Research Paper

Comprehensive bioinformatics analysis of NOX4 as a biomarker for pan-cancer prognosis and immune infiltration

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ABSTRACT

Background: NADPH oxidase 4 (NOX4) has been proven to be associated with the prognosis of tumors in multiple cancers and can serve as a potential immunotherapy target to provide new treatment options for various tumors. In this study, our aim is to conduct an in-depth investigation of NOX4 across a range of cancer types to determine the relationship between NOX4 and tumors.

Methods: Utilizing large-scale transcriptomic and clinical data from public databases, a systematic examination of NOX4 expression patterns was performed in pan-cancer cohorts. Survival analysis, methylation analysis, and correlation studies were employed to assess the diagnostic and prognostic significance of NOX4 in diverse cancer types. Additionally, an exploration of the relationship between NOX4 expression and immune infiltration across various tumors was conducted.

Results: The analyses unveiled a consistent upregulation of NOX4 expression in multiple cancer types relative to normal tissues, indicating its potential as a universal cancer biomarker. Elevated NOX4 expression significantly correlated with poor overall survival in several cancers. Furthermore, the study demonstrated a robust correlation between NOX4 expression and immune cell infiltration, signifying its involvement in the modulation of the tumor microenvironment.

Conclusions: This study imparts valuable insights into the potential applications of NOX4 in cancer research, highlighting its significance as a multifaceted biomarker with diagnostic, prognostic, and immunomodulatory implications across diverse malignancies.

INTRODUCTION

Cancer is a complex group of diseases and remains a major cause of human mortality globally. It continues to pose a significant global health challenge, necessitating urgent research efforts and solutions [1, 2]. The current central focus of cancer research is on identifying effective biomarkers to facilitate early diagnosis of cancer, predict patient prognosis, and select appropriate treatment strategies [3].

NADPH oxidase 4 (NOX4) is one of the seven members of the Nox family (Nox1, Nox2, Nox3, Nox4, Nox5, Duox1, and Duox2), and Nox4 has been identified as one of the major sources of reactive oxygen species (ROS). Increased generation of ROS has been implicated

in the pathogenesis of various diseases such as cancer and cardiovascular disease [4]. NOX4 is widely expressed in many different tissues and has a wider range of biological functions. As a major endogenous ROS source, NOX4 is involved in regulating multiple cellular functions, including cell proliferation, migration, and death [5, 6]. Dysregulation of NOX4 expression in cancer has been observed across a spectrum of malignancies [7, 8]. Initial studies indicate elevated NOX4 expression in tumors compared to adjacent normal tissues, suggesting a potential role in tumorigenesis [9]. The multifaceted engagement of NOX4 in cancer has generated interest in its potential as a diagnostic and prognostic biomarker, as well as its influence on the tumor microenvironment through interactions with the immune system. The diagnostic potential of NOX4 is rooted in its abnormal expression in cancer tissues, including but not limited to breast cancer, lung cancer, colorectal cancer, and pancreatic cancer [10–13]. Although the role of NOX4 has been identified in some tumors, the current study lacks a comprehensive study of NOX4 in all tumors.

The prognostic significance of NOX4 in cancer is also gradually gaining attention. Several studies have proposed a correlation between high NOX4 expression and adverse clinical outcomes, including reduced overall survival (OS) and disease-free survival in cancer patients [14]. However, the consistency of these findings across diverse cancer types and the underlying molecular mechanisms governing NOX4-mediated prognostic effects necessitate further exploration. Further exploration is needed to investigate the interaction between the expression of NOX4 and the immune microenvironment. More and more evidence suggest that NOX4 may influence the immune infiltration of tumors, as well as the recruitment and function of immune cells [15]. Studies have shown that NOX4 is essential for maintaining the immunosuppressive CAF phenotype in tumors. Pharmacological inhibition of NOX4 enhances immunotherapy by overcoming CAF-mediated CD8 T cell exclusion [7]. Understanding the relationship between NOX4 expression and immune infiltration is crucial for unraveling the immunomodulatory aspects of NOX4 in cancer progression. Nevertheless, the extent of NOX4's involvement in different cancer types and its clinical implications have not been systematically explored.

In summary, this study aims to elucidate the multifaceted role of NOX4 in cancer by exploring its diagnostic potential, prognostic significance, and impact on immune infiltration in tumors. It seeks to provide insights for future experimental research and aid in the clinical translation of NOX4 as a tumor biomarker, thereby improving the diagnosis, prognosis, and selection of treatment options for cancers.

MATERIALS AND METHODS

Data acquisition and processing

The assessment of NOX4 expression involved a comprehensive examination across 34 distinct tumors and their corresponding normal tissues, utilizing datasets from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) cohorts. The SangerBox web-based tool (http://sangerbox.com/) facilitated this analysis. In addition, the "Pathological Stage Plot" module of Gene Expression Profiling Interactive Analysis [16] was employed to scrutinize NOX4 expression levels in various pathological stages within selected TCGA tumors.

NOX4-related gene enrichment analysis

For protein-protein interaction network associated with NOX4, the STRING website (https://string-db.org/) [17] was utilized. Additionally, the "Similar Gene Detection" module of GEPIA2 identified the top 100 NOX4-associated genes in TCGA tumors. Subsequently, the correlation analysis module of GEPIA2 explored the correlation between NOX4 and the top five NOX4-associated target genes. Pathway and process enrichment analysis for the identified top 100 NOX4-associated target genes was conducted using the Metascape web-based tool [18], with specific parameters set to $P < 0.01$, a minimum count of three for terms, and an enrichment factor > 1.5 for canonical pathways.

Analysis of tumor immune and immunosuppressive cell infiltration

To assess the correlation between NOX4 expression and the infiltration of various immune cell types, the TIMER2 server [19] was employed. The impact of genetic and epigenetic alterations of NOX4 on dysfunctional T-cell phenotypes was evaluated using the QUERY module of the Tumor Immune Dysfunction and Exclusion (TIDE) algorithm [20].

Epigenetic methylation analysis

The TCGA methylation module within the UALCAN interactive web resource was utilized to investigate differences in NOX4 methylation levels between tumor and paired normal tissues across various TCGA cancer types. Furthermore, the TIDE server was employed to explore the effect of NOX4 methylation on dysfunctional T-cell phenotypes and prognoses.

Statistical analysis

Student's t-test was applied to compare NOX4 expression levels between different groups, and the Wilcoxon rank-sum test was employed for non-normally distributed data. Pearson correlation analysis was used to evaluate the correlation between NOX4 expression and immune infiltration.

Data and materials availability

Data from the TCGA and public databases were utilized and examined in the present investigation. For additional information on this study, please contact the corresponding author. The entire data needed to evaluate the findings can be found within this article.

RESULTS

Pan-cancer analysis of NOX4 expression

A comprehensive pan-cancer analysis of NOX4 expression was conducted using data from TCGA and GTEx cohorts. The analysis revealed elevated NOX4 expression in the majority of tumors compared to their corresponding normal tissues (25 out of 34) (Figure 1A). A consistent pattern was observed when focusing solely on the TCGA database, with increased NOX4 expression evident in 19 out of 26 tumors (Figure 1B). These findings underscore the close association between NOX4 and the initiation and progression of diverse tumors.

Prognostic implications of NOX4 expression

Further exploration of the correlation between NOX4 expression and patient outcomes indicated a consistent association between high NOX4 expression and poorer prognosis across several cancer types, including GBMLGG, LGG, MESO, ACC, STAD, COAD, COADREAD, STES, PAAD, LUAD, WT, and BLCA (Figure 2A). Notably, in SKCM, SKCM-M, KIPAN, KIRC, KIRP, patients with high NOX4 expression exhibited better OS. Additionally, NOX4 expression increased with higher tumor stages in BLCA, SKCM, ESCA, COAD, STAD, THCA, and TGCT (Figure 2B).

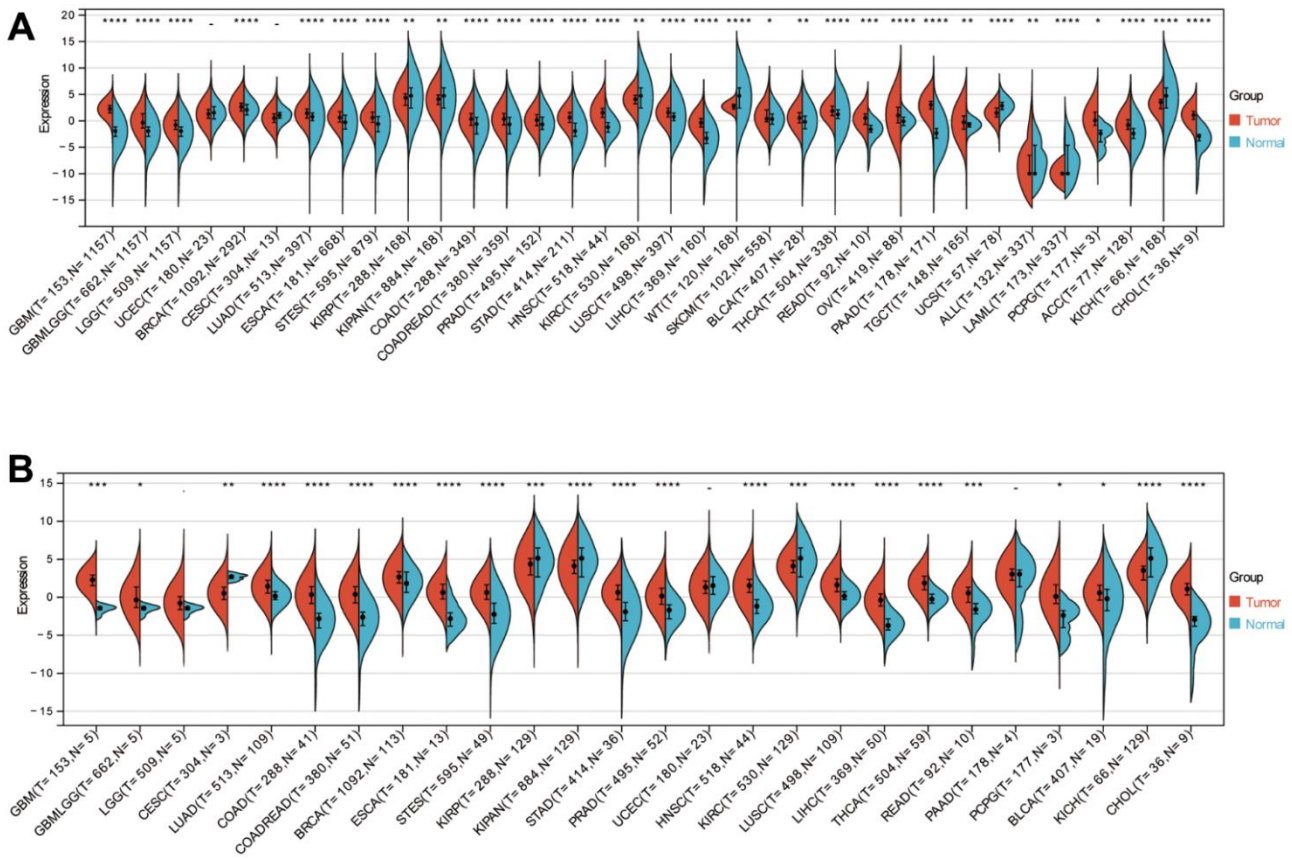


Figure 1. NOX4 expression in cancers. (**A**) The bar plot illustrates NOX4 mRNA expressions in various normal human tissues sourced from the TCGA and GTEx databases. (**B**) NOX4 mRNA expression levels in diverse cancer types based on TCGA databases; (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001).

A detailed investigation highlighted significantly higher NOX4 expression in TGCT and CHOL among patients with metastases (Figure 2C).

DNA methylation analysis

Assessing DNA methylation alterations, crucial for the identification of diagnostic and prognostic biomarkers, revealed NOX4 hypermethylation in various cancer types, including BRCA, LUSC, BLCA, LUAD, PRAD, UCEC, STAD, THCA, and CHOL (Figure 3A). The consequences of NOX4 methylation status varied across cancers, with hypermethylation positively associated with patient risk and correlated with shorter OS in bladder, kidney and endometrial cohorts. Conversely, in brain and stomach cohorts, NOX4 hypermethylation was negatively associated with patient risk and correlated with longer OS (Figure 3B, 3C).

Functional enrichment analysis

Exploring NOX4-binding proteins through the STRING tool identified interactions with CYBB, NOX1, CYBA, DUOX1, TLR4, NOX3, NOX5, NCF1, NCF2, and NCF4 (Figure 4A). Correlation analysis of gene expression data from TCGA highlighted SLC16A4, ENPEP, NAT8, SLC5A10, and SLC22A2 as the top five genes significantly correlated with NOX4 expression (Figure 4B). The top 100 NOX4-associated genes were significantly associated with cancer-related signaling pathways, such as modified amino acid transport, organic anion transport, and SLC-mediated transmembrane transport (Figure 4C). These findings suggest a potential role for NOX4 in modulating cancer metabolism.

NOX4 is related to immunity

Investigating the relationship between NOX4 expression and immune cell infiltration in the tumor microenvironment revealed a significant positive correlation with six immune cell types in most tumor types, excluding DLBC, UVM, THYM, and TGCT (Figure 5A). Additionally, NOX4 expression was positively correlated with the infiltration abundance of cancer-associated fibroblasts (CAFs) in most tumors (Figure 5B). Comparing NOX4 with established

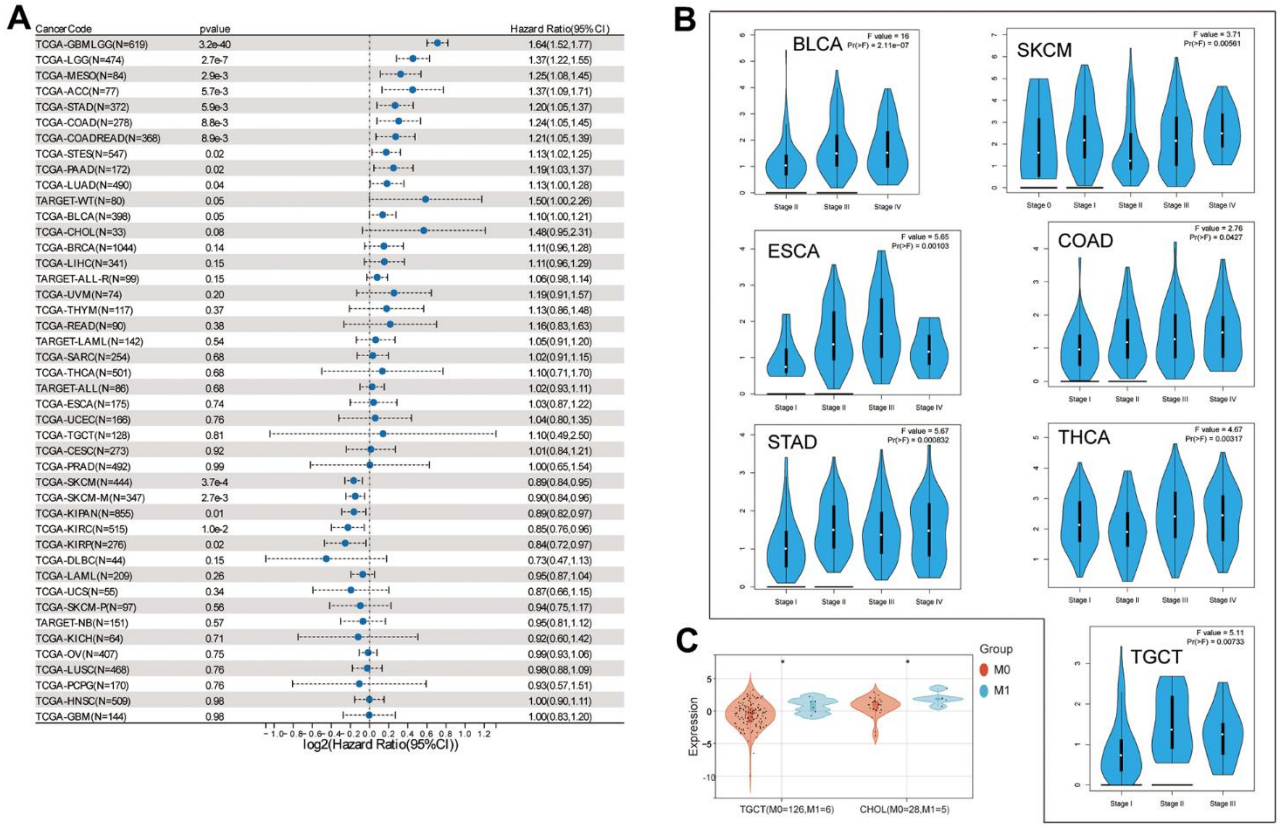


Figure 2. Correlation of NOX4 expression with prognosis and clinical stages. (**A**) Examination of the prognostic association of NOX4 expression in various cancer types utilizing SangerBox. (**B**) Investigation into the association of NOX4 gene expression levels with pathological stages. (**C**) Exploration of the relationship between NOX4 expression and metastasis; (*P < 0.05).

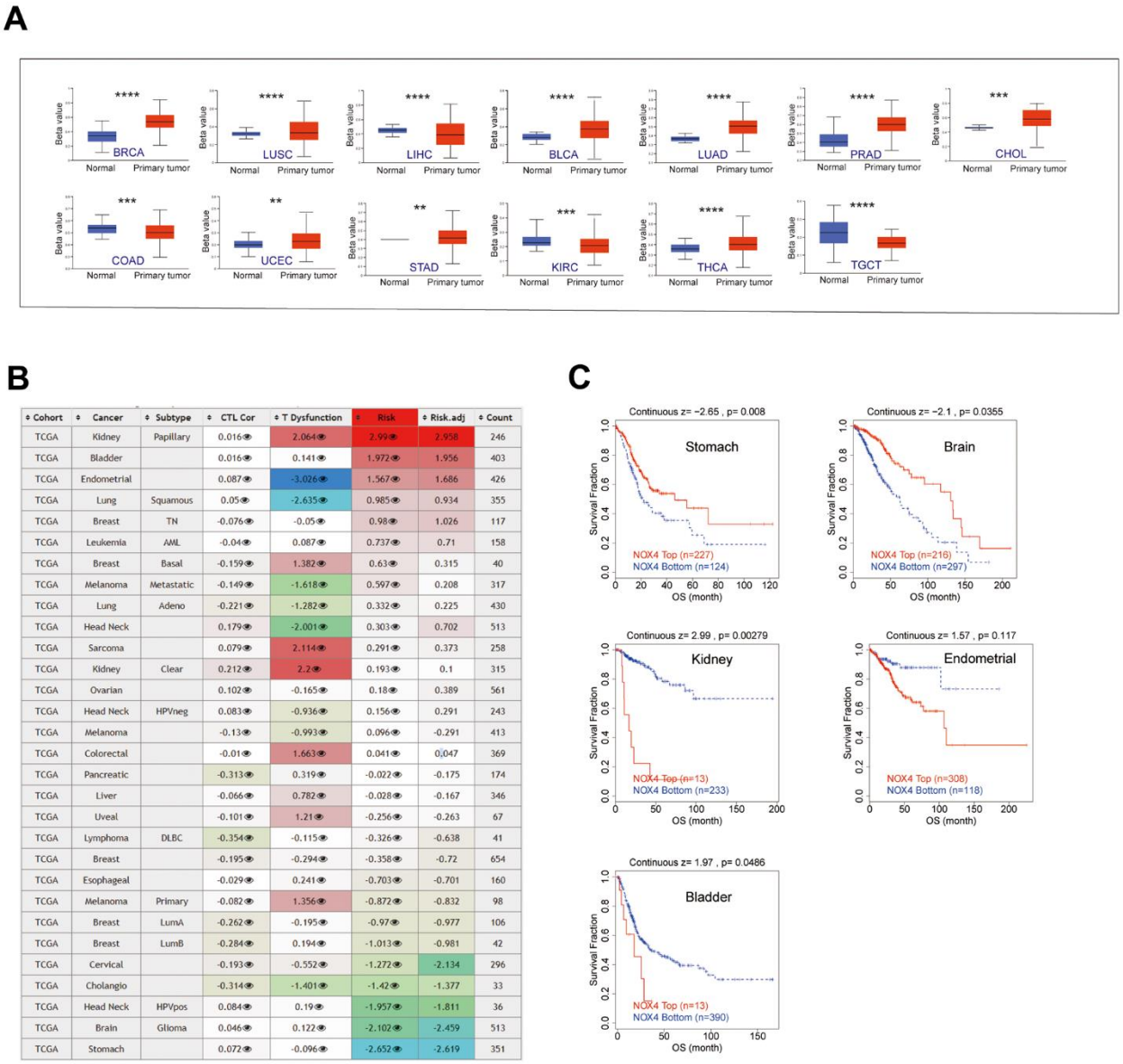
biomarkers for predicting response outcomes and OS in immune checkpoint blockade (ICB) sub-cohorts demonstrated an area under the receiver operating characteristic curve (AUC) of >0.5 in 7 of 18 ICB sub-cohorts (Figure 5C).

Correlation of NOX4 expression with immune checkpoint genes

Further investigation into the correlation between NOX4 expression and immune checkpoint genes across 40 cancer types revealed a close association with common immune checkpoints, such as PD-L1 and CTLA-4 (Figure 6). This implies a potential role for NOX4 in regulating immune checkpoint gene expression, suggesting its involvement in immune evasion mechanisms of cancer cells.

DISCUSSION

Cancer's complex landscape demands continual exploration of novel therapeutic targets to improve current treatment outcomes. As a member of the NADPH oxidase family, NOX4 plays a crucial role in



Cohort	Cancer	Subtype	CTL Cor	T Dysfunction	Risk	Risk.adj	Count
TCGA	Kidney	Papillary	0.016	2.064	2.99	2.958	246
TCGA	Bladder		0.016	0.141	1.972	1.956	403
TCGA	Endometrial		0.087	-3.026	1.567	1.686	426
TCGA	Lung	Squamous	0.05	-2.635	0.985	0.934	355
TCGA	Breast	TN	-0.076	-0.05	0.98	1.026	117
TCGA	Leukemia	AML	-0.04	0.087	0.737	0.71	158
TCGA	Breast	Basal	-0.159	1.382	0.63	0.315	40
TCGA	Melanoma	Metastatic	-0.149	-1.618	0.597	0.208	317
TCGA	Lung	Adeno	-0.221	-1.282	0.332	0.225	430
TCGA	Head Neck		0.179	-2.001	0.303	0.702	513
TCGA	Sarcoma		0.079	2.114	0.291	0.373	258
TCGA	Kidney	Clear	0.212	2.2	0.193	0.1	315
TCGA	Ovarian		0.102	-0.165	0.18	0.389	561
TCGA	Head Neck	HPVneg	0.083	-0.936	0.156	0.291	243
TCGA	Melanoma		-0.13	-0.993	0.096	-0.291	413
TCGA	Colorectal		-0.01	1.663	0.041	0.047	369
TCGA	Pancreatic		-0.313	0.319	-0.022	-0.175	174
TCGA	Liver		-0.066	0.782	-0.028	-0.167	346
TCGA	Uveal		-0.101	1.21	-0.256	-0.263	67
TCGA	Lymphoma	DLBC	-0.354	-0.115	-0.326	-0.638	41
TCGA	Breast		-0.195	-0.294	-0.358	-0.72	654
TCGA	Esophageal		-0.029	0.241	-0.703	-0.701	160
TCGA	Melanoma	Primary	-0.082	1.356	-0.872	-0.832	98
TCGA	Breast	LumA	-0.262	-0.195	-0.97	-0.977	106
TCGA	Breast	LumB	-0.284	0.194	-1.013	-0.981	42
TCGA	Cervical		-0.193	-0.552	-1.272	-2.134	296
TCGA	Cholangio		-0.314	-1.401	-1.42	-1.377	33
TCGA	Head Neck	HPVpos	0.084	0.19	-1.957	-1.811	36
TCGA	Brain	Glioma	0.046	0.122	-2.102	-2.459	513
TCGA	Stomach		0.072	-0.096	-2.652	-2.619	351

Figure 3. Epigenetic methylation analysis. (**A**) Boxplots depict the differential NOX4 methylation levels (beta values) across TCGA cohorts. (**B**) A heatmap displays the impact of NOX4 methylation on cytotoxic T-cell levels (CTLs), dysfunctional T-cell phenotypes, and risk factors within TCGA cohorts. (**C**) Kaplan-Meier curves compare overall survival differences between high and low NOX4 methylation levels, with statistically significant differences depicted; (**P < 0.01, ***P < 0.001, ****P < 0.0001).

the generation and regulation of ROS, making it a potential new target for cancer therapy [21]. Previous studies have shown that aberrant expression of NOX4 in tumor cells leads to elevated cellular ROS levels, promoting tumorigenesis. This abnormal expression is closely associated with accelerated cell proliferation, angiogenesis, and resistance to apoptosis [22]. Therefore, a thorough exploration of the functional significance and specific mechanisms of NOX4 in cancer is crucial for the development of new treatment modalities. By elucidating the role of NOX4 in tumorigenesis, researchers aim to reveal new avenues for precision medicine, offering innovative approaches to address the multifaceted challenges posed by cancer. Studies suggest that targeting NOX4 as a potential druggable target to disrupt redox balance within cancer cells can inhibit cancer growth by mitigating the pro-tumorigenic

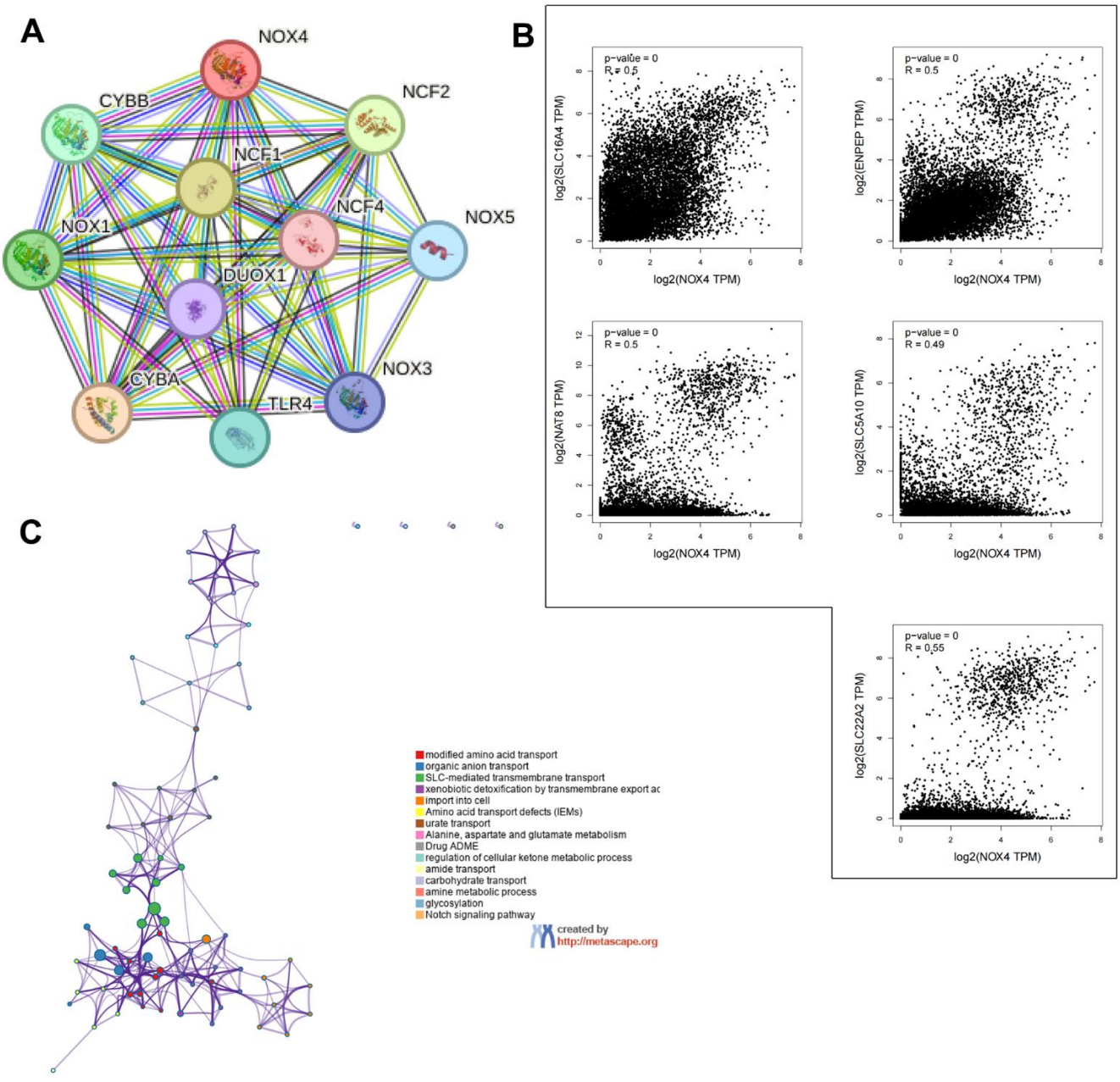


Figure 4. Enrichment analysis of NOX4-related genes across pan-cancers. (**A**) Identification of NOX4-binding proteins using the STRING tool. (**B**) Exploration of the top five NOX4-correlated genes across pan-cancers and their relationships with NOX4 expression analyzed through the GEPIA2 website. (**C**) Potential biological functions analysis of NOX4 by Metascape; presentation of enriched terms with a similarity >0.3 connected by edges.

effects associated with increased ROS production [23]. Continuous overexpression of NOX4 has been observed in breast cancer, colorectal cancer, clear cell renal cell carcinoma, and lung cancer compared to normal tissues, suggesting its potential as a universal cancer biomarker. Moreover, elevated levels of NOX4 expression are associated with poorer prognosis, manifested as shorter OS. Additionally, its correlation with advanced tumor stages across various cancers suggests a role for NOX4 in driving tumor progression.

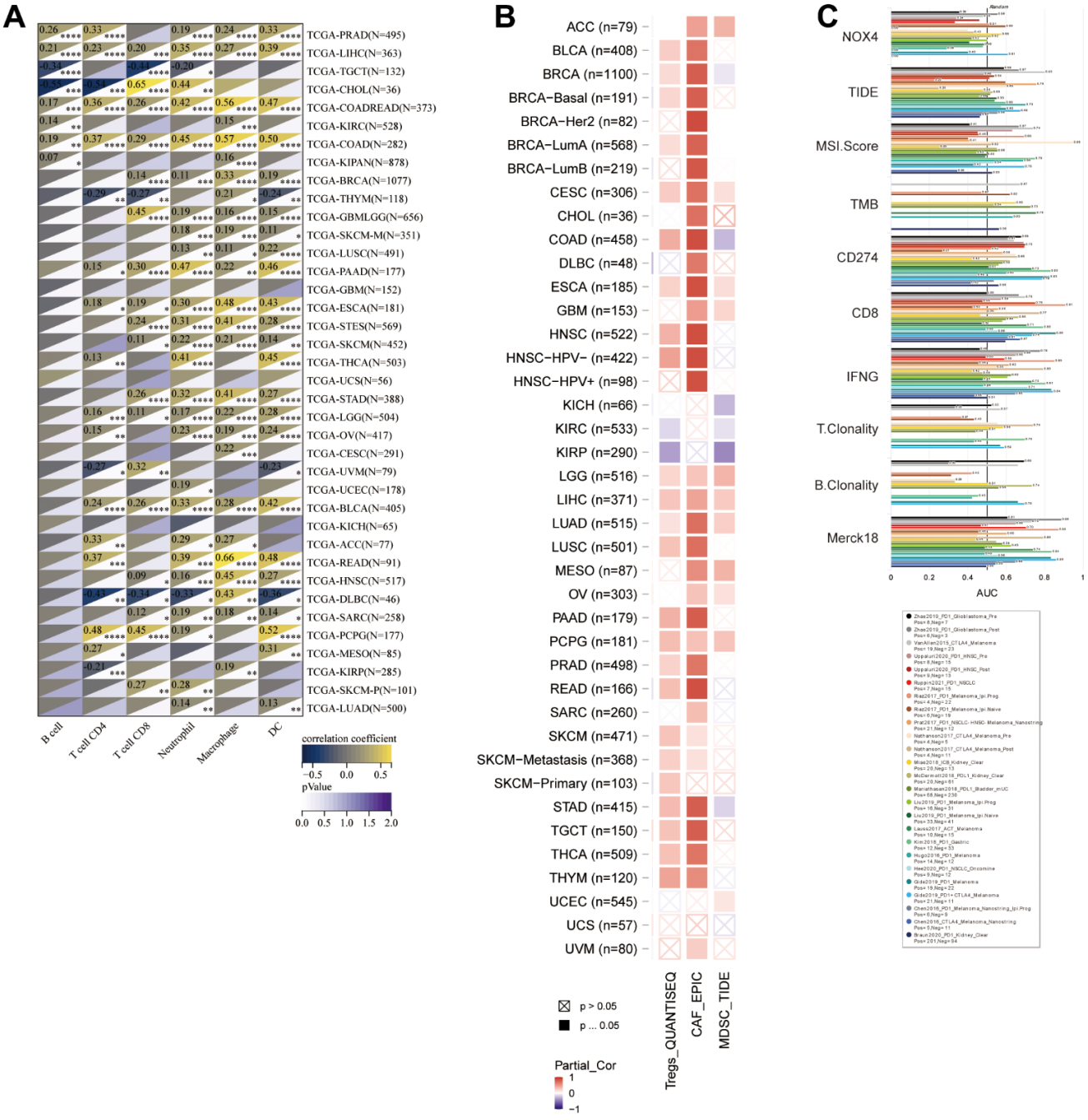


Figure 5. Correlations between NOX4 expression and immune cell infiltration levels. (**A**) A heatmap displays the correlations between NOX4 expression and infiltration levels of six immune cell types in TCGA cohorts. (**B**) Another heatmap depicts the correlations between NOX4 expression and infiltration levels of three immunosuppressive cell types in TCGA cohorts. (**C**) A bar plot compares the biomarker relevance of NOX4 with standardized cancer immune evasion biomarkers in immune checkpoint blockade (ICB) subcohorts, including cancer-associated fibroblasts (CAFs), myeloid-derived suppressor cells (MDSCs), and regulatory T cells (Tregs); (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001).

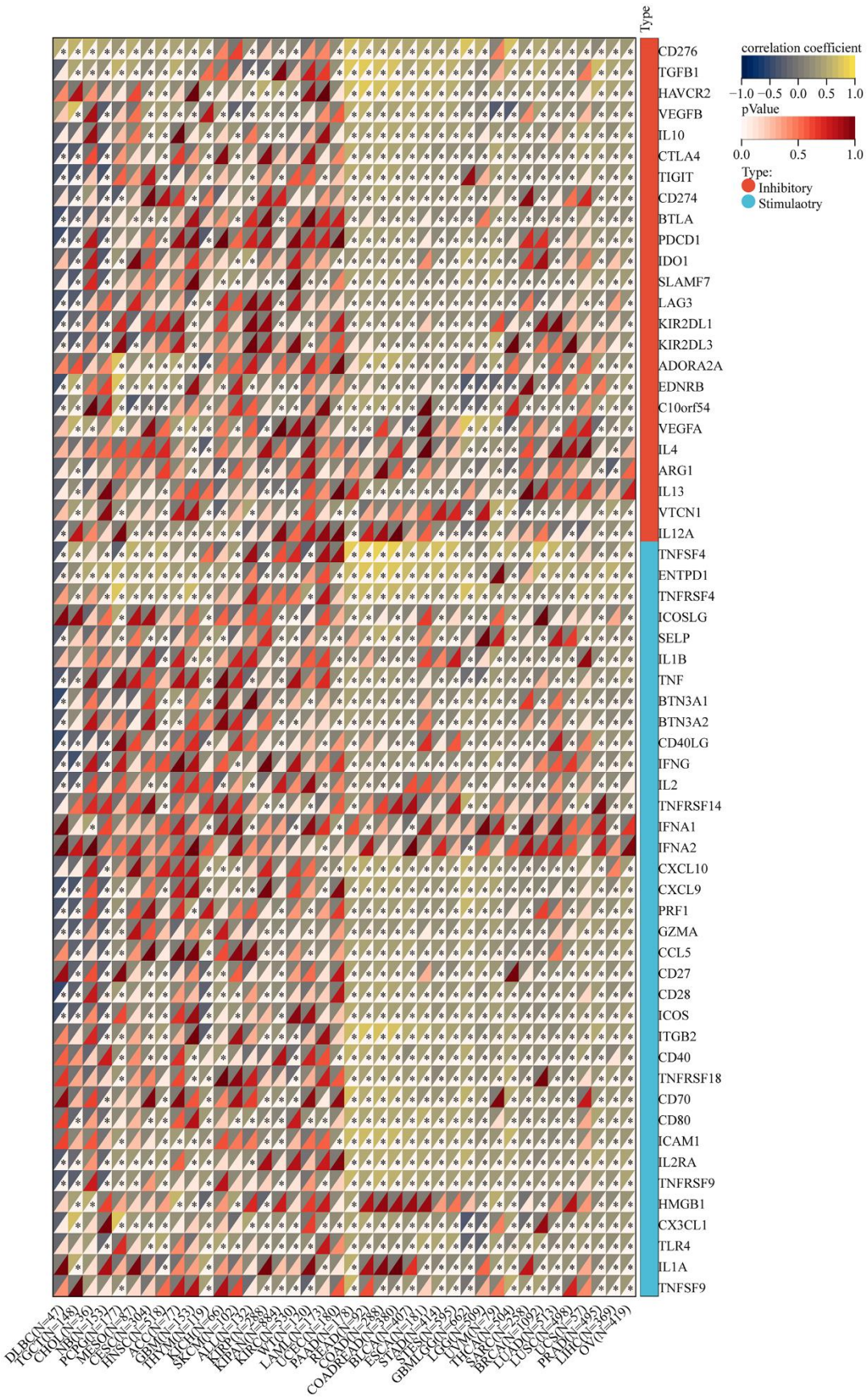


Figure 6. Relationship between NOX4 mRNA expression and immune checkpoints in multiple cancers; (*P < 0.05).

Exploration of NOX4's epigenetic regulation through DNA methylation analysis provides valuable insights. Hypomethylation of NOX4 has been observed in various cancer types, including breast cancer, lung squamous cell carcinoma, bladder cancer, lung adenocarcinoma, prostate cancer, endometrial cancer, gastric cancer, thyroid cancer, and gallbladder cancer, suggesting that epigenetic modifications may contribute to its dysregulation in cancer. Intriguingly, hypermethylation of NOX4 is associated with a lower risk and longer OS in specific cancer cohorts, highlighting the complex interplay between epigenetic regulation and clinical outcomes.

Functional enrichment analysis reveals potential roles for NOX4 in modulating cancer metabolism through its interaction with proteins involved in cancer-related signaling pathways such as CYBB, NOX1, CYB1, DUOX1, TLR4, NOX3, NOX5, NCF1, NCF2, and NCF4. This suggests that NOX4 may have multi-functional roles beyond its canonical function in redox homeostasis. The positive correlation between NOX4 expression and immune cell infiltration levels in the tumor microenvironment adds complexity to its role in cancer progression. Furthermore, the association with established biomarkers for predicting immune checkpoint blockade response outcomes positions NOX4 within the intricate network of immune responses in cancer.

In conclusion, this comprehensive analysis deepens our understanding of NOX4 in cancer, highlighting its diverse roles in gene expression, diagnostic potential, prognostic implications, epigenetic regulation, functional associations, and immune. The multifaceted involvement of NOX4 in cancer underscores its significance as a potential target for further research and clinical exploration.

Abbreviations

ACC: Adrenocortical carcinoma; BLCA: Bladder urothelial carcinoma; BRCA: Breast invasive carcinoma; CESC: Cervical squamous cell carcinoma and endo-cervical 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; GBMLGG: GBM: Glioblastoma multiforme; 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.

AUTHOR CONTRIBUTIONS

The research studies were designed by LQH, and conducted by YYL, HH, and XJY. Data analysis was performed by DHH, XWW, and MYY, and the manuscript was written by YYL and HH. Supervision was provided by LQH, and all authors provided comments on the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021; 71:209–49. https://doi.org/10.3322/caac.21660 PMID:33538338

2. Xia C, Dong X, Li H, Cao M, Sun D, He S, Yang F, Yan X, Zhang S, Li N, Chen W. Cancer statistics in China and United States, 2022: profiles, trends, and determinants. Chin Med J (Engl). 2022; 135:584–90. https://doi.org/10.1097/CM9.0000000000002108 PMID:35143424

3. Tsimberidou AM, Fountzilas E, Nikanjam M, Kurzrock R. Review of precision cancer medicine: Evolution of the treatment paradigm. Cancer Treat Rev. 2020; 86:102019.

https://doi.org/10.1016/j.ctrv.2020.102019 PMID:32251926

4. Brandes RP, Weissmann N, Schröder K. Nox family NADPH oxidases: Molecular mechanisms of activation. Free Radic Biol Med. 2014; 76:208–26. https://doi.org/10.1016/j.freeradbiomed.2014.07.046 PMID:25157786

5. Ogboo BC, Grabovyy UV, Maini A, Scouten S, van der Vliet A, Mattevi A, Heppner DE. Architecture of the NADPH oxidase family of enzymes. Redox Biol. 2022; 52:102298. https://doi.org/10.1016/j.redox.2022.102298 PMID:35334249

6. Vermot A, Petit-Härtlein I, Smith SME, Fieschi F. NADPH Oxidases (NOX): An Overview from Discovery, Molecular Mechanisms to Physiology and Pathology. Antioxidants (Basel). 2021; 10:890. https://doi.org/10.3390/antiox10060890 PMID:34205998

7. Ford K, Hanley CJ, Mellone M, Szyndralewiez C, Heitz F, Wiesel P, Wood O, Machado M, Lopez MA, Ganesan AP, Wang C, Chakravarthy A, Fenton TR, et al. NOX4 Inhibition Potentiates Immunotherapy by Overcoming Cancer-Associated Fibroblast-Mediated CD8 T-cell Exclusion from Tumors. Cancer Res. 2020; 80:1846–60. https://doi.org/10.1158/0008-5472.CAN-19-3158 PMID:32122909

8. Yang X, Yu Y, Wang Z, Wu P, Su X, Wu Z, Gan J, Zhang D. NOX4 has the potential to be a biomarker associated with colon cancer ferroptosis and immune infiltration based on bioinformatics analysis. Front Oncol. 2022; 12:968043. https://doi.org/10.3389/fonc.2022.968043 PMID:36249057

9. Eun HS, Chun K, Song IS, Oh CH, Seong IO, Yeo MK, Kim KH. High nuclear NADPH oxidase 4 expression levels are correlated with cancer development and poor prognosis in hepatocellular carcinoma. Pathology. 2019; 51:579–85. https://doi.org/10.1016/j.pathol.2019.05.004 PMID:31443922

10. Liu ZZ, Duan XX, Yuan MC, Yu J, Hu X, Han X, Lan L, Liu BW, Wang Y, Qin JF. Glucagon-like peptide-1 receptor activation by liraglutide promotes breast cancer through NOX4/ROS/VEGF pathway. Life Sci. 2022; 294:120370. https://doi.org/10.1016/j.lfs.2022.120370 PMID:35124000

11. Hanley CJ, Mellone M, Ford K, Thirdborough SM, Mellows T, Frampton SJ, Smith DM, Harden E, Szyndralewiez C, Bullock M, Noble F, Moutasim KA, King EV, et al. Targeting the Myofibroblastic Cancer-Associated Fibroblast Phenotype Through Inhibition of NOX4. J Natl Cancer Inst. 2018; 110:109–20. https://doi.org/10.1093/jnci/djx121 PMID:28922779

12. Shen CJ, Chang KY, Lin BW, Lin WT, Su CM, Tsai JP, Liao YH, Hung LY, Chang WC, Chen BK. Oleic acid-induced NOX4 is dependent on ANGPTL4 expression to promote human colorectal cancer metastasis. Theranostics. 2020; 10:7083–99. https://doi.org/10.7150/thno.44744 PMID:32641980

13. Bi Y, Lei X, Chai N, Linghu E. NOX4: a potential therapeutic target for pancreatic cancer and its mechanism. J Transl Med. 2021; 19:515. https://doi.org/10.1186/s12967-021-03182-w PMID:34930338

14. Pecchillo Cimmino T, Ammendola R, Cattaneo F, Esposito G. NOX Dependent ROS Generation and Cell Metabolism. Int J Mol Sci. 2023; 24:2086. https://doi.org/10.3390/ijms24032086 PMID:36768405

15. Moon JS, Nakahira K, Chung KP, DeNicola GM, Koo MJ, Pabón MA, Rooney KT, Yoon JH, Ryter SW, Stout-Delgado H, Choi AM. NOX4-dependent fatty acid oxidation promotes NLRP3 inflammasome activation in macrophages. Nat Med. 2016; 22:1002–12. https://doi.org/10.1038/nm.4153 PMID:27455510

16. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017; 45:W98–102. https://doi.org/10.1093/nar/gkx247 PMID:28407145

17. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, Doncheva NT, Legeay M, Fang T, Bork P, Jensen LJ, von Mering C. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res. 2021; 49:D605–12. https://doi.org/10.1093/nar/gkaa1074 PMID:33237311

18. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, Benner C, Chanda SK. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun. 2019; 10:1523. https://doi.org/10.1038/s41467-019-09234-6 PMID:30944313

19. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, Li B, Liu XS. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res. 2020; 48:W509–14.

https://doi.org/10.1093/nar/gkaa407
PMID:32442275

20. Fu J, Li K, Zhang W, Wan C, Zhang J, Jiang P, Liu XS. Large-scale public data reuse to model immunotherapy response and resistance. Genome Med. 2020; 12:21. https://doi.org/10.1186/s13073-020-0721-z PMID:32102694

21. Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev. 2007; 87:245–313. https://doi.org/10.1152/physrev.00044.2005 PMID:17237347

22. Larson-Casey JL, Gu L, Kang J, Dhyani A, Carter AB. NOX4 regulates macrophage apoptosis resistance to induce fibrotic progression. J Biol Chem. 2021; 297:100810. https://doi.org/10.1016/j.jbc.2021.100810 PMID:34023385

23. Zhang J, Li H, Wu Q, Chen Y, Deng Y, Yang Z, Zhang L, Liu B. Tumoral NOX4 recruits M2 tumor-associated macrophages via ROS/PI3K signaling-dependent various cytokine production to promote NSCLC growth. Redox Biol. 2019; 22:101116. https://doi.org/10.1016/j.redox.2019.101116 PMID:30769285