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ORIGINAL RESEARCH

Prognostic and Immunological Significance of NMNAT1 in Colorectal and Pan-Cancer Contexts

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Introduction: Nicotinamide plays a critical role in the prevention and treatment of tumors, and its metabolism is closely associated with tumor progression. The aim of this study was to understand the prognostic and immunological significance of nicotinamide metabolism-related genes in pan-cancer.

Methods: We downloaded The Cancer Genome Atlas and Genotype Tissue Expression pan-cancer datasets for NMNAT1 from the UCSC database. We analyzed the differential expression, prognosis, genetic alterations, DNA methylation, immune infiltration, and co-expression with RNA modification-related genes and immune checkpoint-related genes. Genes with expression patterns similar to NMNAT1 were identified using the GEPIA library. The GSCA database was used to investigate the correlation between gene expression and drug sensitivity, as assessed by GDSC and CTRP. The CancerSEA database was employed to examine the association of NMNAT1 expression at the single-cell level across different tumors and its relation to 14 functional states. Immunohistochemistry was performed to assess the clinical significance of NMNAT1 expression.

Results: NMNAT1 exhibited differential expression across 25 tumor types, including colorectal cancer (CRC), and its expression was significantly associated with the prognosis of 11 tumors. Furthermore, NMNAT1 expression correlated significantly with clinicopathological features. NMNAT1 was strongly associated with immune cells, RNA modification-related genes, and immune checkpoint-related genes in most tumors, affecting immune responses. The expression of NMNAT1 also correlated with sensitivity and resistance to several drugs. Single-cell analysis revealed that NMNAT1 is involved in the progression of retinoblastoma, uveal melanoma, and CRC. Immunohistochemical analysis confirmed that NMNAT1 expression is an independent prognostic factor in patients with CRC.

Conclusion: NMNAT1 is a crucial prognostic and immune marker gene for nicotinamide metabolism, particularly in CRC. It has potential as a clinical biomarker and a therapeutic target for cancer treatment.

Keywords: NMNAT1, nicotinamide metabolism, pan-cancer analysis, prognosis, immune infiltration, colorectal cancer

Introduction

Colorectal cancer (CRC) is a prevalent malignant tumor, with more than 1.92 million new cases and 900,000 deaths globally in 2022. It has the third highest incidence and second highest mortality rate among all tumors.[1] In recent years, with the concept of precision therapy, the treatment approach for CRC has gradually evolved from traditional surgical treatment and systemic chemotherapy to molecular marker-guided biotargeted drugs or immunotherapy.[2–4] Although the 5-year survival rate of patients with early-stage CRC is relatively satisfactory, the treatment outcomes for advanced-stage patients are still unsatisfactory.[5] Therefore, identifying new biomarkers to guide individualized immunotherapy and targeted therapy in patients with cancer is crucial.[6]

Metabolic reprogramming is a hallmark of malignancy, and reprogrammed metabolic activity can be utilized for the diagnosis and treatment of tumors.[7] Nicotinamide (niacinamide) is the amide form of the water-soluble vitamin B3.

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Nicotinamide metabolism is strongly associated with aging and cancer.[8] Nicotinamide and its metabolites, such as nicotinamide adenine dinucleotide (NAD+), reduced NAD+ (NADH), NAD+ phosphate (NADP+), and NADH phosphate (NADPH), are pyridine compounds required for cellular bioenergy production and metabolism, and they are regulators of important biochemical processes in cells.[9,10] The nicotinamide metabolite NAD is involved in a variety of intracellular redox reactions, and high NAD levels can inhibit reactive oxygen species and prevent oxidative stress.[11] Previous studies have shown that nicotinamide can reduce tumor hypoxia and thus improve the efficacy of radiotherapy, as well as inhibit the expression of SIRT1 or PARP1 to enhance sensitivity to chemotherapy.[12–14] While increased NAD+ levels increase the effectiveness of anti-PD-L1 antibodies against immunotherapy-resistant tumors, tumors with high NAMPT expression are more responsive to anti-PD-L1 therapy.[15] Based on these studies, we hypothesized that nicotinamide metabolism is closely related to tumor progression and that nicotinamide metabolism-related genes (NMRGs) hold great promise for prognostic, targeted, and immunotherapeutic use in tumors.

In this study, 42 NMRGs were subjected to differential expression analysis and survival analysis based on The Cancer Genome Atlas (TCGA)-COAD database, and the key gene NMNAT1, which is closely associated with tumors, was screened out. To further elucidate the biological functions of NMNAT1 and its roles in cancers, we conducted pan-cancer analyses and systematically explored its differential expression, survival, gene mutation characteristics, and protein methylation modification. We thoroughly analyzed the correlation between NMNAT1, the tumor immune microenvironment, and immune checkpoint gene expression. Through functional enrichment and single-cell analyses, we further elucidated the relevant signaling pathways of NMNAT1 involved in tumorigenesis and development, as well as its potential molecular mechanisms. In addition, we verified the expression of NMNAT1 in patients by immunohistochemistry and evaluated its clinical prognostic value. These results suggest that NMNAT1 not only has a significant prognostic role in pan-cancer but may also play an important role in cancer progression by regulating the tumor immune microenvironment. In conclusion, NMNAT1 is an important prognostic marker and immune-related molecule, particularly in CRC, and a potential target for the treatment of patients with tumors.

Materials and Methods

Data Acquisition

For patients with colon cancer, gene expression data and associated clinical information were obtained from TCGA. Differentially expressed genes (DEGs) were identified by performing LIMMA analysis on genes from tumors and paracancerous tissues using R software.[16] These DEGs were then intersected with NMRGs obtained in previous studies to identify nicotinamide-related differentially expressed genes (NMRDEGs).[17] A univariate Cox regression model was applied to identify prognostic genes. For the pan-cancer analysis, we downloaded the expression data and clinical information of 34 cancer types from the UCSC database (https://xenabrowser.net/), which includes TCGA TARGET Genotype Tissue Expression dataset (PANCAN, N = 19,131, G = 60,499). We filtered the ENSG00000173614 (NMNAT1) gene expression data and performed a log2 (x + 0.001) transformation. Additionally, we conducted a pan-cancer analysis of NMNAT1 using online tools such as SangerBox 3.0 (http://sangerbox.com/) and TIMER 2.0 (TIMER2.0).[18,19]

Differential Expression and Prognostic Assessment of Pan-Cancer

Differential analysis of the expression of target genes in cancer and paracancerous tissues from 34 tumor samples was performed using R software. Statistical methods, including unpaired Wilcoxon rank-sum and signed-rank tests, were used for the analysis. Based on clinical information, including overall survival (OS), the Cox proportional hazards regression model was used to analyze the expression of NMNAT1 in the pan-cancer prognostic role. The results were visualized using forest plots when $P < 0.05$, which was considered statistically significant.

Correlation Analysis of Gene Expression With Staging and Grading of Cancer

We extracted NMNAT1 expression data and clinical T-, N-, M-, and grade-stage data from the pan-cancer database. Two-by-two significance analyses were performed using unpaired Student's t-tests, and differences in multiple samples were tested using ANOVA.

Genetic Alteration and DNA Methylation Analysis of NMNAT1

The cBioPortal database (https://www.cbioportal.org/) was used to analyze the type and frequency of mutations in NMNAT1. GSCA (https://guolab.wchscu.cn/GSCA/#/) was used to investigate the relationship between NMNAT1 expression and methylation levels. Differential analyses were performed using Spearman's rank correlation coefficient, and the results were visualized using bubble plots.[20,21]

Immune Infiltration Correlation Analysis

Based on information from the pan-cancer database, we assessed the correlation between NMNAT1 expression and immune infiltration scores using the Pearson correlation coefficient. Immune infiltration-related scores, including stromal, immune, and ESTIMATE scores, were calculated using the R software package ESTIMATE.[21] The correlation between gene expression levels and the six immune cells in different tumors was further re-evaluated using the timer method from the R package IOBR.[22]

Analysis of RNA Modification-Related Genes and Immune Checkpoint-Related Genes

We extracted the ENSG00000173614 (NMNAT1) gene, 44 marker genes for three classes of RNA modification (m1A (10), m5C (13), m6A (21)) genes, and 60 genes for two classes of the immune checkpoint pathway (inhibitory (24) and stimulatory (36)) from the pan-cancer dataset. The Pearson correlations between NMNAT1 and these marker genes were calculated and visualized using heatmaps.[23]

Functional Enrichment Analysis and Protein–Protein Interaction Analysis

Overall, 100 genes with similar expression patterns to NMNAT1 were obtained from the "Similar Genes Taction" module of the GEPIA repository. To explore the potential functions of NMNAT1, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed based on genes co-expressed with NMNAT1. The results were visualized using the R package ClusterProfiler (version 3.14.3). Additionally, the STRING database was used to construct a protein–protein interaction (PPI) network to explore the molecular mechanisms of NMNAT1 gene in tumorigenesis.

Drug Sensitivity Analyses

The GSCA drug sensitivity module of GSCA (https://guolab.wchscu.cn/GSCA/#/) was used to explore the correlation between gene expression and GDSC and CTRP drug sensitivity in pan-cancer.[21]

Single-Cell Sequencing

We explored the association between NMNAT1 expression and 14 functional states at the single-cell level in different tumors using the CancerSEA database (http://biocc.hrbmu.edu.cn/CancerSEA/goSearch). To obtain statistically significant results, we set filter values for correlation strength ≥ 0.3 and P value < 0.05.[24]

Immunohistochemical Analysis

Surgical samples from 123 patients with colorectal adenocarcinoma who underwent radical surgery were collected at Guangdong Provincial People's Hospital. Patients without complete follow-up data or poor-quality tissue samples were excluded. The Ethics Committee of Guangdong Provincial People's Hospital approved this study, and each participant provided signed informed consent. Protein expression in CRC tissues was detected according to the manufacturer's instructions of the immunohistochemistry kit. Primary antibodies for NMNAT1 (cat. No.: 11,399-1-AP, Proteintech, 1:200) were used. The pathological results were analyzed separately by two senior specialists. Hematoxylin-stained nuclei were blue in color, and DAB showed positive expression in a brownish-yellow color. The criteria for evaluation of staining intensity were as follows: 0, no staining; 1, pale yellow (weakly positive); 2, brownish yellow (moderately positive); and 3, brown (strongly positive). According to the proportion of positive cells in the total number of cells, ≤25% is 1 point, 26–50% is 2 points, 51–75% is 3 points, and 76–100% is 4 points. The percentage of positive results and staining intensity score were multiplied to calculate the final staining index.

Statistical Analysis

Independent prognostic factors were identified using the Cox regression model, and the prognostic value was evaluated using Kaplan–Meier analysis with the Log rank test. Kruskal–Wallis or Wilcoxon two-sample tests were used to analyze data with non-parametric features. Chi-squared and Fisher's exact tests were used to examine clinical features. R software was used for the statistical analysis and visualization, and a P-value < 0.05 (*) was considered as significant.

Results

Five thousand one hundred fifty-eight differential genes were identified based on LIMMA analysis (Figure 1a) of genes expressed in tumors and paracancerous tissues (P < 0.01, fold change > 1.5). By intersecting 42 NMRGs with the 5158 DEGs, we identified eight NMRDEGs[17] (Figure 1b). NMNAT1 was the only nicotinamide metabolism-related prognostic gene in COAD according to the univariate Cox regression analysis of NMRDEGs (Figure 1c). The Kaplan–Meier curve showed that OS was significantly better in the high-expression group than in the low-expression group (Figure 1d).

Differential Expression and Prognostic Assessment of Pan-Cancer

To investigate the expression characteristics of NMNAT1 in pan-cancer cells, a comprehensive differential expression analysis was performed in 34 different cancer types. The results of the analysis showed that NMNAT1 exhibited significant expression differences in 25 cancer types compared to the corresponding normal tissues, whereas no significant expression changes were observed in the remaining nine cancer types. Particularly, the expression of NMNAT1 was significantly upregulated in 11 tumor types and significantly downregulated in 14 other tumors, implying that it plays an important role in the development of these cancers (Figure 2a). Univariate Cox regression analyses were performed using OS information from 44 cancer types to better explore the prognostic value of NMNAT1 in pan-cancers. The results showed that four tumor types with high expression had a poor prognosis, whereas seven tumor types with low expression had a poor prognosis (Figure 3). In addition, we used the survival analysis module of the GEPIA2 database to obtain the Kaplan–Meier curve of NMNAT1 expression in patients with pan-cancer and found that patients with low NMNAT1 expression had better OS (Figure 2b) and disease-free survival (Figure 2c).[25]

Correlation Analysis of Gene Expression With Staging and Grading of Cancer

In this study, we investigated the association between NMNAT1 expression and clinicopathological features in pan-cancer and showed that marker gene expression was significantly correlated with grade-stage (Figure 4d) in five tumors, T-stage in eight tumors (Figure 4a), N-stage in five tumors (Figure 4b), and M-stage in five tumors (Figure 4c).

Genetic Alteration and DNA Methylation Analysis

Because epigenetics can influence the mRNA expression level of a gene, we used the cBioPortal and GSCA datasets to examine the genetic alterations and DNA methylation levels of NMNAT1. Lung cancer, mature B-cell neoplasms, cervical cancer, esophageal cancer, and ovarian cancer showed the highest frequency of NMNAT1 alterations (Figure 5a). Furthermore, amplification was the most common form of genetic modification of NMNAT1 (Figure 5a). In most cancers, NMNAT1 expression negatively correlated with DNA methylation levels, particularly in PCPG, LGG, and BRCA (Figure 5b).

Immune Infiltration Correlation Analysis

To further investigate the association of NMNAT1 with tumor immunity, we calculated the pan-cancer Pearson's correlation coefficient between NMNAT1 and immune infiltration scores. The results showed that the immune infiltration scores were strongly correlated with the expression of this gene in a wide range of malignant tumors, with 10 significantly positive and five significantly negative correlations. NMNAT1 was highly correlated with the degree of immune infiltration in KIPAN, THCA, GBMLGG, and LGG (Figure 6b–e). In addition, according to the TIMER algorithm, we obtained the infiltration scores of six types of immune cells (B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells) in different cancer types and found that the expression of

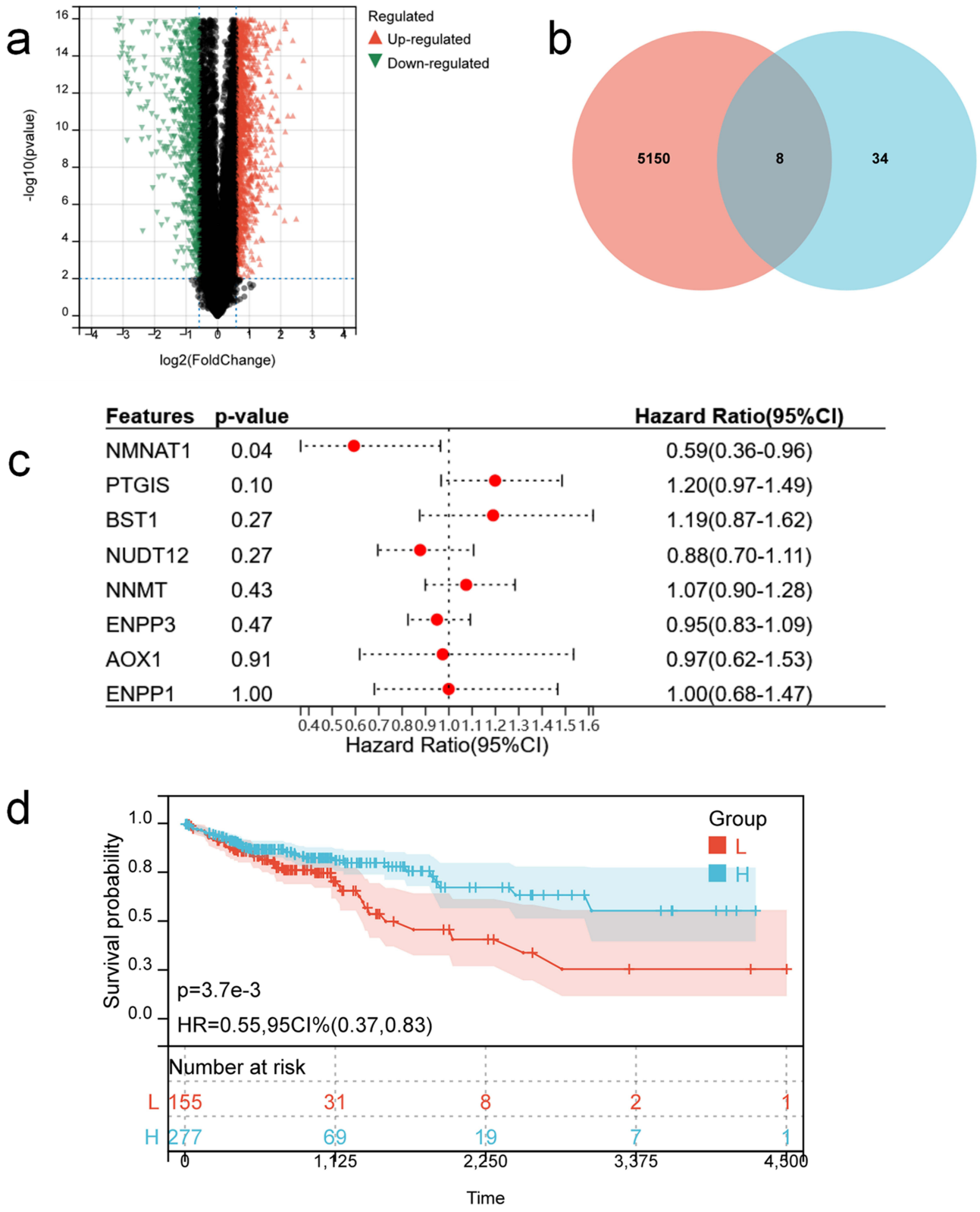

Figure 1 Identification of prognostic genes from nicotinamide metabolism genes, including NMNAT1. (**a**) A total of 5158 differentially expressed genes (DEGs) identified from LIMMA analysis of cancerous and paracancerous tissues. (**b**) Intersection of nicotinamide metabolism-related genes (NMRGs) and DEGs. (**c**) Univariate Cox regression analysis of nicotinamide-related differentially expressed genes (NMRDEGs). (**d**) Kaplan–Meier curve for OS based on NMNAT1 expression in CRC.

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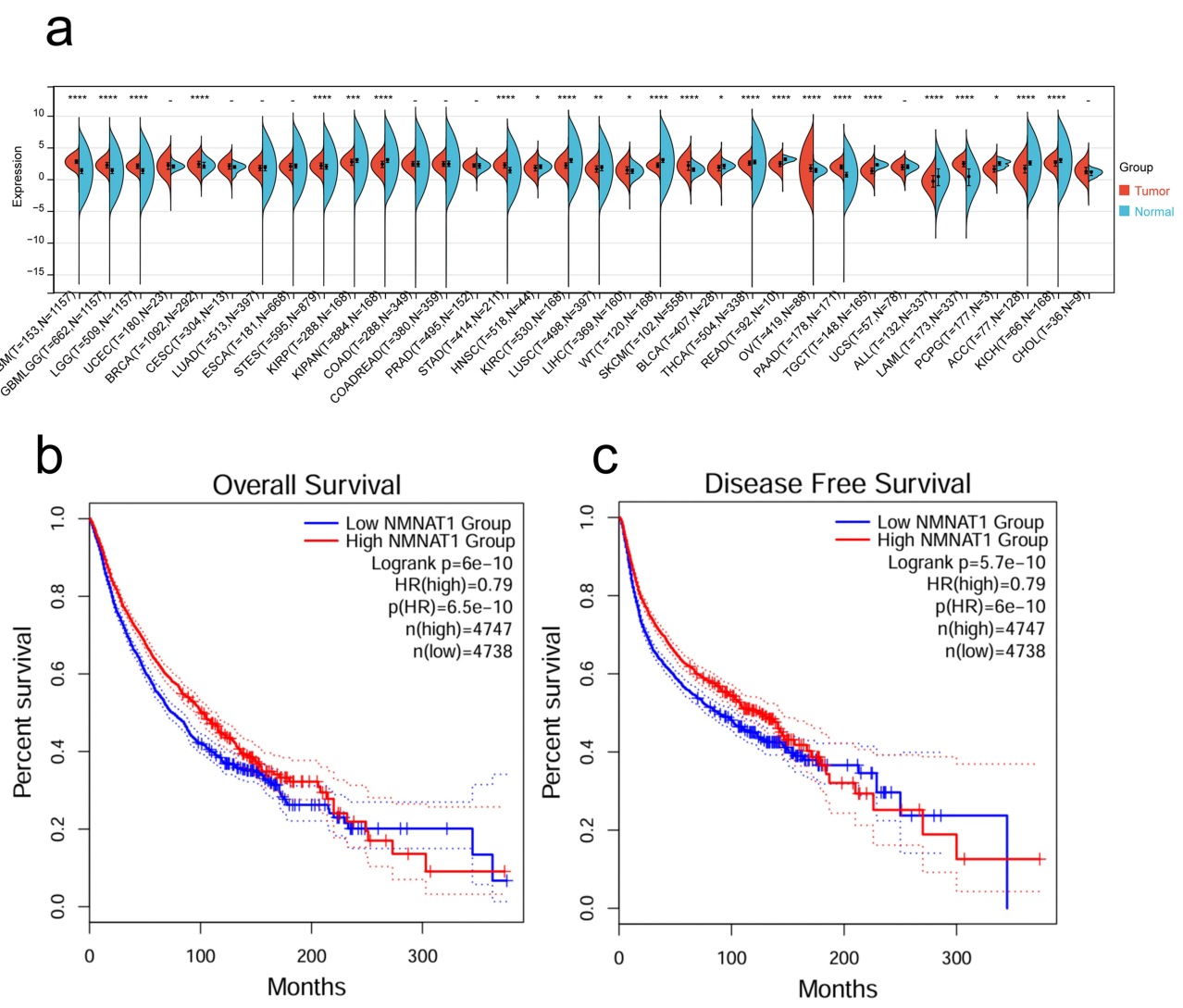

Figure 2 (**a**) Differential analysis of the expression of target genes in cancerous and paracancerous tissues across 34 tumor samples. (**b** and **c**) Kaplan–Meier curve for OS and DFS (****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05).

this gene in most cancers was positively correlated with these six types of immune cells (Figure 6a). These results suggest a potential link between NMNAT1 and tumor immunity.

Analysis of RNA Modification-Related Genes and Immune Checkpoint-Related Genes

A comparison of NMNAT1 with RNA modification-related genes, including m1A (10), m5C (13), and m6A (21), revealed that these three gene families were strongly correlated with NMNAT1 expression in most tumor types (Figure 7). In malignancies, such as CHOL, MESO, NB, TGCT, UCS, and READ, this correlation was more pronounced in all three gene families, with an overall positive correlation. When NMNAT1 was analyzed using immune checkpoint-related genes, both types of genes were positively correlated with NMNAT1 expression in most tumor types, and only negatively correlated with the expression in THCA, KIRP, and CHOL (Figure 8).

Functional Enrichment Analysis and PPI Analysis

GO analysis showed that NMNAT1-related genes were mainly involved in the actin cytoskeleton, cellular protein metabolic processes, perinuclear region of the cytoplasm, catalytic complex, U2-type catalytic step 1 spliceosome, and other biological processes (Figure 9a). KEGG analysis showed that NMNAT1 is involved in tumor progression through

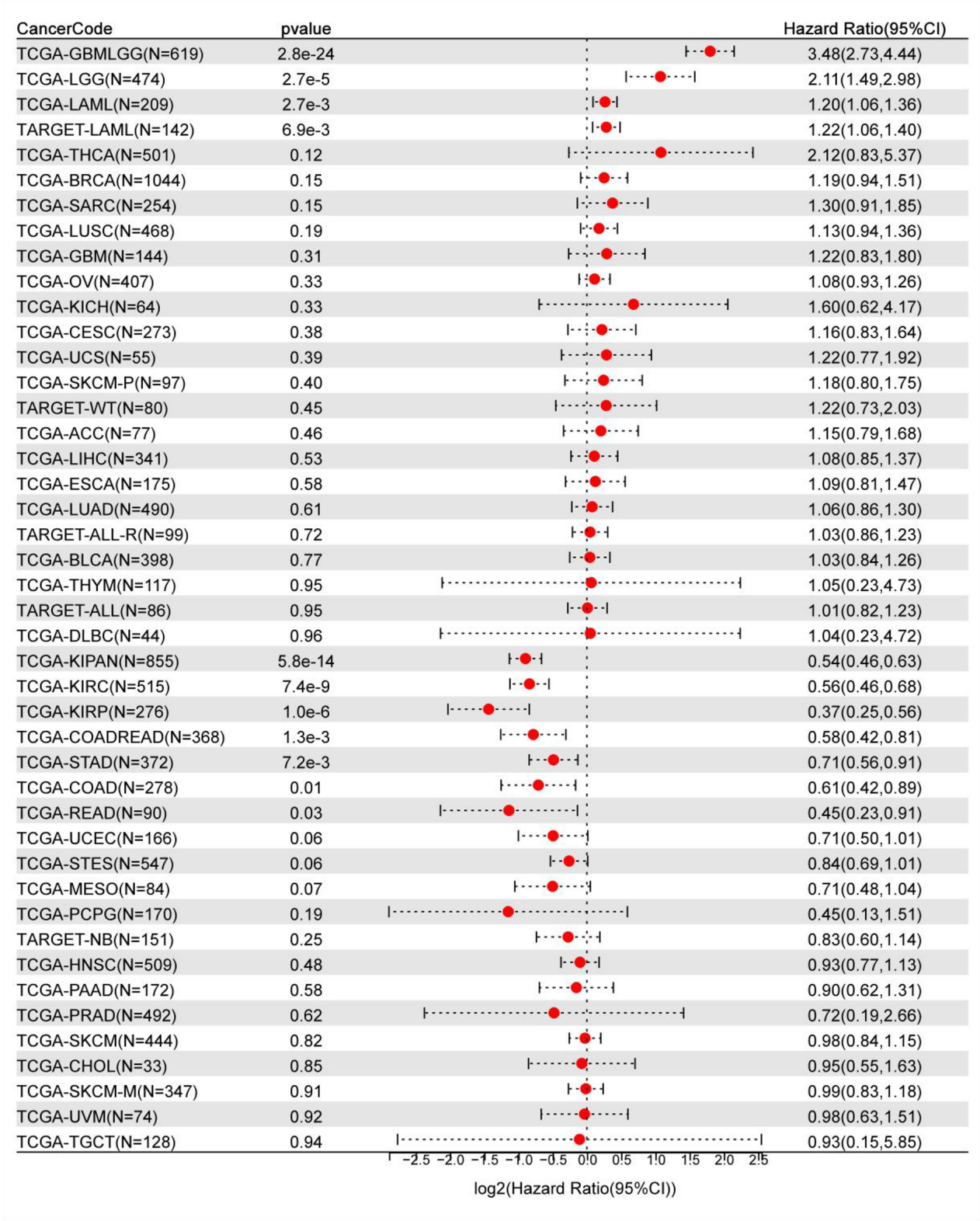

Figure 3 Cox proportional hazards regression model based on survival data, including OS, to evaluate the prognostic value of NMNAT1 in pan-cancer.

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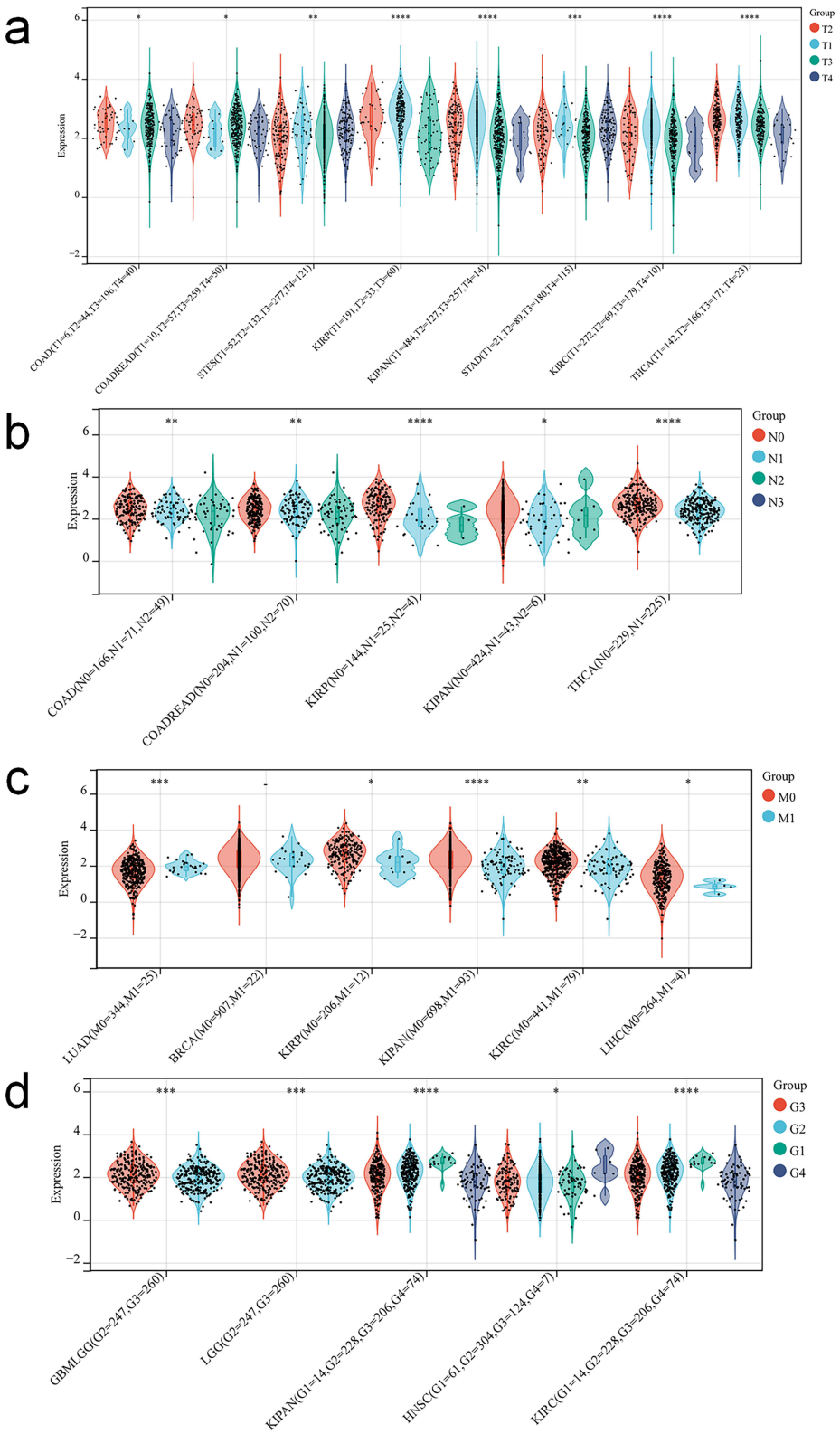

Figure 4 Correlation analysis of NMNAT1 gene expression with tumor staging and grading. NMNAT1 expression was significantly associated with T-stage in eight tumors (**a**), N-stage in five tumors (**b**), M-stage in five tumors (**c**), and grade-stage in five tumors (**d**) (****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05).

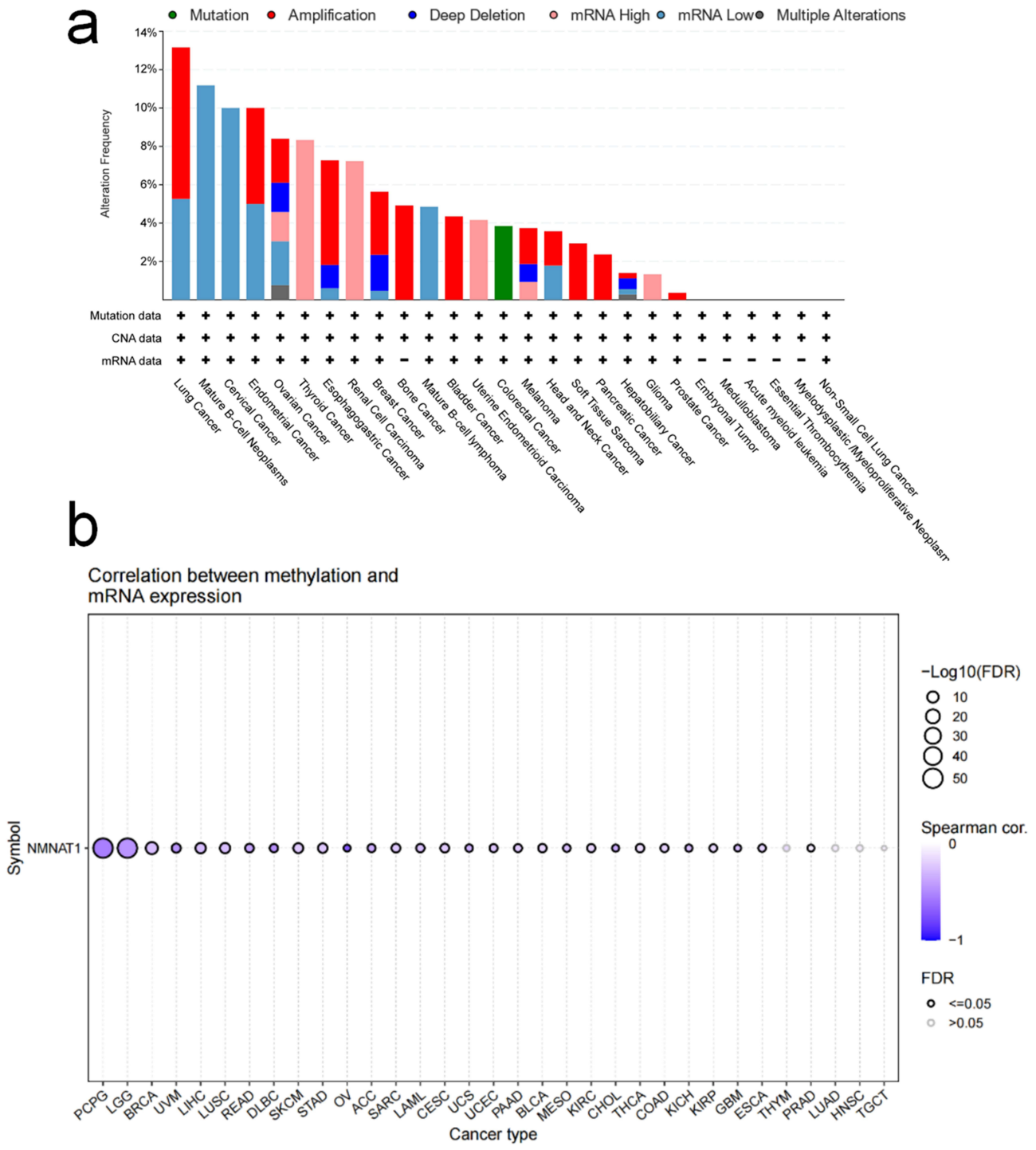

Figure 5 Analysis of genetic alterations (**a**) and DNA methylation (**b**).

tyrosine metabolism, whereas ABC transporters are associated with resistance to anticancer drugs (Figure 9b). In addition, to deeply explore the molecules interacting with NMNAT1 protein, we constructed a PPI network using the STRING database (Figure 9c).

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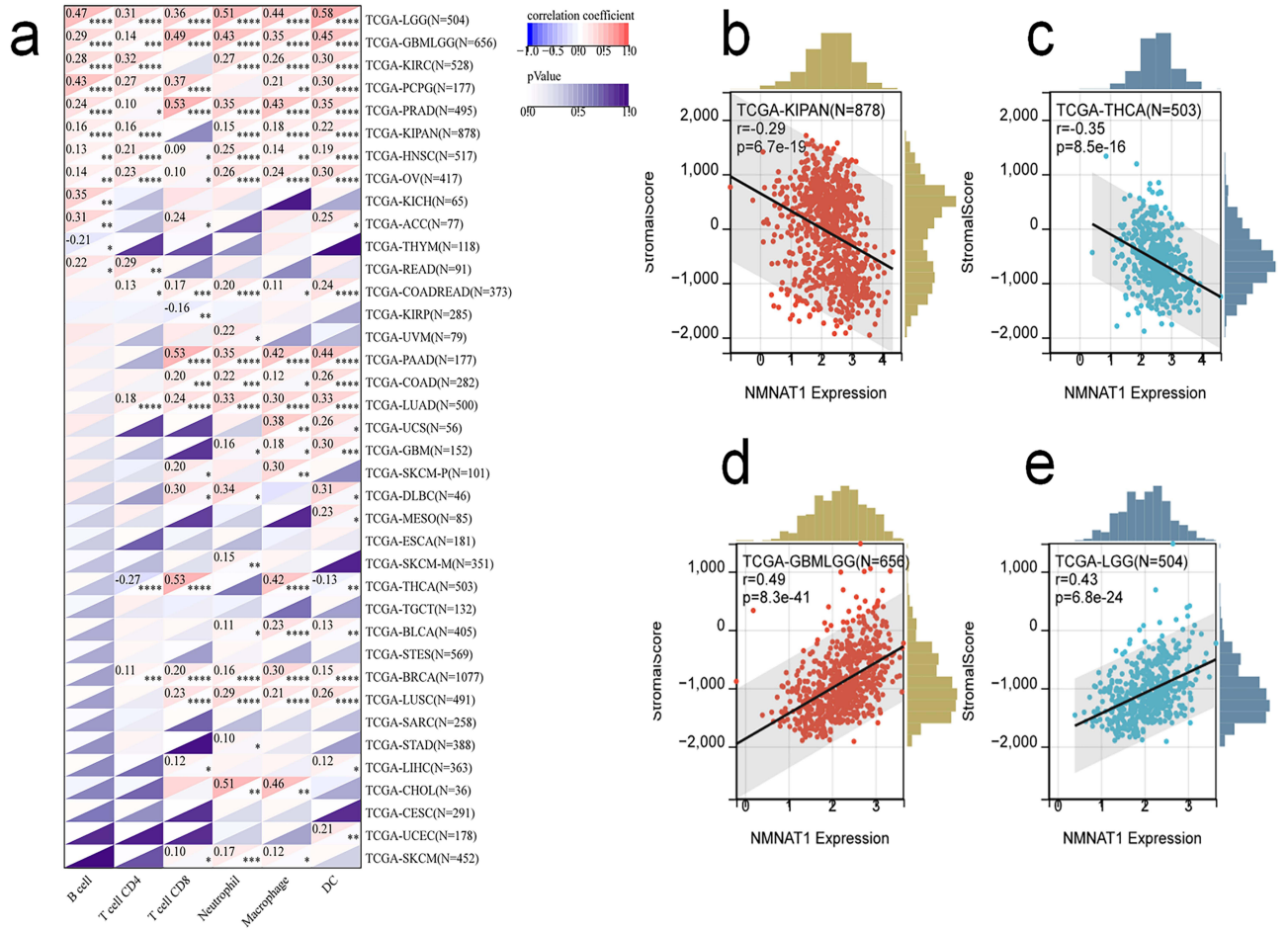

Figure 6 Immune infiltration correlation analysis. (**a**) Correlation between gene expression levels and six immune cells in various tumors. NMNAT1 showed particularly strong correlations with the degree of immune infiltration in KIPAN (**b**), THCA (**c**), GBMLGG (**d**), LGG (**e**), based on Pearson correlation coefficients (****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05).

Drug Sensitivity Analysis

Analysis of the GDSC database showed that high NMNAT1 expression was associated with sensitivity to most chemotherapies, including 17-AGG, PLX720, FH535, and WZ3105, and resistance to a few drugs, such as MP470 and Navitoclax (Figure 10a). Analysis of the CTRP database indicated that high NMNAT1 expression may lead to drug resistance in 3-CL-AHPC with CD-437 (Figure 10b).

Single-Cell Sequencing

It is believed that 14 key biological processes are linked to the development, progression, and metastasis of tumors. We used CancerSEA to preliminarily investigate whether NMNAT1 is involved in some basic cancer-related functional states and found that NMNAT1 was negatively correlated with DNA repair, epithelial–mesenchymal transition (EMT), DNA damage, and invasion, whereas it was positively correlated with angiogenesis, inflammation, and stemness in different tumors (Figure 11a). Based on correlation strength ≥ 0.3 and P value < 0.05, we also observed that NMNAT1 was positively correlated with differentiation, angiogenesis, inflammation, and metastasis and negatively correlated with DNA repair, cell cycle, and DNA damage in retinoblastoma (RB) (Figure 11c). Moreover, NMNAT1 expression negatively correlated with EMT in CRC (Figure 11b) and with DNA repair, apoptosis, DNA damage, invasion, and metastasis in uveal melanoma (UM) (Figure 11d). T-SNE plots were used to show the expression profiles of NMNAT1 in CRC (Figure 12a), UM (Figure 12b), and RB (Figure 12c) cells at the single-cell level.

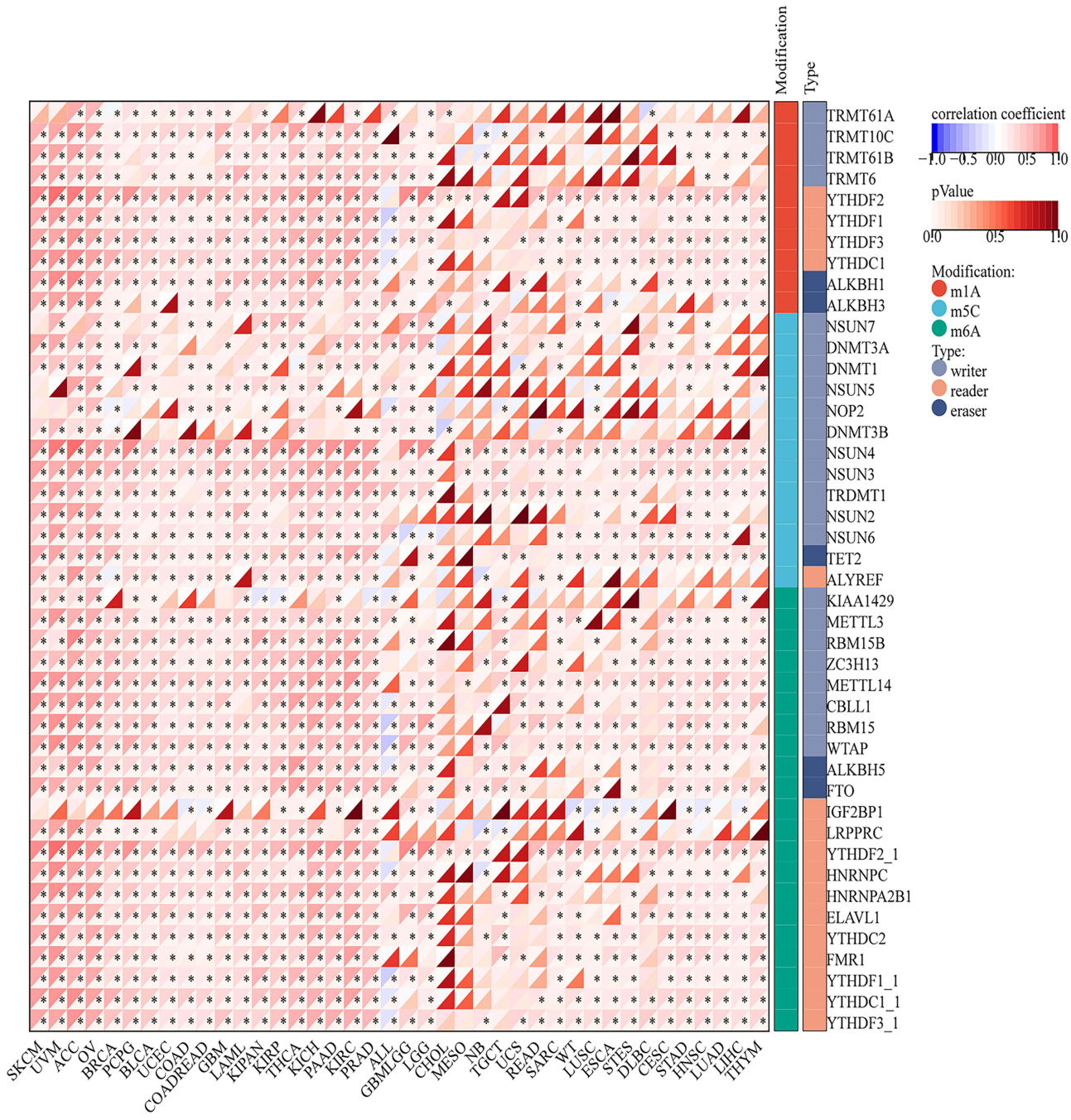

Figure 7 Correlation between NMNAT1 expression and RNA modification-related genes.

Immunohistochemical Analysis

We analyzed the clinical significance of NMNAT1 expression in patients with CRC using immunohistochemical techniques. The results of the chi-square test suggested that although the expression of NMNAT1 in tumors was not significantly associated with clinical characteristics (Table 1), expression in paraneoplastic tissues was significantly associated with TNM stage and tumor differentiation (Table 2). To further understand the relationship between NMNAT1 expression and prognosis, we analyzed the correlation between NMNAT1 expression and cancer-specific survival (CSS) and OS using Kaplan-Meier (KM) survival curves. In tumor tissues, CSS (Figure 13a) was better in the low-expression group than in the high-expression group, whereas OS (Figure 13b) was not significantly different between the high- and

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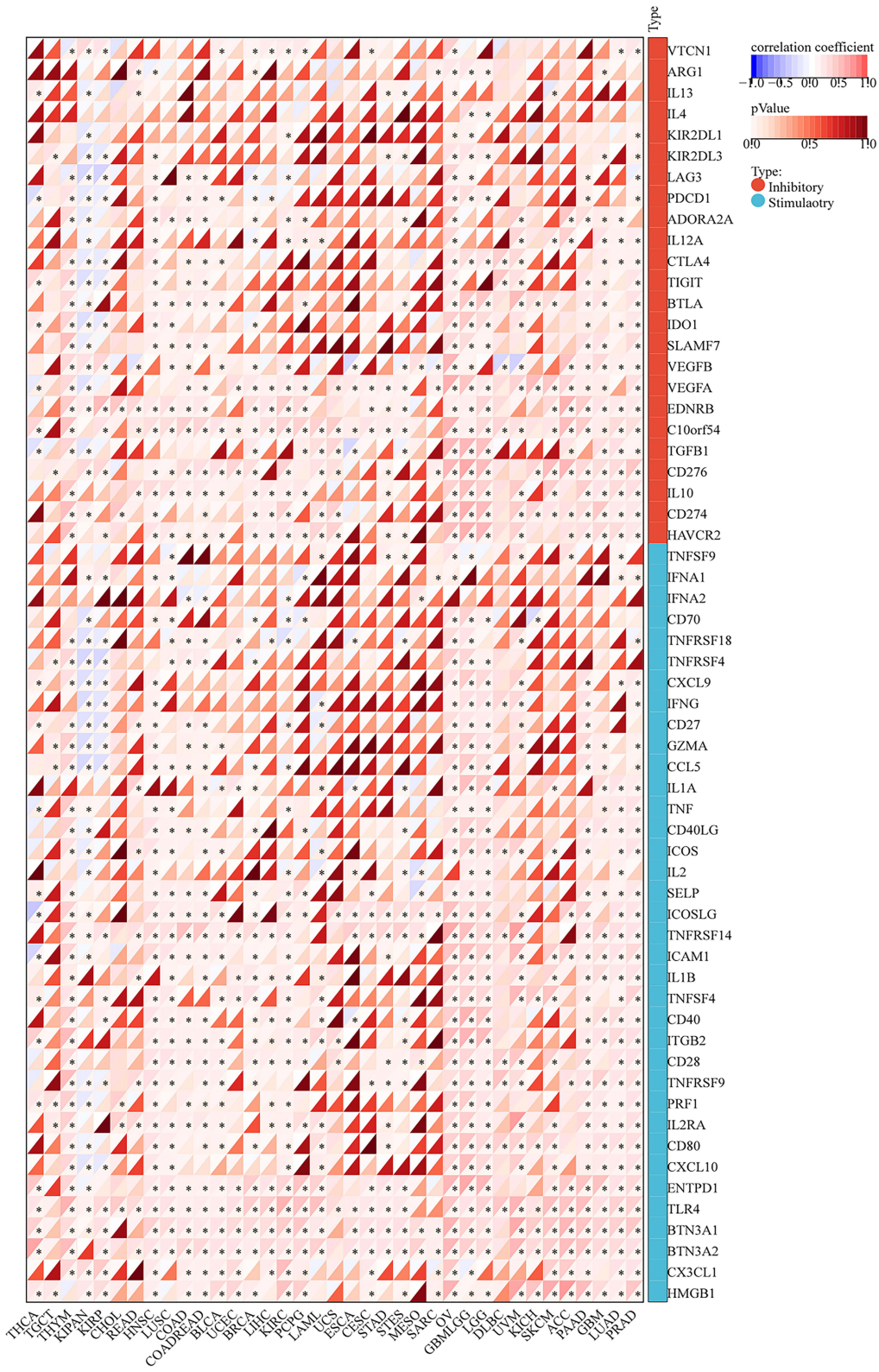

Figure 8 Correlation between NMNAT1 expression and immune checkpoint-related genes.

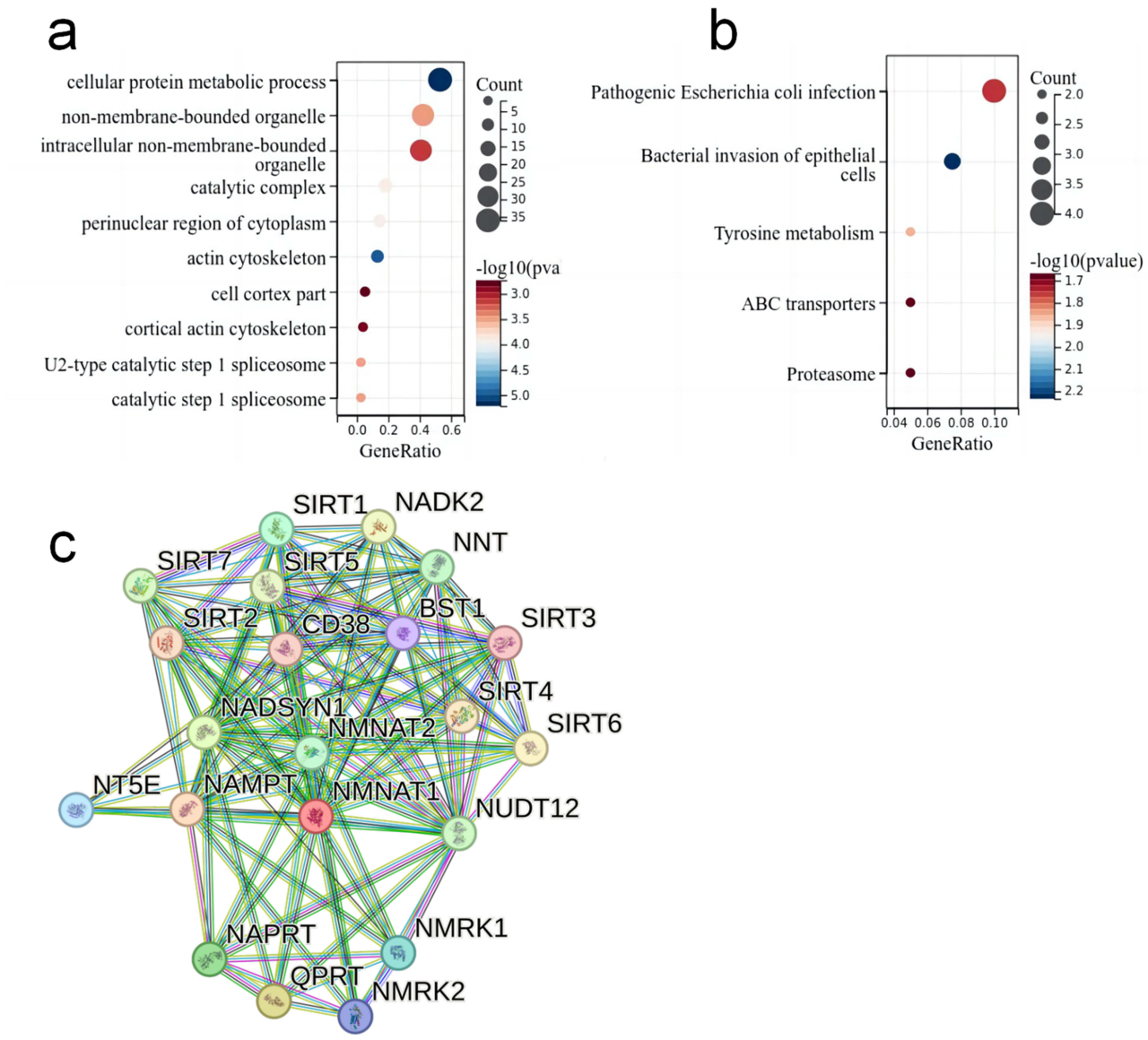

Figure 9 Functional enrichment and protein–protein interaction analyses. GO (**a**) and KEGG (**b**) enrichment analyses of NMNAT1 co-expressed genes. (**c**) Protein–protein interaction analysis using the STRING database.

low-expression groups. Further analysis of gene expression in paraneoplastic tissues showed that both OS (Figure 13f) and CSS (Figure 13e) were significantly better in the low-expression group than in the high-expression group. Non-parametric tests showed that the expression of NMNAT1 correlated with TNM stage and differentiation, and this correlation was more significant in paracancerous tissues (Figure 13g and h) than in tumor tissues (Figure 13c and d). In addition, using the Kaplan-Meier curve, we found that the CSS (Figure 13a) of the low-expression group was better than that of the high-expression group in tumor tissues, whereas the OS (Figure 13f) and CSS (Figure 13e) of the low-expression group were better than those of the high-expression group in paracancerous tissues. In summary, we found that NMNAT1 expression correlated with patient prognosis, and the predictive ability of paracancerous tissues was better than that of tumor tissues. We further investigated the effect of NMNAT1 expression in paraneoplastic tissues on OS and CSS in patients with CRC using univariate and multivariate Cox regression models. The results of univariate analysis indicated that the target gene, tumor stage, and differentiation were associated with OS (Figure 14a) and CSS (Figure 14c), while multivariate analysis revealed that the expression of this marker gene was an independent factor affecting CSS (Figure 14d) of CRC patients, independent of OS (Figure 14b).

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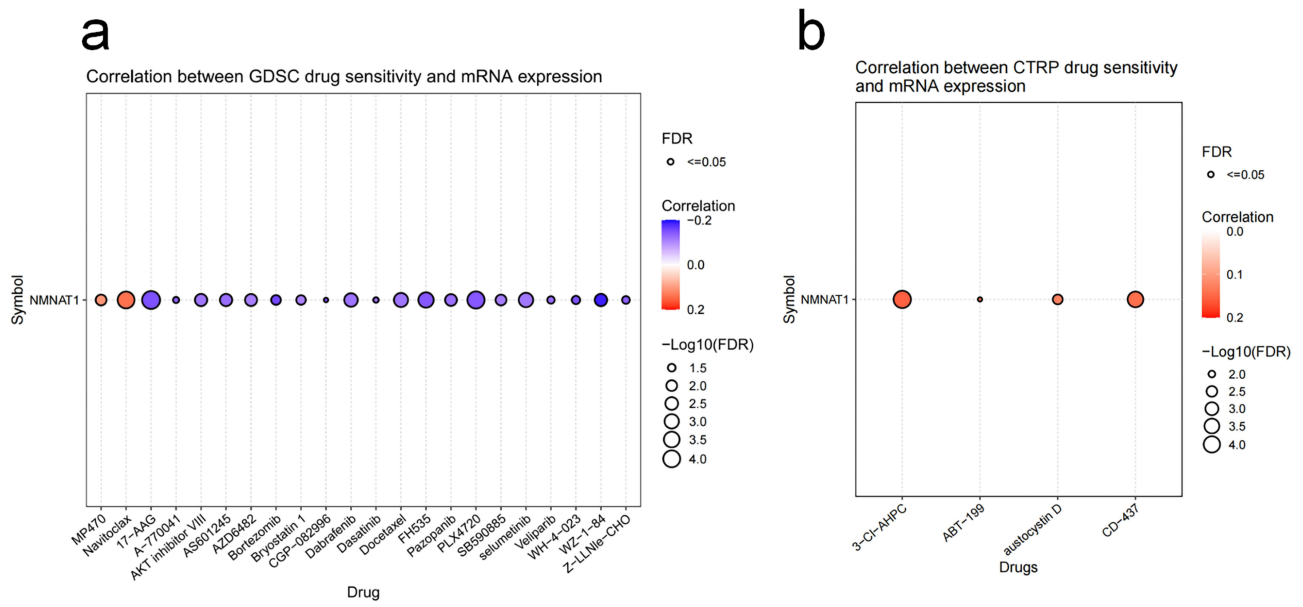

Figure 10 Correlation between gene expression and drug sensitivity based on GDSC (**a**) and CTRP (**b**) databases.

Discussion

There is growing evidence that nicotinamide is of great value for the prevention and treatment of tumors. Nicotinamide is a potential radiosensitizer and vasoactive agent that reduces perfusion-limited hypoxia in head and neck cancers.[14,26] NAM inhibits tumor cell proliferation, cell cycle progression, and DNA replication, while enhancing tumor cell apoptosis and inhibiting DNA damage repair by inhibiting PARP1.[27–29] Previous studies have shown that tumor progression is closely related to nicotinamide metabolism. Tumor cells use the Warburg effect to produce higher NAD+/NADH and NADP+/NADPH ratios to adapt to the reprogrammed metabolic state, and nicotinamide metabolism and its associated products play important translocation roles here.[30] Moreover, the roles of genes related to nicotinamide metabolism in tumors have been studied in detail. By increasing the NAD+ pool, NAMPT promotes tumor cell growth and accelerates the formation and spread of malignancies. Although NAPRT overexpression allows PARP activation, leading to resistance to DNA-damaging pharmacological therapies, NAPRT inhibition promotes epithelial-mesenchymal transition (EMT) in gastric cancer.[31–33] The oncogenic effects of SIRT1, such as triggering the MAPK pathway to promote cancer cell growth, depend on the NAD+ pool and NAMPT activity.[34,35] However, the role of another key gene in nicotinamide metabolism, NMNAT1, in tumors has been less studied.

In this study, we used bioinformatics to explore the role of NMNAT1, a key gene in nicotinamide metabolism, in pan-cancer. Using a pan-cancer database, we found that NMNAT1 was differentially expressed in a variety of tumor cells compared to normal cells, and further survival analyses showed that NMNAT1 was associated with the prognosis of a variety of tumors. Therefore, we hypothesized that NMNAT1 can be used as a prognostic biomarker for cancer.

To further explore the potential mechanisms of this marker gene in cancer, GO analysis of NMNAT1 co-expressed genes showed that the target genes were mainly associated with mitochondrial metabolism. KEGG enrichment analysis revealed that NMNAT1 co-expressed genes play roles in pathways such as tyrosine metabolism and ABC transporters, which have previously been shown to be involved in tumor progression and chemoresistance. PPI analysis indicated that NMNAT1 mainly interacts with other nicotinamide metabolism genes.

The mRNA expression of NMNAT1 was negatively correlated with methylation in a variety of tumors, which is consistent with the trend of changes in tumor DNA methylation that manifests as a decrease in the overall methylation level of the genome.[36] Mutations in NMNAT1 have been reported to be closely associated with the progression of Leber congenital amaurosis.[37] However, studies on NMNAT1 gene alterations in human malignancies are relatively rare. In this

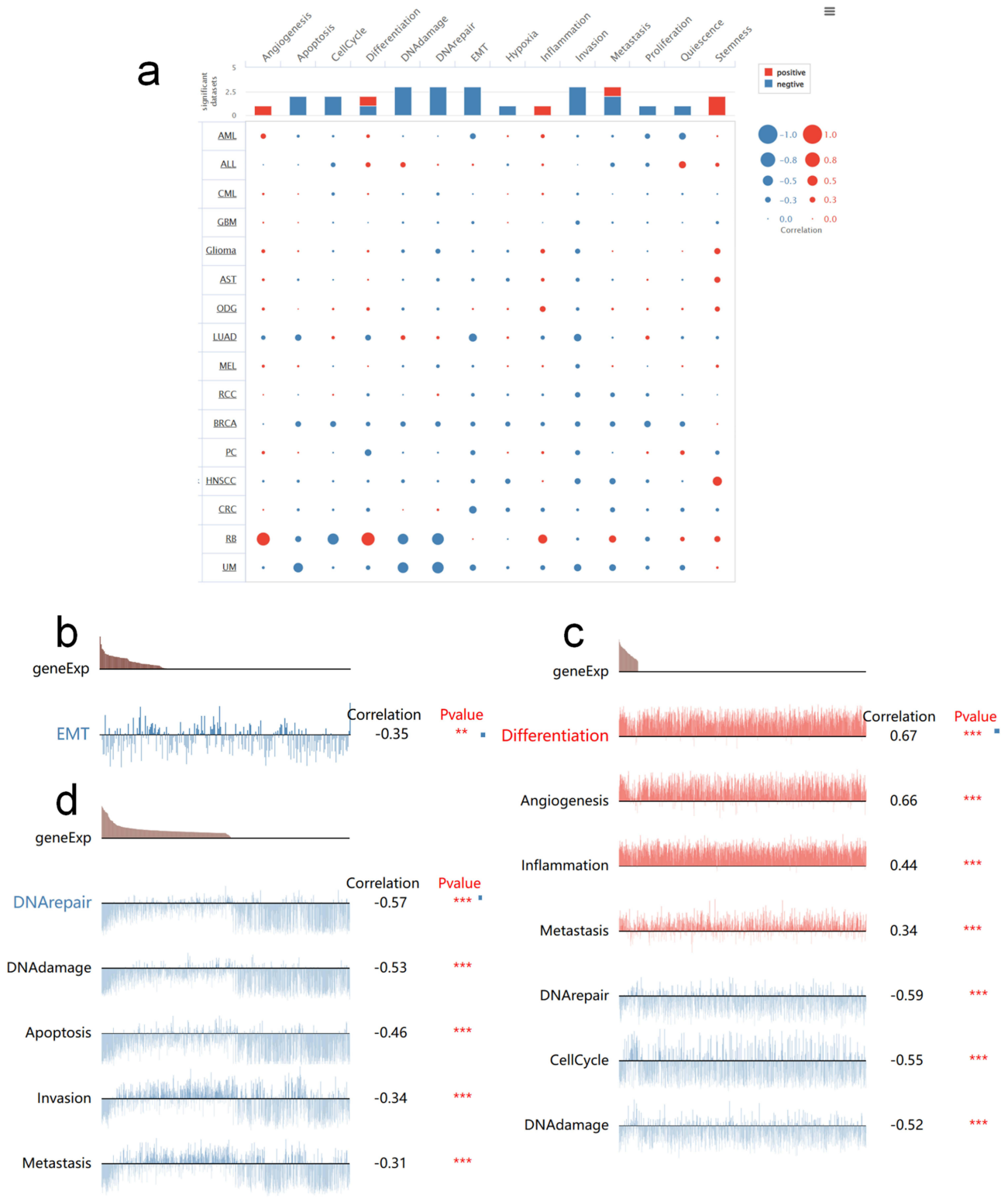

Figure 11 Association of NMNAT1 with 14 cancer-related functional states in pan-cancer (**a**), including CRC (**b**), RB (**c**), and UM (**d**).
Abbreviations: AML, Acute Myeloid Leukemia; ALL, Acute Lymphoblastic Leukemia; CML, Chronic Myeloid Leukemia; GBM, Glioblastoma Multiforme; Glioma, Glioma; AST, Astrocytoma; ODC, Oligodendroglioma; LUAD, Lung Adenocarcinoma; MEL, Melanoma; RCC, Renal Cell Carcinoma; BRCA, Breast Cancer; PC, Prostate Cancer; HNSCC, Head and Neck Squamous Cell Carcinoma; CRC, Colorectal Cancer; RB, Retinoblastoma; UM, Uveal Melanoma.

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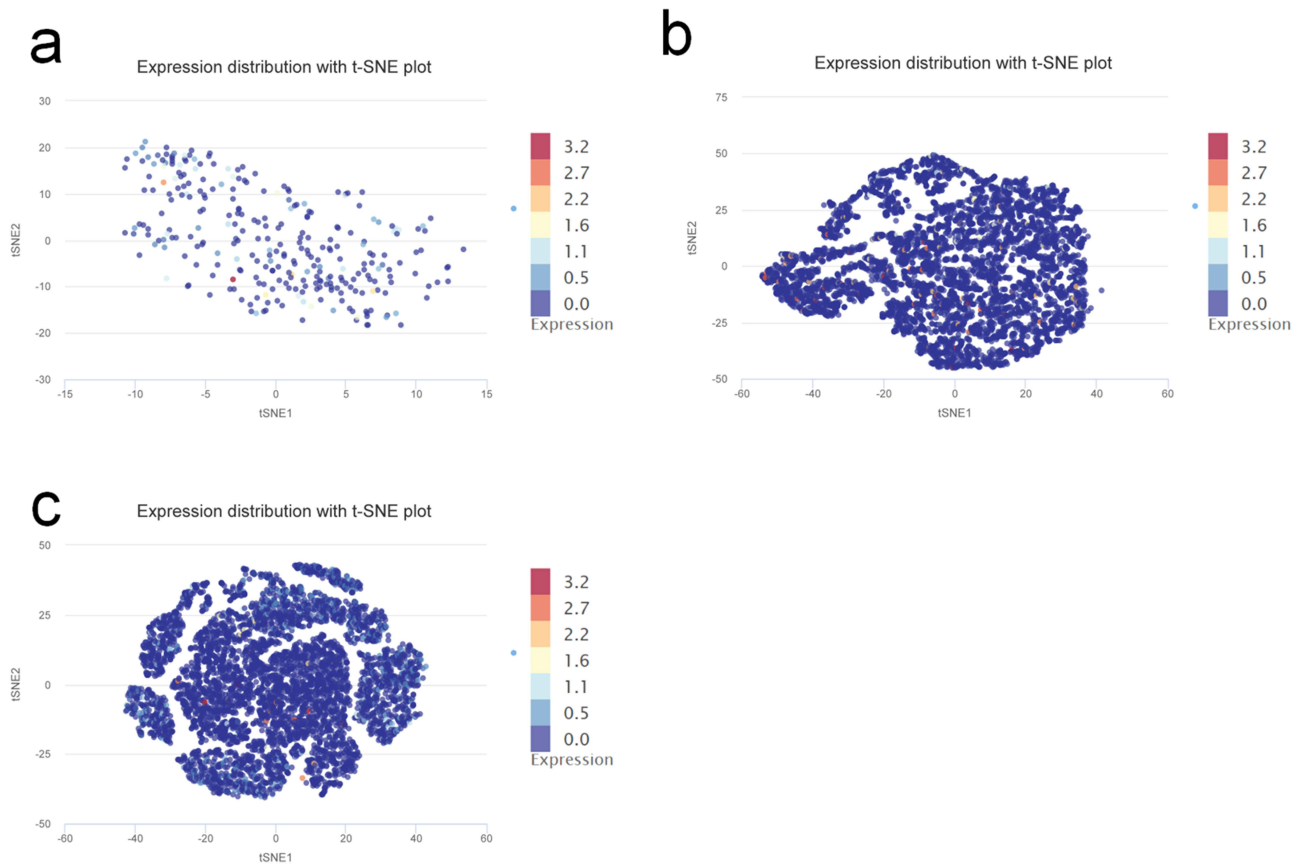

Figure 12 T-SNE plots depicting the expression profile of NMNAT1 in CRC (**a**), UM (**b**), and RB (**c**) at the single-cell level.

study, the NMNAT1 gene was found to be altered in a variety of tumors, mainly by amplification and mutation. However, these are bioinformatics data, and more mechanistic studies are needed to investigate genetic alterations in NMNAT1.

The tumor microenvironment plays an important role in tumor initiation, development, metastasis, and therapeutic response, and immune cells, as an important part of the tumor microenvironment, influence the immune response and immunotherapy.[38–40] We analyzed the correlation between NMNAT1 expression and immune cells, and the heatmap

Table 1 Analysis of the Correlation Between NMNAT1 Expression and Clinical Characteristics of Colorectal Cancer in Tumor Tissue

Group	n	%	NMNAT1 Expression					
			Low Expression	%	High Expression	%	*P*	FDR
Total	123							
Gender								
Female	53	43.09	11	36.67	42	45.16	0.55	1
Male	70	56.91	19	63.33	51	54.84		
Age								
<60	36	29.27	6	20.00	30	32.26	0.29	1
≥60	87	70.73	24	80.00	63	67.74		
Tumor Location								
Colon	95	77.24	23	76.67	72	77.42	1	1
Rectum	28	22.76	7	23.33	21	22.58		

(*Continued*)

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Table 1 (Continued).

Group	n	%	NMNAT1 Expression					
			Low Expression	%	High Expression	%	*P*	FDR
Tumor Stage (TNM Stage)								
I + II	89	72.36	22	73.33	67	72.04	1	1
III + IV	34	27.64	8	26.67	26	27.96		
Tumor Differentiation								
Median or Well	48	39.02	16	53.33	32	34.41	0.1	0.62
Poor	75	60.98	14	46.67	61	65.59		

Abbreviation: FDR, False Discovery Rate.

Table 2 Analysis of the Correlation Between NMNAT1 Expression and Clinical Characteristics of Colorectal Cancer in Paracancerous Tissue

Group	n	%	NMNAT1 Expression					
			Low Expression	%	High Expression	%	*P*	FDR
Total	123							
Gender								
Female	53	43.09	20	35.71	33	49.25	0.18	0.74
Male	70	56.91	36	64.29	34	50.75		
Age								
<60	36	29.27	17	30.36	19	28.36	0.97	0.97
≥60	87	70.73	39	69.64	48	71.64		
Tumor Location								
Colon	95	77.24	46	82.14	49	73.13	0.33	0.74
Rectum	28	22.76	10	17.86	18	26.87		
Tumor Stage (TNM Stage)								
I + II	89	72.36	47	83.93	42	62.69	0.02	0.09
III + IV	34	27.64	9	16.07	25	37.31		
Tumor Differentiation								
Median or Well	48	39.02	28	50.00	20	29.85	0.04	0.18
Poor	75	60.98	28	50.00	47	70.15		

results showed that this gene was significantly associated with six types of immune cell infiltration in pan-cancer. In addition, NMNAT1 was strongly associated with immune infiltration scores in many cancers, particularly TIPAN, THCA, GBMLGG, and LGG. NMNAT1 is associated with immune checkpoint-related and RNA modification-related genes expressed in most tumors. Overall, NMNAT1 plays a key role in immune infiltration of tumors, suggesting that immunotherapy targeting NMNAT1 has potential clinical value.

To further understand the mechanism of action of NMNAT1 in tumors, we used single-cell sequencing technology and found that NMNAT1 is associated with several major cellular activities related to cancer at the single-cell level, such as a negative correlation with DNA repair, EMT, DNA damage, and invasion, whereas a positive correlation with angiogenesis, inflammation, and stemness. Studies have also reported that a similar gene, NAMPT, promotes stemness and dedifferentiation by activating OSKM factors.[41,42] Combined with the results of single-cell analysis, we hypothesized that NMNAT1 influences tumorigenesis, progression, drug resistance, and metastasis by interfering with cancer stem cells and DNA damage repair, thereby affecting tumor development, progression, drug resistance, and metastasis.[43]

To validate the clinical significance of NMNAT1 in tumors, we analyzed the correlation between the expression of this gene and clinicopathology based on pan-cancer data, which showed that NMNAT1 was associated with TNM stage

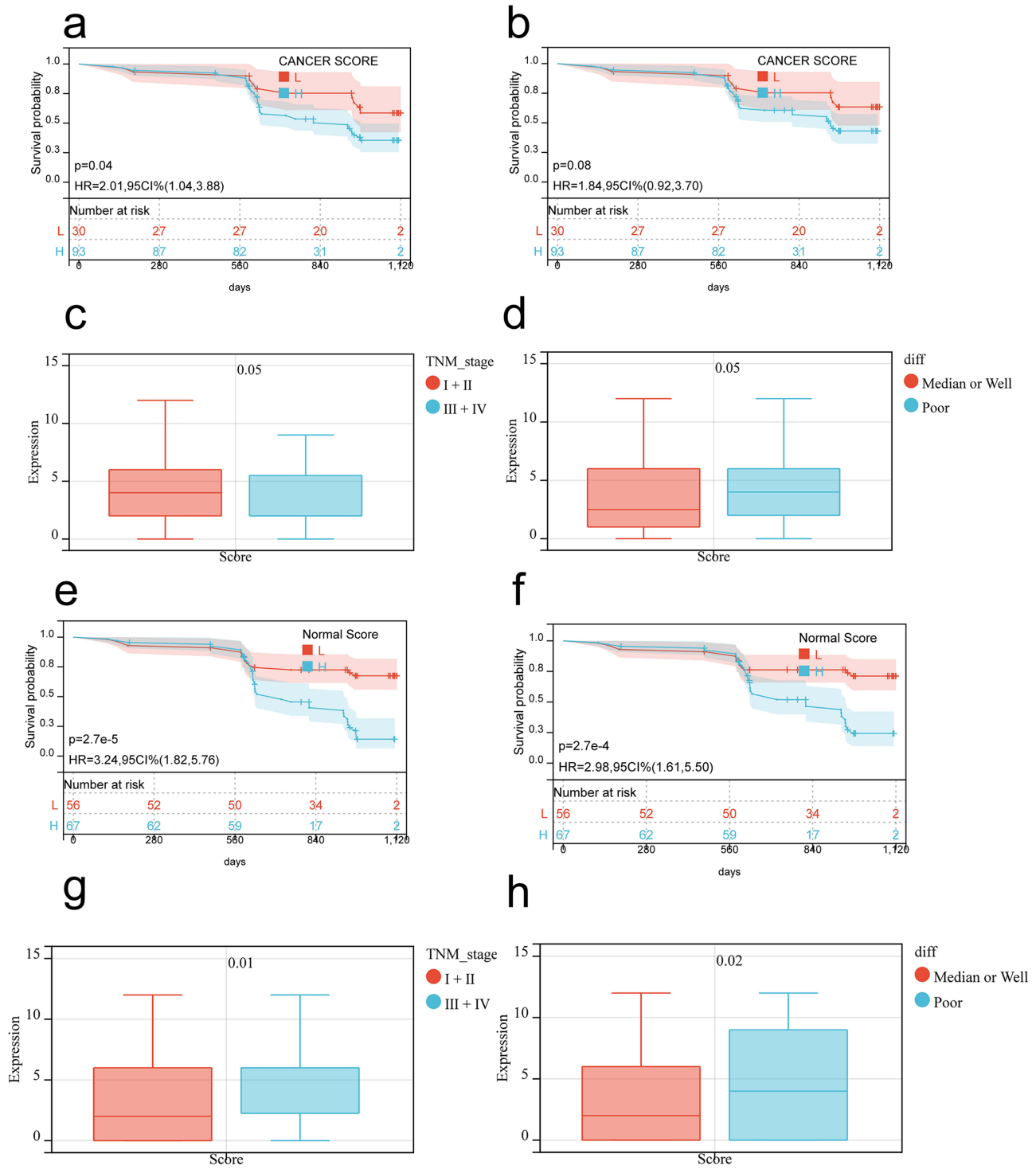

Figure 13 Immunohistochemistry identifies the prognostic role of NMNAT1. Kaplan–Meier curve of CSS and OS for NMNAT1 expression based on staining index (SI) in tumors (**a** and **b**) and paracancerous tissues (**e** and **f**). Non-parametric test validated the association of clinicopathological features with NMNAT1 in tumors (**c** and **d**) and paracancerous tissues (**g** and **h**).

and differentiation in a variety of tumors. We also analyzed NMNAT1 expression using immunohistochemistry in samples from 123 patients who had undergone radical surgery. Although the chi-squared test results indicated that NMNAT1 expression in tumor tissues was not significantly associated with clinical features, expression in paraneoplastic tissues was significantly associated with tumor differentiation and stage. Further analysis of target gene expression in

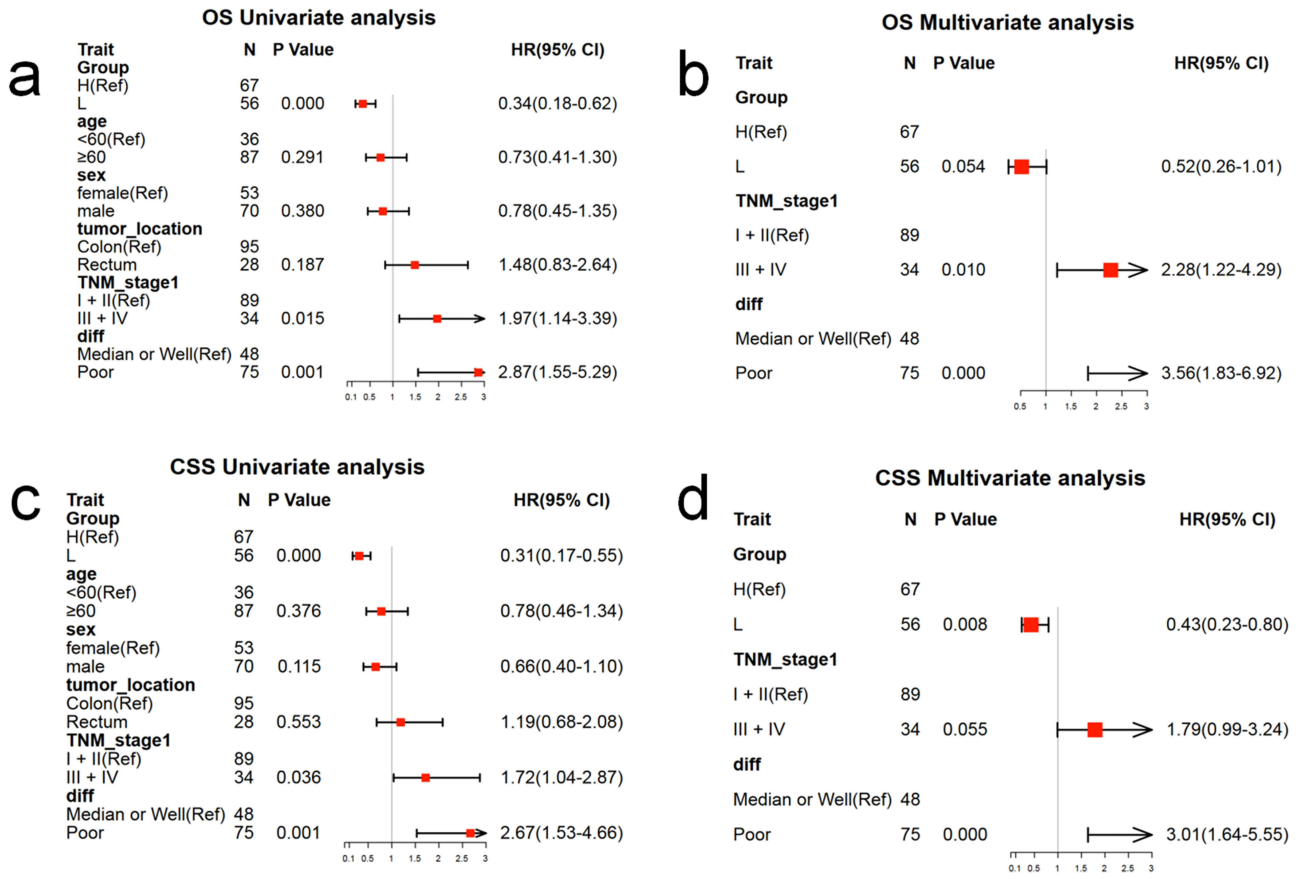

Figure 14 Univariate and multivariate Cox regression analyses investigate NMNAT1 expression in paracancerous tissues. Univariate Cox regression models analyzed the factors affecting OS (**a**) and CSS (**c**) in patients with CRC; multivariate Cox regression models analyzed the factors affecting OS (**b**) and CSS (**d**) in patients with CRC.

paraneoplastic tissues and univariate and multivariate Cox regression analyses suggested that NMNAT1 was significantly associated with patient prognosis. Unfortunately, the gene has a positive prognostic outcome in paraneoplastic tissues and a poorer prognostic ability in tumor tissues. The results of our experiments differ slightly from the results of the pan-cancer data, which may be related to the insufficient size of our samples as well as the widespread low expression of NMNAT1.

Finally, drug sensitivity analysis of the GSVA database suggested that NMNAT1 expression was correlated with sensitivity and resistance to a wide range of chemotherapeutic agents, which is consistent with the previously reported ability of nicotinamide to downregulate the SIRT1/Akt pathway, thereby increasing sensitivity to chemotherapeutic or radiotherapeutic agents.[12,13]

This study systematically revealed the key role of NMNAT1 in tumorigenesis and cancer development. These results indicate that NMNAT1 is an important oncogene that is closely associated with the progression of multiple cancer types and has significant prognostic value. It shows broad application prospects in clinical translational applications such as immunotherapy response assessment and drug sensitivity prediction. The present study elucidates the role of NMNAT1 in tumorigenesis from multiple perspectives and provides a basis for further research on the biological role of NMNAT1.

Our study had several limitations. First, we did not maintain a balanced sample size in the pan-cancer analysis, which was low in some cancer types. Second, this study was dedicated to bioinformatics analysis and lacked in vivo and in vitro experiments to validate the specific mechanisms. Third, the surgical specimens we collected for immunohistochemistry studies were retrospective, and in the future, we could recruit a prospective multicenter cohort to study the prognostic role of NMNAT1.

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Conclusion

NMNAT1, a key enzyme in the nicotinamide metabolic pathway, has demonstrated significant prognostic and immunolabelling potential in pan-cancer studies, particularly in CRC, and is expected to be used as a clinical biomarker and potential therapeutic target in CRC patients, providing a new strategy and direction for the precise diagnosis and individualized treatment of CRC.

Abbreviations

ACC, Adrenocortical carcinoma;; BLCA, Bladder Urothelial Carcinoma;; BRCA, breast invasive carcinoma;; CESC, Cervical squamous cell carcinoma and endocervical adenocarcinoma;; CHOL, Cholangiocarcinoma;; COAD, colon adenocarcinoma;; COADREAD, colon adenocarcinoma/rectum adenocarcinoma Esophageal carcinoma;; DLBC, Lymphoid Neoplasm Diffuse Large B-cell Lymphoma;; ESCA, Esophageal carcinoma;; FPPP, FFPE Pilot Phase II;; GBM, Glioblastoma multiforme;; GBMLGG, Glioma;; HNSC, Head and Neck squamous cell carcinoma;; KICH, Kidney Chromophobe;; KIPAN, Pan-kidney cohort (KICH+KIRC+KIRP);; KIRC, Kidney renal clear cell carcinoma;; KIRP, Kidney renal papillary cell carcinoma;; LAML, Acute Myeloid Leukemia;; LGG, Brain Lower Grade Glioma;; LIHC, Liver hepatocellular carcinoma;; LUAD, Lung adenocarcinoma;; LUSC, Lung squamous cell carcinoma;; MESO, Mesothelioma;; OV, Ovarian serous cystadenocarcinoma;; PAAD, Pancreatic adenocarcinoma;; PCPG, Pheochromocytoma, and Paraganglioma;; PRAD, Prostate adenocarcinoma;; READ, Rectum adenocarcinoma;; SARC, Sarcoma;; STAD, Stomach adenocarcinoma;; SKCM, Skin Cutaneous Melanoma;; STES, Stomach and Esophageal carcinoma;; TGCT, Testicular Germ Cell Tumors;; THCA, Thyroid carcinoma;; THYM, Thymoma;; UCEC, Uterine Corpus Endometrial Carcinoma;; UCS, Uterine Carcinosarcoma;; UVM, Uveal Melanoma;; OS, Osteosarcoma;; ALL, Acute Lymphoblastic Leukemia;; NB, Neuroblastoma;; WT, High-Risk Wilms Tumor.

Data Sharing Statement

The Cancer Genome Atlas database, accessible at https://portal.gdc.cancer.gov/, contained the gene expression data used in this investigation. The pan-cancer dataset was downloaded from UCSC (https://xenabrowser.net/).

Ethics Approval and Consent to Participate

The Declaration of Helsinki guidelines were followed in this study. All research participants and/or their legal guardians provided informed consent to participate in this study. The Guangdong Provincial People's Hospital Institutional Review Board approved all the experimental protocols, and the ethical approval number for this study was GDREC2019504H(R2).

Consent for Publication

The study was published with the consent of all participants.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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