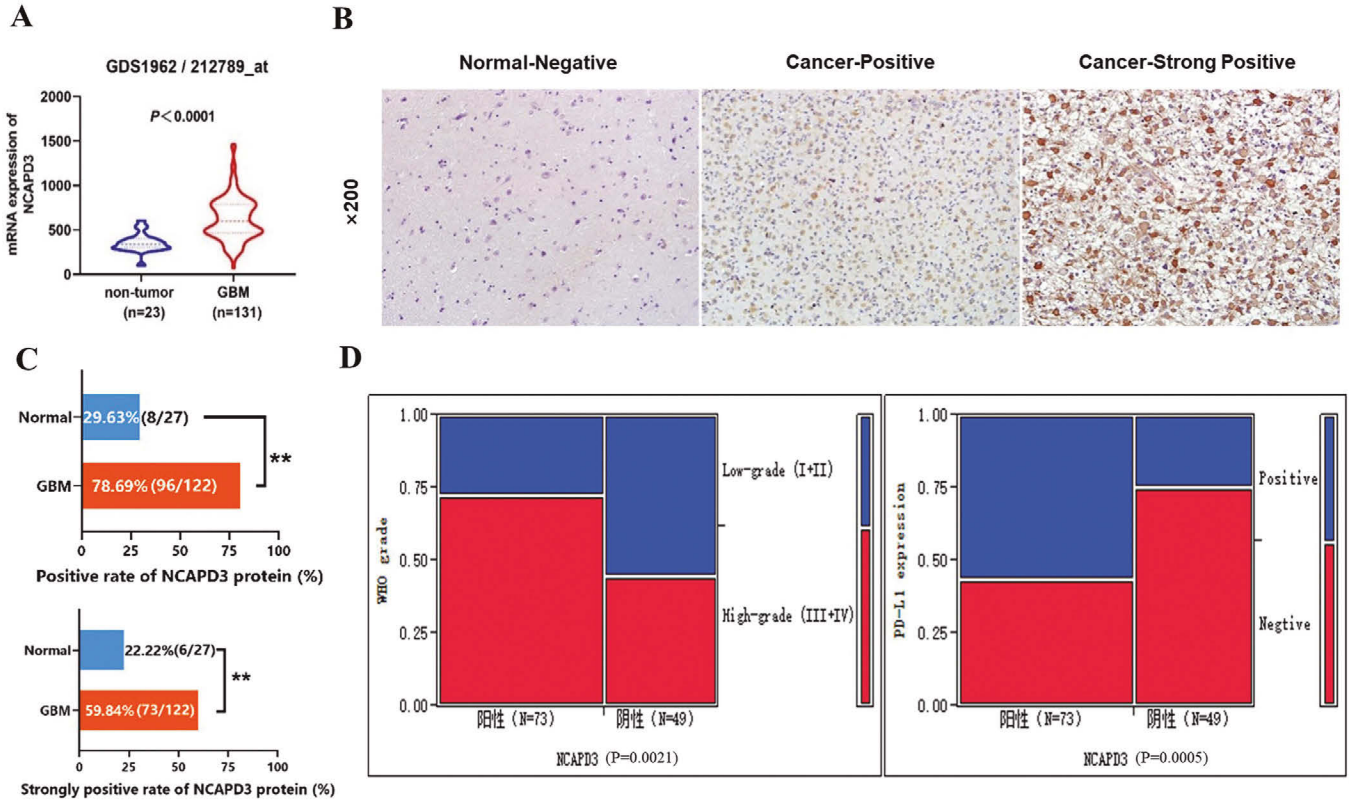

Fig. 2. High level of NCAPD3 expression was closely associated with the malignancy degree of glioma. **A.** Violin plots derived from gene expression data in GEO DataSet (GDS1962/212789_at) comparing the mRNA expression of NCAPD3 in normal and GBM tissues ($P<0.0001$). **B.** NCAPD3 expression in normal and GBM tissues as examined by IHC. **C.** Statistical results of IHC showed that NCAPD3 protein expression had positive and strongly positive staining rates in GBM tissues. **D.** NCAPD3 overexpression rates in different WHO grade of glioma and NCAPD3 overexpression rates in PD-L1 expression of glioma. × 200.

Table 1. NCAPD3 expression and clinicopathological features in GBM.

Features	No.	NCAPD3 positive expression	χ^2	*P*-value
Gender	73		0.043	0.835
Male	81	49 (60.49%)		
Female	41	24 (58.54%)		
Age (years)			0.114	0.736
<50	80	47 (58.75%)		
≥50	42	26 (61.90%)		
Tumor size			0.770	0.380
<4 cm	43	28 (65.12%)		
≥4cm	79	45 (56.96%)		
Tumor location			1.381	0.501
Left	49	28 (57.14%)		
Right	57	36 (63.16%)		
Other	12	9 (75.00%)		
WHO grade			9.502	**0.002**
Low-grade (I+II)	47	20 (42.55%)		
High-grade (III+IV)	75	53 (70.67%)		
PD-L1 expression			11.972	**0.001**
Positive	53	41 (77.36%)		
Negative	69	32 (46.38%)		

expression had a connection with an unsatisfactory overall survival. (Fig. 3A; *P*=0.0051). In recurrent glioma patients (n=253), it was discovered that patients with high NCAPD3 expression had a considerably lower OS than those with low NCAPD3 expression (Fig. 3B; *P*=0.024). Similarly, increased NCAPD3 expression was associated with a poorer OS in grade III glioma (Fig. 3C; *P*=0.0032). In addition, Univariate Cox regression shown NCAPD3 expression (*P*<0.001), 1p/19q codeletion (*P*<0.001), IDH status (*P*<0.001), recurrence (*P*<0.001), WHO grade (*P*<0.001), age (*P*<0.001), radioactive status (*P*=0.005) and chemotherapy status (*P*=0.014) were associated with the prognosis of glioma (Fig. 3D). The multivariate Cox regression analysis revealed that NCAPD3 expression (*P*=0.040), WHO grade (*P*<0.001), 1p/19q codeletion (*P*<0.001), recurrence (*P*<0.001), age (*P*=0.023), and chemotherapy status (*P*=0.001) were independent prognostic factors for patients with glioma (Fig. 3E). These findings suggested that NCAPD3 overexpression was associated with a poor outcome in glioma patients.

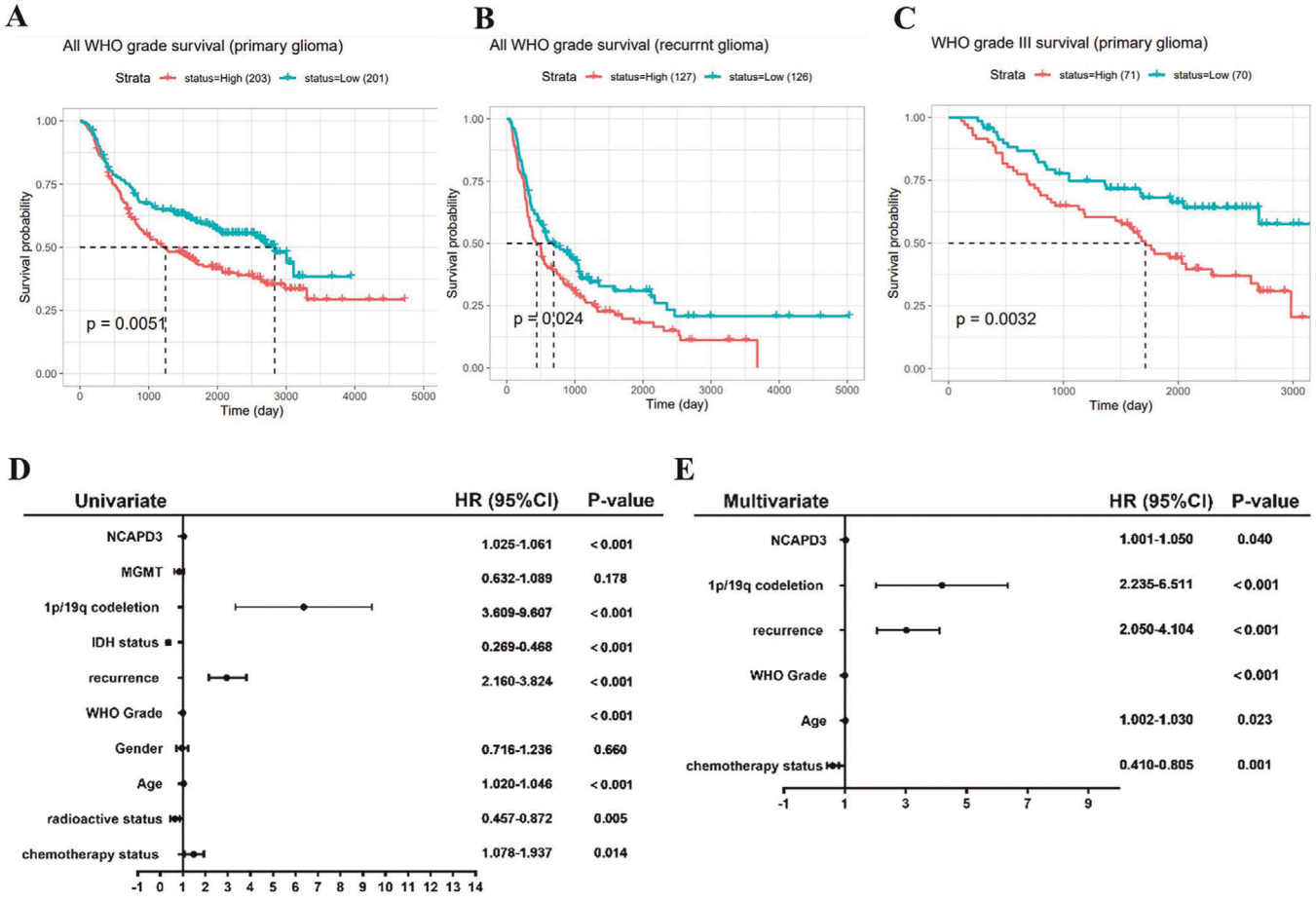

Fig. 3. In glioma patients, high levels of NCAPD3 expression were associated with a poor prognosis. **A-C.** Kaplan-Meier examination of the association between NCAPD3 and survival probability in CGGA datasets for all WHO-grade primary, recurrent, and grade III glioma. **D, E.** Forest plots showed the results of univariate **(D)** and multivariable **(E)** Cox regression analysis.

Gene function and enrichment analysis

The genes co-expressed with NCAPD3 in glioma patients in the TCGA dataset were first examined by LinkedOmics to further investigate the mechanism of NCAPD3 in glioma. The findings of the study revealed that 9234 genes have the significant correlations with NCAPD3 in glioma (FDR<0.05, P<0.05). Out of these 9234 genes, in 4216 of them a link was discovered between the expression of NCAPD3 and these factors, whereas 5018 showed a negative correlation (Fig. 4A). A heat map was employed to visually represent the 50

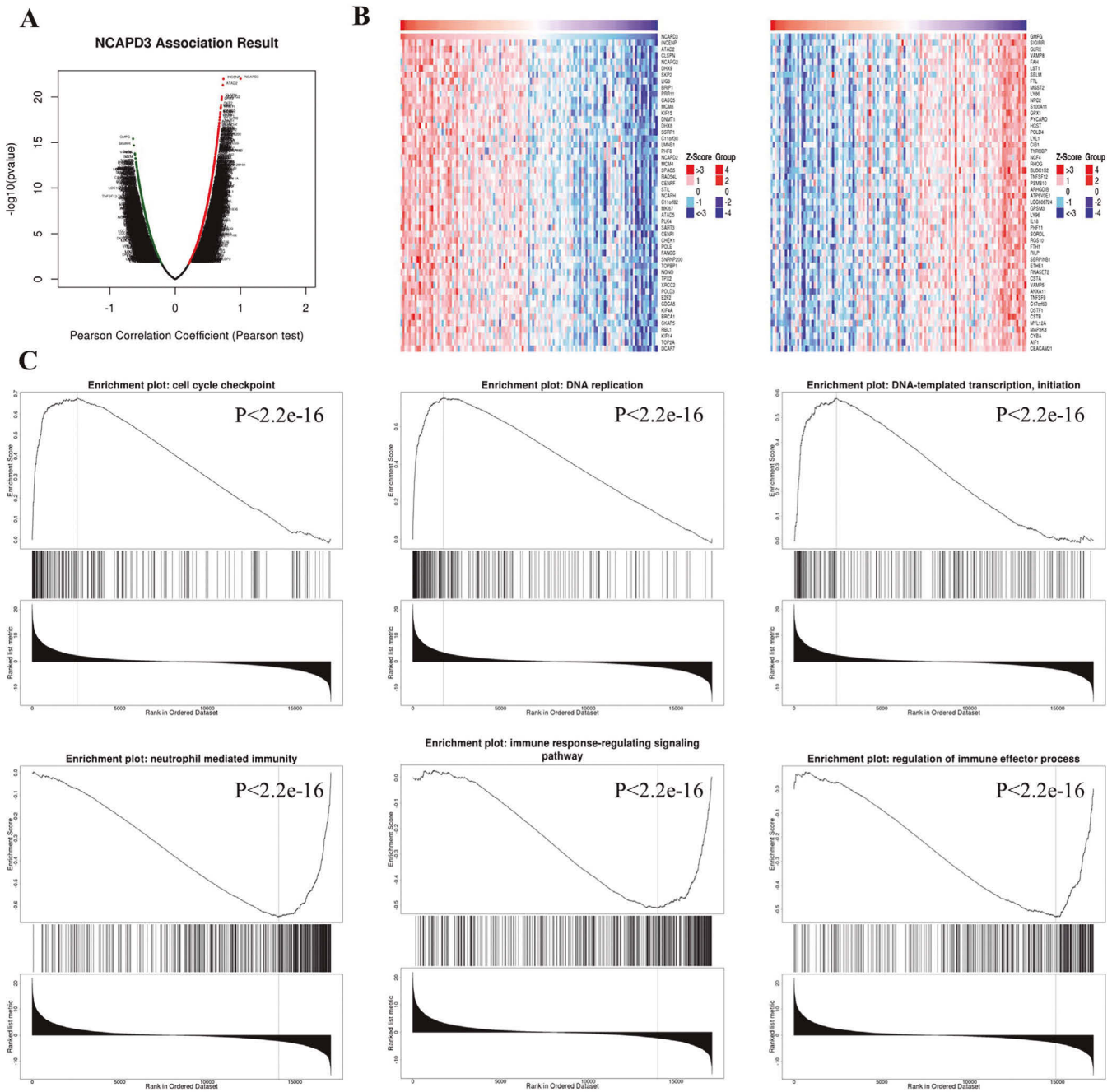

Fig. 4. The LinkedOmics database and enrichment analysis were used to examine NCAPD3 coexpression genes in GBM. **A.** Pearson test revealed highly associated NCAPD3 genes in a GBM cohort. **B.** The most significant 50 genes positively and negatively correlated to NCAPD3. **C.** The findings of the GO enrichment analysis.

genes that had positive and negative correlations with NCAPD3 (Fig. 4B). As for enrichment analysis, NCAPD3 was significantly associated with cell cycle checkpoint, DNA replication, DNA-templated transcription, initiation, neutrophil mediated immunity, immune response-regulating signaling pathway, and regulation of immune effector process in glioma (Fig. 4C). Hence, it may be inferred that NCAPD3 exhibits a significant association with both the cell cycle and immune response in glioma.

NCAPD3 expression levels affects immune cell infiltration in glioma

We analysed the association between NCAPD3 expression and immune process in glioma. A negative connection was seen between the expression of NCAPD3 mRNA and the ESTIMATE score, immune score, and stromal score (Fig. 5A, all $P<0.05$). We further explored the association between the expression level of NCAPD3 and immune cell infiltration level in GBM using Sanger Box. The results showed that NCAPD3 expression was positively correlated with infiltration abundances of B cells, Monocytes, NK cells, CD4 T cells and DCs, and negatively correlated with that of Macrophages (Fig. 5B). The levels of DCs infiltration seemed to be associated with alterations in the number of copies of NCAPD3 (Fig. 5C). Furthermore, we examined how NCAPD3 levels and immune cells present in tumours affect the survival rate of patients with glioma. As shown in Figure 5D, low expression of NCAPD3 in B cells led to a poor prognosis in patients with glioma ($P=0.043$), but high expression of NCAPD3 in DCs led to a poor prognosis in patients with glioma ($P=0.002$). Taken together, these findings suggested that NCAPD3 could be an immune related biomarker and regulate the immune process via DCs in glioma.

Discussion

The NCAPD3 protein plays a role in controlling Condensin II and is involved in many important tasks within cells, such as repairing DNA, separating sister chromatids, regulating gene expression, causing cellular senescence, and modifying histones (Csankovszki et al., 2009; Liu et al., 2010; Shintomi and Hirano, 2011; Samoshkin et al., 2012; Floyd et al., 2013; Yokoyama et al., 2015). It is reported that NCAPD3 helps to remove bacteria from cells in the lining of the human intestine (Schuster et al., 2015). Notably, elevated levels and frequent mutations of NCAPD3 are found in many somatic cancers (Leiserson et al., 2015). There is a significant negative correlation between the expression level of NCAPD3 and overall survival in pancreatic ductal adenocarcinoma (Dawkins et al., 2016). Jing et al. demonstrated that NCAPD3 enhances the progression of prostate cancer (PCa) by up-regulating EZH2 and MALAT1 (Jing et al., 2022a). NCAPD3 was overexpressed in colorectal cancer (CRC) tissues and positively correlated with poor prognosis of CRC patients (Jing et al., 2022b). Consistent with our results, we identified that the mRNA levels of NCAPD3 expression were significantly higher in human pan-cancer including COAD, rectum adenocarcinoma (READ) and prostate adenocarcinoma (PRAD). These discovered functions indicated that NCAPD3 plays an indispensable role in tumorigenesis and cancer progression.

Then, we focused on the level and prognostic value of NCAPD3 expression in glioma. We found that NCAPD3 was substantially increased in glioma tissues, and positively correlated with WHO grade ($P=0.002$), and PD-L1 expression ($P=0.001$). Similarly, a high level of NCAPG expression is associated with the tumor grade in patients with glioma (Zheng et al., 2022). The findings of this study indicate a positive association between elevated levels of NCAPD3 expression with both the presence of malignancy degree and the potential effectiveness of immunotherapy in glioma. Notably, prognostic value of NCAPD3 is found in many somatic cancers, such as colorectal cancer and pancreatic ductal adenocarcinoma (Dawkins et al., 2016; Jing et al., 2022b). In this research, we discovered that a high level of NCAPD3 expression was related to worse survival and was recognised as an autonomous predictor in glioma. The findings are additionally corroborated by previous studies that have examined the prognostic importance of abnormally elevated expression of NCAPD3 in prostate cancer (PCa). These studies have revealed high levels of NCAPD3 expression as predictive factors for survival (Yin et al., 2022). The results of this research indicate that NCAPD3 exerts a substantial influence on the grade and prognosis of glioma.

Many studies about biological function and signaling pathways of glioma have emerged recently. Wang et al. and Li et al. reported that enhanced activity of NF-Kb signaling pathway promoted growth of GBM *in vivo* and induced ferroptosis of glioma cell lines (Li et al., 2021). Esemen et al. reported that the p53 pathway was highly deregulated in GBM, the mutational status of TP53 was related with GBM growth (Esemen et al., 2022). Through GSEA, our study revealed that cell cycle checkpoint, DNA replication, DNA-templated transcription, initiation, neutrophil mediated immunity, immune response-regulating signaling pathway, and regulation of immune effector process were differentially enriched in glioma patients with high NCAPD3 expression phenotype, indicating that NCAPD3 may promote glioma cell growth and migration via these pathways. Those all suggest that NCAPD3 may serve as a potential therapeutic target in glioma.

Tumor-infiltrating immune cells (TIICs) are an indispensable component of the tumor micro-environment (TME), and play an important role in the growth and progression of tumors. However, the

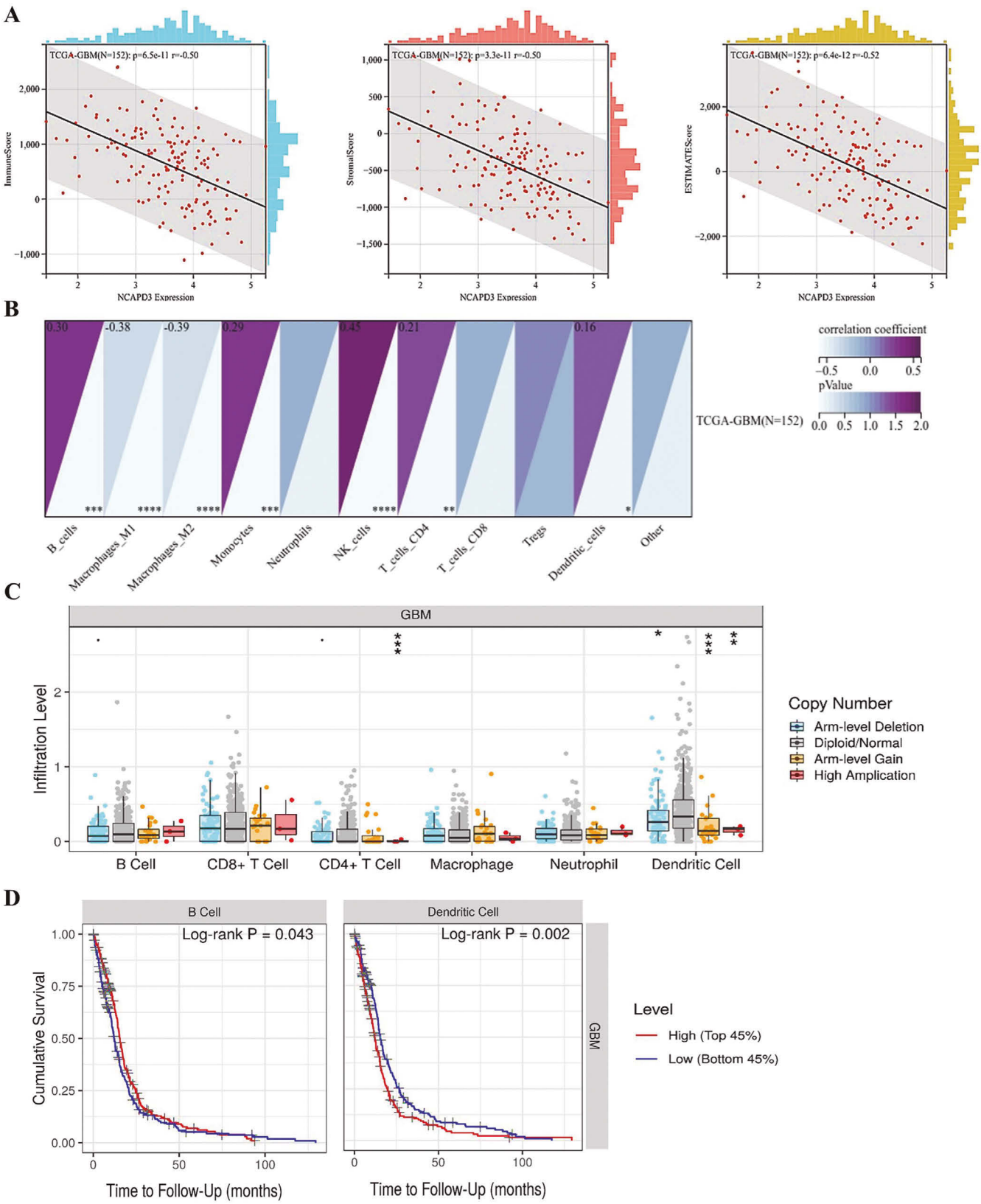

Fig. 5. The correlation involving NCAPD3 and immune infiltration in GBM. **A.** Pearson's method was used to evaluate the Immune Score, Stromal Score and ESTIMATE Score of NCAPD3 mRNA expression in GBM. **B.** The SangerBox database shows an association between NCAPD3 and immune types of cells in GBM (**P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001). **C.** NCAPD3 has an effect on the infiltration levels of CD4+ T cells and dendritic cells in GBM (**P*<0.05, ***P*<0.01, ****P*<0.001). **D.** Association of tumor cell immune infiltration with survival in patients with GBM.

correlation between NCAPD3 expression and immune cell infiltration in glioma has not been investigated. In the present study, the results revealed that NCAPD3 expression was positively correlated with infiltration abundances of B cells, Monocytes, NK cells, CD4 T cells and DCs, and negatively correlated with that of Macrophages. These correlations could be indicative of a potential mechanism by which NCAPD3 inhibits the function of Macrophages, and subsequently promotes the function of B cells, Monocytes, NK cells, CD4 T cells and DCs, which are responsible for maintaining the immunosuppresssive local microenvironment of tumor, and thus contribute to the development and progression of the tumor. In recent years, immunotherapy for GBM has made considerable achievements, particularly in DCs immunotherapy (Wang and Wang, 2022). DCs encompass a heterogeneous population of specialized antigen-presenting cells that play crucial roles in the initiation and modulation of both innate and adaptive immune responses (Powles et al., 1977; Wan and Dupasquier, 2005; Palucka and Banchereau, 2012). In this study, we found a close linkage between NCAPD3 and the immunity of GBM. It has been found that the infiltration of DCs and the expression of NCAPD3 are positively correlated. Additionally, a high level of NCAPD3 expression in DCs indicates a poor prognosis for patients with glioma. These findings suggest that NCAPD3 may be a potentially promising target by regulating its interaction with infiltrating immune cells in glioma patients. However, the detailed underlying mechanisms still need to be further explored.

Although the present study suggested some correlations between NCAPD3 expression and glioma, there are still some limitations in this study. Firstly, the number of healthy samples used as controls differed significantly from that of tumor samples, hence additional studies were required to maintain a balance of sample size. Secondly, the current study was performed primarily based on bioinformatic analyses and *in vitro* experiments, further more clinical samples of glioma patients are required to verify the abnormal expression of NCAPD3. Meanwhile, it is necessary to further elucidate the biological functions of NCAPD3 in glioma and the underlying molecular mechanisms in subsequent experiments. Last but not least, retrospective studies still have their own bias caused by potential confounding factors because of data from public databases, especially non-uniform intervening measures and lack of some detailed information; therefore, a prospective study should be performed in the future to avoid analysis bias.

Conclusions

The findings of our study indicate a notable association between elevated levels of NCAPD3 and an unfavorable prognosis, as well as increased immune infiltration in glioma. This suggests that NCAPD3 may contribute to the development of glioma by inducing aberrant inflammatory and immunological responses in affected patients. Additionally, the analysis of NCAPD3 shows a significant increase in different signal pathways, such as the cell cycle and immune system pathways. Therefore NCAPD3 may be involved in the progression of glioma. In aggregate, our findings indicate that NCAPD3 has the potential to function as a promising prognostic biomarker in glioblastoma. Furthermore, it exhibits a correlation with dendritic cell infiltration, so presenting itself as a prospective target for immune therapy in the context of glioma.

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Declaration of interests. The authors declare that they have no competing interests.

Authors contributions. Linzhuo Qu, Huiying Che and Hongjian Guan conceived this study and take responsibility for the quality of the data. Linzhuo Qu, Jingyu Zhao, Xin Lu and Zijun Ren prepared all figures. Linzhuo Qu, and Huiying Che participated in the tissue sample selection and experiments. Hongjian Guan and Huiying Che acquired data and played an important role in interpreting the results. Linzhuo Qu, Huiying Che, Yu Wu and Qian Liu performed the data analysis and wrote the manuscript. All authors read and approved the final manuscript.

References

Bleeker F.E., Molenaar R.J. and Leenstra S. (2012). Recent advances in the molecular understanding of glioblastoma. J. Neurooncol. 108, 11-27.

Baergen A.K., Jeusset L.M., Lichtensztejn Z. and McManus K.J. (2019). Diminished condensin gene expression drives chromosome instability that may contribute to colorectal cancer pathogenesis. Cancers 11, 1066

Cuddapah V.A., Robel S., Watkins S. and Sontheimer H. (2014). A neurocentric perspective on glioma invasion. Nat. Rev. Neurosci. 15, 455-465.

Csankovszki G., Collette K., Spahl K., Carey J., Snyder M., Petty E., Patel U., Tabuchi T., Liu H., McLeod I., Thompson J., Sarkeshik A., Yates J., Meyer B.J. and Hagstrom K. (2009). Three distinct condensin complexes control C. elegans chromosome dynamics. Curr. Biol. 19, 9-19.

Danen E.H., van Rheenen J., Franken W., Huveneers S., Sonneveld P., Jalink K. and Sonnenberg A. (2005). Integrins control motile strategy through a Rho-cofilin pathway. J Cell Biol. 169, 515-526.

Dawkins J.B., Wang J., Maniati E., Heward J.A., Koniali L., Kocher H.M., Martin S.A., Chelala C., Balkwill F.R., Fitzgibbon J. and Grose R.P. (2016). Reduced expression of histone methyltransferases KMT2C and KMT2D correlates with improved outcome in pancreatic ductal adenocarcinoma. Cancer Res. 76, 4861-4871.

de Gooijer M.C., Guillen Navarro M., Bernards R., Wurdinger T. and van Tellingen O. (2018). An experimenter's guide to glioblastoma invasion pathways. Trends Mol. Med. 24, 763-780.

Dong X., Liu T., Li Z. and Zhai Y. (2023). Non-SMC condensin I complex subunit D2 (NCAPD2) reveals its prognostic and immunologic features in human cancers. Aging 15, 7237-7257.

Esemen Y., Awan M., Parwez R., Baig A., Rahman S., Masala I., Franchini S. and Giakoumettis D. (2022). Molecular pathogenesis of glioblastoma in adults and future perspectives: A systematic review.

Int. J. Mol. Sci. 23, 2607.

Floyd S.R., Pacold M.E., Huang Q, Clarke S.M., Lam F.C., Cannell I.G., Bryson B.D., Rameseder J., Lee M.J., Blake E.J., Fydrych A., Ho R., Greenberger B.A., Chen G.C., Maffa A., Del Rosario A.M., Root D.E., Carpenter A.E., Hahn W.C., Sabatini D.M., Chen C.C., White F.M., Bradner J.E. and Yaffe M.B. (2013). The bromodomain protein Brd4 insulates chromatin from DNA damage signalling. Nature 498, 246-250.

Huang B., Li X., Li Y., Zhang J., Zong Z. and Zhang H. (2020). Current immunotherapies for glioblastoma multiforme. Front. Immunol. 11, 603911.

Jing Z., Liu Q., Xie W., Wei Y., Liu J., Zhang Y., Zuo W., Lu S., Zhu Q. and Liu P. (2022a). NCAPD3 promotes prostate cancer progression by up-regulating EZH2 and MALAT1 through STAT3 and E2F1. Cell. Signal. 92, 110265.

Jing Z., Liu Q., He X., Jia Z., Xu Z., Yang B. and Liu P. (2022b). NCAPD3 enhances Warburg effect through c-myc and E2F1 and promotes the occurrence and progression of colorectal cancer. J. Exp. Clin. Cancer Res. 41, 198.

Jiang L., Zhou J., Zhong D., Zhou Y., Zhang W., Wu W., Zhao Z., Wang W., Xu W., He L., Ma Y., Hu Y., Zhang W. and Li J. (2022). Correction: Overexpression of SMC4 activates TGFbeta/Smad signaling and promotes aggressive phenotype in glioma cells. Oncogenesis 11, 68.

Kschonsak M., Merkel F., Bisht S., Metz J., Rybin V., Hassler M. and Haering C.H. (2017). Structural basis for a safety-belt mechanism that anchors condensin to chromosomes. Cell 171, 588-600.e24.

Leiserson M.D.M., Vandin F., Wu H.T., Dobson J.R., Eldridge J.V., Thomas J.L., Papoutsaki A., Kim Y., Niu B., McLellan M., Lawrence M.S., Gonzalez-Perez A., Tamborero D., Cheng Y., Ryslik G.A., Lopez-Bigas N., Getz G., Ding L. and Raphael B.J. (2015). Pan-cancer network analysis identifies combinations of rare somatic mutations across pathways and protein complexes. Nat. Genet. 47, 106-114.

Li T., Fan J., Wang B., Traugh N., Chen Q., Liu J.S., Li B. and Liu X.S. (2017). TIMER: A web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res. 77, e108-e110.

Li S., He Y., Chen K., Sun J., Zhang L., He Y., Yu H. and Li Q. (2021). RSL3 drives ferroptosis through NF-κB pathway activation and GPX4 depletion in glioblastoma. Oxid. Med. Cell. Longev. 2021, 2915019.

Li H., Jiang Y., Hu J., Xu J., Chen L., Zhang G., Zhao J., Zong S., Guo Z., Li X., Zhao X. and Jing Z. (2023). The U2AF65/circNCAPG/RREB1 feedback loop promotes malignant phenotypes of glioma stem cells through activating the TGF-β pathway. Cell Death Dis. 14, 23.

Liu W., Tanasa B., Tyurina O.V., Zhou T.Y., Gassmann R., Liu W.T., Ohgi K.A., Benner C., Garcia-Bassets I., Aggarwal A.K., Desai A., Dorrestein P.C., Glass C.K. and Rosenfeld M.G. (2010). PHF8 mediates histone H4 lysine 20 demethylation events involved in cell cycle progression. Nature 466, 508-512.

Louis D.N., Perry A., Reifenberger G., von Deimling A., Figarella-Branger D., Cavenee W.K., Ohgaki H., Wiestler O.D., Kleihues P. and Ellison D.W. (2016). The 2016 World Health Organization classification of tumors of the central nervous system: A summary. Acta Neuropathol. 131, 803-820.

Nduom E.K., Weller M. and Heimberger A.B. (2015). Immunosuppressive mechanisms in glioblastoma. Neuro. Oncol. 17 (Suppl. 7), vii9-vii14.

Ohgaki H. and Kleihues P. (2005). Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. J. Neuropathol. Exp. Neurol. 64, 479-489.

Ono T., Losada A., Hirano M., Myers M.P., Neuwald A.F. and Hirano T. (2003). Differential contributions of condensin I and condensin II to mitotic chromosome architecture in vertebrate cells. Cell 115, 109-121.

Ono T., Yamashita D. and Hirano T. (2013). Condensin II initiates sister chromatid resolution during S phase. J. Cell Biol. 200, 429-441.

Powles R.L., Russell J., Lister T.A., Oliver T., Whitehouse J.M., Malpas J., Chapuis B., Crowther D. and Alexander P. (1977). Immunotherapy for acute myelogenous leukaemia: a controlled clinical study 2 1/2 years after entry of the last patient. Br. J. Cancer 35, 265-272.

Palucka K. and Banchereau J. (2012). Cancer immunotherapy via dendritic cells. Nat. Rev. Cancer 12, 265-277.

Qin S., Yuan Y., Liu H., Pu Y., Chen K., Wu Y. and Su Z. (2023). Identification and characterization of sex-dependent gene expression profile in glioblastoma. Neuropathology 43, 72-83.

Sung H., Ferlay J., Siegel R.L., Laversanne M., Soerjomataram I., Jemal A. and Bray F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J. Clin. 71, 209-249.

Samoshkin A., Dulev S., Loukinov D., Rosenfeld J.A. and Strunnikov A.V. (2012). Condensin dysfunction in human cells induces nonrandom chromosomal breaks in anaphase, with distinct patterns for both unique and repeated genomic regions. Chromosoma 121, 191-199.

Shintomi K. and Hirano T. (2011). The relative ratio of condensin I to II determines chromosome shapes. Genes Dev. 25, 1464-1469.

Schuster A.T., Homer C.R., Kemp J.R., Nickerson K.P., Deutschman E., Kim Y., West G., Sadler T., Stylianou E., Krokowski D., Hatzoglou M., de la Motte C., Rubin B.P., Fiocchi C., McDonald C. and Longworth M.S. (2015). Chromosome-associated protein D3 promotes bacterial clearance in human intestinal epithelial cells by repressing expression of amino acid transporters. Gastroenterology 148, 1405-1416.e3.

Tang Z., Kang B., Li C., Chen T. and Zhang Z. (2019). GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res. 47, W556-W560.

Wu J., Li L., Jiang G., Zhan H., Zhu X. and Yang W. (2021). NCAPG2 facilitates glioblastoma cells' malignancy and xenograft tumor growth via HBO1 activation by phosphorylation. Cell Tissue Res. 383, 693-706.

Wan H. and Dupasquier M. (2005). Dendritic cells *in vivo* and *in vitro*. Cell. Mol. Immunol. 2, 28-35.

Wang G. and Wang W. (2022). Advanced cell therapies for glioblastoma. Front. Immunol. 13, 904133.

Yokoyama Y., Zhu H., Zhang R. and Noma K. (2015). A novel role for the condensin II complex in cellular senescence. Cell Cycle 14, 2160-2170.

Yin Y., Liu Q., Shao Y., He X., Zhu Q., Lu S. and Liu P. (2022). Regulatory mechanism of androgen receptor on NCAPD3 gene expression in prostate cancer. Prostate 82, 26-40.

Zhao Z., Zhang K.N., Wang Q., Li G., Zeng F., Zhang Y., Wu F., Chai R., Wang Z., Zhang C., Zhang W., Bao Z. and Jiang T. (2021). Chinese Glioma Genome Atlas (CGGA): A comprehensive resource with functional genomic data from chinese glioma patients.

Genomics Proteomics Bioinformatics 19, 1-12.
Zheng G., Han T., Hu X., Yang Z., Wang J., Wen Z., Li H. and Wang H. (2022). NCAPG promotes tumor progression and modulates immune cell infiltration in glioma. Front. Oncol. 12, 770628.

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