NEK7 Promotes Gastric Cancer Progression as a Cell Proliferation Regulator

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Primary research

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Abstract

Background: Gastric cancer is one of the most common malignant tumors of alimentary canal. However, its targeted therapy develops at a slow pace. Thus, exploring the mechanism of gastric cancer cells' malignant behavior is of crucial importance to its therapeutic method exploitation. Mammalian NIMA-related kinases are considered to play a significant role in cell proliferation. However, NIMA family proteins have not been reported in gastric cancer yet.

Methods: Bioinformatics analysis was used to clarify the expression patterns of NEK1-NEK11 and their effects on prognosis. We also analyzed the effect on immune infiltration and related pathways of NEK7. At cell level, EdU assay, cell cycle assay and CCK-8 assay were utilized to clarify the NEK7 effect on gastric cancer cell proliferation. Mice subcutaneous model revealed the regulating effect on gastric cancer cell proliferation of NEK7 in vivo.

Results: Bioinformatics analysis revealed that NEK7 upregulates in gastric cancer and is related to poor prognosis. Meanwhile, NEK7 is related to T-stage, which has closely related to cell proliferation. Further analysis showed that NEK7 has correlation with multiple immune cells infiltration as well as related pathways of gastric cancer. Cell experiments indicated the promoting effect of NEK7 to cell proliferation, while lack of NEK7 could result in gastric cancer proliferation inhibition and G1/S arrest.

Conclusion: NEK7 exert the regulatory effect during cell proliferation and is closely related to tumor immune infiltration

Background

Cancer is known as the leading factor to cause death and a huge barrier of extending life expectancy all around the world. According to the statistics published on CA by American Cancer Society in 2021, there were about 19.3 million new cases of cancer (except for non-melanoma skin cancer) in 2020, while nearly 10 million deaths were reported because of that. Thereinto, gastric cancer is the 5th leading cause of death, the 4th of male death while the 7th of female[1]. Immortalization is the most basic characteristics of tumor so target cell proliferation is the essential idea of cancer therapy for decades. However, the targeted therapy of gastric cancer develops slowly. Therefore, shedding new light on the mechanism of gastric cancer cell proliferation is of vital significance to develop new therapeutic methods and early screening. The stability of cell cycle is maintained by many regulatory proteins, especially by kinases[2]. Abnormal expression of cell cycle-related kinases could lead to acceleration of cell cycle and improper proliferation. Mammalian NIMA-related kinases (NEK proteins)is a group of positive regulatory proteins of cell cycle which could regulate microtubule and promote mitosis. They also play an important role in regulating normal cell cycle. In many types of malignancies, NEK proteins have higher expression, more ectopic expression, and genetic variation than normal tissue. These unusual expression of NEK proteins could lead to cell cycle dysregulation which would induce cancer eventually[3].

In 1975, Morris found Apergillus nidulans never goes into mitosis A through the research of mitotic mutants of Aspergillus nidulans. NIMA kinases were named after nidulans protein kinases which encoded by NIMA gene. They are a kind of serine-threonine kinase which is essential for going through mitosis[4][5]. Since being discovered, 11 kinds NIMA kinases which are of genetically different were identified in most of eukaryotes, including human, and named NEK1-NEK11. These 11 members have similar sequence of amino catalytic region with NIMA and they differentiate because of the different length of carboxyl terminal. As for the function of NEK proteins, previous studies have showed that NEK2, NEK6, NEK7 and NEK9 mainly participate in G2-M key point regulation, promote the maturity of centrosome and influence chromosome condensation as well as spindles formation in mitosis, while NEK1, NEK10 and NEK11 play a role in DNA damage responses[6][7]. We analyzed the expression and their effects on prognosis of NIMA kinases (NEK1-NEK11) synthetically and found that only NEK7 is upregulated in gastric cancer and has a significant effect on the gastric cancer prognosis.

Previous research showed that NEK7 is activated by direct connection of NEK9 through allosteric and non-allosteric mechanisms in mitosis. Then NEK7 controls phosphorylation of kinesin KIF11 and recruitment to centrosome. As a result, the centrosome separates[8]. NEK7 also takes a part in nuclear membrane disruption. Whereafter, NEK7 participate in spindle assembly through phosphorylated heat shock protein NUP98 and regulate motile kinesin Mklp2 as well as kinesin KIF14 to control cytokinesis[9]. Salem found that lack of NEK7 would cause death in late embryonic and early postnatal period as well as severe developmental retardation through construction and analysis of NEK7 defected mouse. Meanwhile, mouse embryonic fibroblasts (MEF) trend to present lagging chromosome, micronucleus formation and failed cytokinesis[10]. NEK7 is highly expressed under pathological conditions. It actives inflammasome NLRP3 to produce a lot of polykaryocyte and apoptotic cells which are closely related to inflammation, then causes inflammation in body.[11][12]. Besides, for NEK7, its effects on diabetic retinal degeneration, systemic lupus erythematosus and gout have already been studied[13][14][15][16]. Meanwhile, Nada H. Eisa et al. found that the expression of NEK7 could promote cell division in cancer[17]. Zhang etc. found the frequent upregulation of NEK7 in retinoblastoma cell lines, while knock down NEK7 by virus-mediated RNA interference could significantly inhibit cell growth as well as colony formation, and cause arrest in G0/G1 phase[18]. Moreover, Zhou etc. found that in hepatoma cell lines, the expression of NEK7 is significantly higher than that in normal liver cell lines. Furthermore, virus mediated NEK7 silencing could inhibit the growth of hepatocellular carcinoma cell lines and the growth of tumor on the xenotransplantation model onto immunodeficient mice[19]. However, NEK7 in gastric cancer has not yet reported.

Methods

Cell culture

Human gastric cancer cell line MKN-45, SGC-7901, HEK-293T were purchased from Beyotime Biotechnology (Shang Hai, China). Thereinto, MKN-45, SGC-7901 and HEK293T were cultured in 90%DMEM + 10%FBS +1%P/S medium.

Construction of NEK7 knockdown cell line

Plasmid expressed shRNA-1 (CATTCTCGAAGAGTCATGCATAGAGATATAAAACCAGCTAA) and shRNA-2 (GAAGGCCTTACGACCGGATATGGGCTATAATACATTAGCCA) were designed.

After screening, we used shRNA-1, which is more effective, to construct stable knockdown cell lines. The lentivirus packaging kit (Gmeasy-40, Genomeditech) were utilized.

Protein extraction

Cells were cultured in 100mm petri dish until their density reached to 70% to 90%. RIPA-medium (Beyotime Biotechnology, P00103C) were used to extract total protein from cultured cells. Then boiled for ten minutes after adding loading buffer(CoWin Biosciences).

RT-PCR

MKN-45 and SGC-7901 were treated with Trizol and RNA extracted following the manufacturer's instructions. Dissolved the RNA in 10-100 ul DEPC-treated water and did proper dilution when quantification. The RNA was measured via a spectrophotometer (UV) and reverse transcribed into cDNA using reverse transcription kit.

RNA expression was assayed by Real-Time PCR set to 95℃ 30s, 55℃ 30s, 72℃ 7min and repeat for 40 circulation. GAPDH was utilized as an endogenous control. All RT-qPCR reactions were performed 3 times independently. The relative RNA expression levels were calculated using the 2-△△Ct method.

Western blot

We used protease inhibition to extract total protein from cell lysis of MKN45 and SGC-6901. Bicinchoninic acid (BCA) protein assay kit was used to measure protein concentration. Protein was separated by SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) with proper concentration and transformed onto polyvinylidene fluoride (PVDF) membranes. After blocked for 1h at 4℃ using TBST brewed skim milk powder, the membrane was incubated all night with the anti-NEK7 (ABACM: ab13514)antibody and anti-GAPDH (ABCAM: ab8245) antibody which was diluted to appropriate concentration. Then after washing, the membrane was incubated with the second antibody at 4℃ for at least 1h and washed by TBST for 3 times. Signals were detected using a chemiluminescence system and analyzed using Image Lab Software.

5-Ethynyl-2-Deoxyuridine (EdU) to stain proliferating cells

EdU staining was utilized to analysis MKN-45 and SGC-7901 cell with normal and down regulation of NEK7 expression. EdU buffer and the cell medium were mixed in a ratio of 1:1000 and added into the plate (2ml in each well) then incubated at 37℃ for 2 hours. The medium was discarded and after washing, PBS with 4% paraformaldehyde was added (2 ml each well) for cell fixation (37℃, 30min). Then permeabilized the cells with 0.5% Triton X-100 and cultured for 10 min. EdU staining solution was

added and then staining the nuclei with DAPI. The results could be visualized under a fluorescence microscope.

CCK-8 assay

MKN-45 and SGC-7901 cells were suspended and seeded into 96-well plates. After incubated at 37℃ for 24 hours, 10ul cell counting kit-8 (CCK-8) solution was added in each well. The absorbance was measured by a microplate reader at 450nm after incubated for 4 hours in dark.

Flow-cytometry

The treated cells were collected and fixed with chilled 75% ethanol at -20 °C overnight or longer. After being ethanol discarded, cells were washed twice with PBS and then stained with cell cycle and apoptosis kit (UE, C6031) at room temperature for 30 min. Cell cycle analysis was performed on the flow cytometry (FACS LSRII, BD Bioscience, China).

Animal studies

shNC and shNEK7 cell were collected and suspended in pre-cooled PBS and subcutaneously injected into mice ($1X10^7$/100μl per mouse). The mice were sacrificed at the end of the experiment (26 days). The removed tumors were used in IHC staining and WB.

Bioinformatics analysis and statistics

The differential gene expression in tumor and normal tissue, and the correlation between proteins expression and clinical prognosis of GC patients were both analyzed by GEPIA tool. The correlation between NEK7 expression and immune infiltration level were performed using TIMER 2.0. GraphPad Prism 7 software was also used to analyze the results.

Results

NEK7 is highly expressed in gastric cancer and may be implicated in poor prognosis

We synthetically analyzed the expression of NIMA kinases (NEK1-NEK11) and the correlation between NEK proteins expression and clinical prognosis of GC patients. The results showed that only NEK7 expression level was upregulated in gastric cancer ($|LogFC|>1$, $P<0.01$ was significant) and predicts poor survival prognosis.

NEK7 is related to staging of gastric cancer

We obtained RNA-seq data and the corresponding clinical information of 375 gastric cancer samples in the TCGA STAD dataset to analysis the relationship between NEK7 expression level and clinical pathological grading and staging. The results indicated that the high expression of NEK7 may be

implicated in late T stage and high pathological grade. The complete clinicopathological information in details is listed in Table 1.

NEK7 is associated with immune cell infiltration

TIMER 2.0 was used to analyze the effect of NEK7 on immune infiltration. The expression of NEK7 is positively related to the infiltration of Treg cell, macrophages, monocytes, neutrophils and M2 macrophages in gastric cancer, though it has no distinct correlation with infiltration of M1 macrophages.

NEK7 is related to gastric cancer cell immunization and important pathway of cell proliferation

According to RNA-seq data of gastric cancer from TCGA, we analyzed genetic correlation and got the gene map of positive and negative associated with NEK7. Then the enrichment analysis on GO-BP and KEGG was performed. The results of GO-BP analysis indicate that NEK7 is closely related to cell-cell adhesion via plasma-membrane adhesion molecules pathway and multicellular organismal signaling pathway. Besides, NEK7 is positively related to cell junction pathway. These results demonstrate that NEK7 is significant in regulating multicellular signaling and intracellular proliferation-related pathway. Moreover, we clarified the relationship between NEK7 and pathways above by GSEA analysis.

NEK7 could promote proliferation of gastric cancer cells in vitro

NEK7 down-regulated gastric cancer models in vitro were established basing on MGC-803 and MKN-45. Western blot and qRT-PCR analysis were conducted to detect the effect of NEK7 silencing. Then we utilized CCK-8 assay, EDU assay and flow cytometry to investigate the effect on gastric cancer cell proliferation of NEK7. The result showed that down-regulation of NEK7 could inhibit cell proliferation of gastric cancer, reduce the proportion of neoplastic gastric cancer cells, and lead to cell cycle G1/S arrest.

NEK7 could promote gastric cancer proliferation in vivo

Next, we took MKN45 cell as the object of study and did subcutaneous injection with MKN45-shNC and MKN45-shNEK7 in mice. The growth of subcutaneous tumor was under detection. The mice were killed and dissected after 26 days. Weighing the removed subcutaneous tumors. Then, we did western blotting to detect the expression level of NEK7. Moreover, the IHC was utilized to detect the expression level of NEK7 and MKI-67. The results indicated that the tumor volume and mass of the experimental group injected with stable MKN45-shNEK7 cells were significantly lower compared with that of the control group injected MKN45-shNC cells. WB and IHC showed that the NEK7 expression level of the experimental group injected with stable MKN45-shNEK7 cells were significantly lower compared with that of the control group injected MKN45-shNC cells.

Discussion

Gastric cancer is a malignancy whose new cases ranked 5th all around world while its death toll numbered 4th. When it comes to the development of targeted therapy, gastric cancer is more limited than

non-small cell lung cancer, chronic myelogenous leukemia, and liver cancer. Though HER-2 targeted pathway, VEGF pathway and immune checkpoint are already widely used, the overall prognosis of gastric cancer patients haven't been revolutionized. Cell proliferation is a basic strategy of targeted therapy so that its mechanism and its relationship with immune regulatory are clinically worth exploring.

NIMA-related kinases (NEKs) are a group of protein whose domains shows identity with NIMA (never-in-mitosis A). A few members of NEKs were reported to relate to tumor progression, such as breast cancer and colorectal cancer. We did bioinformatic analysis and the results showed that among NEK-family, the expression of NEK2, NEK6 and NEK7 is upregulated whereas NEK3 and NEK7 is related to poor prognosis of gastric cancer. Thus, we chose the one shows differences both in the expression level and the prognosis, NEK7, as the research object.

The cGMP-PKG pathway and the Hedgehog signaling pathway could influence tumor cell fate determination and is closely related to the development of tumor[20][21][22]. According to Xiang T, the cGMP-PKG pathway is related to the gastric cancer which induced by Helicobacter pylori[23]. Besides, the research of Lv Y et al. shows that cGMP-PKG pathway could enhance breast cancer stemness and metastasis[20]. The Hedgehog signaling pathway is considered to be able to promote tumor angiogenesis, metastasis, and stemness. Our data indicates that NEK7 participates in cancer cell proliferation and it is related to clinical stage as well as pathological grade. Also, NEK7 has potential regulatory function of the cGMP-PKG pathway and the Hedgehog signaling pathway.

Immune infiltration analysis shows that NEK7 is closely related to the infiltration of macrophage, especially M2 macrophage. Whereas M2 macrophage could promote gastric cancer metastasis, cell proliferation promote tumor progression[24]. NEK7 could interact with NLRP3 and plays an important role in inflammatory response and macrophage fate determination[25]. We hold the view that NEK7 could promote gastric cancer progression not only by regulating cancer cell proliferation directly, but also through cell interaction which could regulate immune infiltration and changes of immune cell subsets. In addition, GO and KEGG also indicate that NEK7 has a close relationship with a lot intercellular signaling pathways and matrix-related signaling pathways.

Ultimately, we revealed how NEK7 promotes gastric cancer proliferation and analyzed the mechanism of promoting progression of gastric cancer.

Abbreviations

NIMA never-in-mitosis A

GC gastric cancer

EdU 5-Ethynyl-2-Deoxyuridine

CCK-8 cell counting kit-8

STAD	Stomach adenocarcinoma
RIP	RNA Immunoprecipitation
acRIP	ac4C-RNA Immunoprecipitation
TCGA	The Cancer Genome Atlas
GEPIA	gene expression profiling and interactive analyses
shRNA	short hairpin RNA
qRT-PCR	Quantitative real-time PCR
WB	Western blot
IHC	Immunohistochemistry
GO-BP	Gene Ontology Biological Process
GSEA	Gene Set Enrichment Analysis

Declarations

Availability of data and materials

The datasets used and/or analyzed during the current study can be acquired from the corresponding author upon reasonable request.

Authors' contributions

Weilin Jin, Yike Li and Xiaoran Zhu conceived and designed the study. Yike Li carried out the experiments. Xiaoran Zhu performed statistical analysis of the pathological data and drafted the manuscript. The authors marked with "#" have equal contribution. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This research was approved by the Ethics Committee of the First Hospital of Lanzhou University. The GC tissue microarrays were purchased from Shanghai Outdo Biotech.

Consent for publication

All subjects have written informed consent.

Competing interests

The authors declare that they have no competing interests.

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References

1. Siegel RL, Miller KD, Fuchs HE, et al. Cancer Statistics, 2021[J]. CA: A Cancer Journal for Clinicians, 2021, 71(1).
2. Grallert B, Boye E. The Gcn2 Kinase as a Cell Cycle Regulator[J]. Cell Cycle. 2007;6(22):2768–72.
3. Cell cycle regulation. by the NEK family of protein kinases[J]. J Cell Sci. 2012;125(19):4423–33.
4. Ronald N. Morris. Mitotic mutants of Aspergillus nidulans[J]. Genetics Research. 1975;26(3):237–54.
5. Morris B. A Mutation in Aspergillus nidulans That Blocks the Transition from Interphase to Prophase[J]. The Journal of Cell Biology. 1983;96(4):1155–8.
6. Fry AM, Richard B, Joan R. Mitotic Regulation by NEK Kinase Networks[J]. Frontiers in Cell Developmental Biology. 2017;5:102.
7. Matossian MD, Wells CI, Zuercher WJ, et al. Targeting Never-In-Mitosis-A Related Kinase 5 in Cancer: A Review[J]. Current Medicinal Chemistry, 2021, 28.
8. Sun Z, Gong W, Zhang Y, et al. Physiological and Pathological Roles of Mammalian NEK7[J]. Frontiers in Physiology, 2020, 11.
9. Cullati SN, Kabeche L, Kettenbach AN, et al. A bifurcated signaling cascade of NIMA-related kinases controls distinct kinesins in anaphase[J]. The Journal of Cell Biology, 2017: jcb.201512055.
10. Salem H, Rachmin I, Yissachar N, et al. Nek7 kinase targeting leads to early mortality, cytokinesis disturbance and polyploidy[J]. Oncogene. 2010;29(28):4046–57.
11. Jin X, Lu L, Li L. NEK7: a novel promising therapy target for NLRP3-related inflammatory diseases[J]. Acta Biochim Biophys Sin (Shanghai). 2016;48(010):966–8.
12. Sharif H, Wang L, Wang WL, et al. Structural mechanism for NEK7-licensed activation of NLRP3 inflammasome[J]. Nature. 2019;570(7761):338.
13. Cai HQ, Wang P, Zhang B, et al. Expression of the NEK7/NLRP3 inflammasome pathway in patients with diabetic lower extremity arterial disease[J]. BMJ Open Diabetes Research Care. 2020;8(2):e001808.

14. Yi Z, Lv X, Hu Z, et al. Protection of Mcc950 against high-glucose-induced human retinal endothelial cell dysfunction[J]. Cell Death Dis. 2017;8(7):e2941.
15. Ma ZZ, Sun HS, Lv JC, et al. Expression and clinical significance of the NEK7-NLRP3 inflammasome signaling pathway in patients with systemic lupus erythematosus[J]. Journal of Inflammation, 2018, 15(1).
16. Lin Y, Luo T, Weng A, et al. Gallic Acid Alleviates Gouty Arthritis by Inhibiting NLRP3 Inflammasome Activation and Pyroptosis Through Enhancing Nrf2 Signaling[J]. Frontiers in immunology, 2020, 11:580593.
17. Eisa NH, Jilani Y, Kainth K, et al. The co-chaperone UNC45A is essential for the expression of mitotic kinase NEK7 and tumorigenesis[J]. Journal of Biological Chemistry, 2019.
18. Zhang W. Zhang. Downregulation of NIMA-related kinase-7 inhibits cell proliferation by inducing cell cycle arrest in human retinoblastoma cells[J]. Experimental Therapeutic Medicine. 2018;15(2):1360–6.
19. Lei Z. Zhixin, et al. Nek7 is overexpressed in hepatocellular carcinoma and promotes hepatocellular carcinoma cell proliferation in vitro and in vivo[J]. Oncotarget, 2016.
20. Lv Y, Wang X, Li X, et al. Nucleotide de novo synthesis increases breast cancer stemness and metastasis via cGMP-PKG-MAPK signaling pathway[J]. PLoS Biol. 2020;18(11):e3000872.
21. Kong X, Wang J, Yang H. Upregulation of lncRNA DARS-AS1 accelerates tumor malignancy in cervical cancer by activating cGMP-PKG pathway[J]. Journal of Biochemical and Molecular Toxicology; 2021.
22. Jiang J. Hedgehog signaling mechanism and role in cancer[J]. Seminars in Cancer Biology, 2021(23).
23. Xiang T, Yuan C, Guo X, et al. The novel ZEB1-upregulated protein PRTG induced by Helicobacter pylori infection promotes gastric carcinogenesis through the cGMP/PKG signaling pathway[J]. Cell Death & Disease, 2021, 12(2).
24. Yang C. Shu, et al. Exosomal miR-487a derived from m2 macrophage promotes the progression of gastric cancer[J]. Cell Cycle; 2021.
25. Schmid-Burgk JL, Chauhan D, Schmidt T, et al. A Genome-wide CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) Screen Identifies NEK7 as an Essential Component of NLRP3 Inflammasome Activation[J]. Journal of Biological Chemistry, 2016, 291.

Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

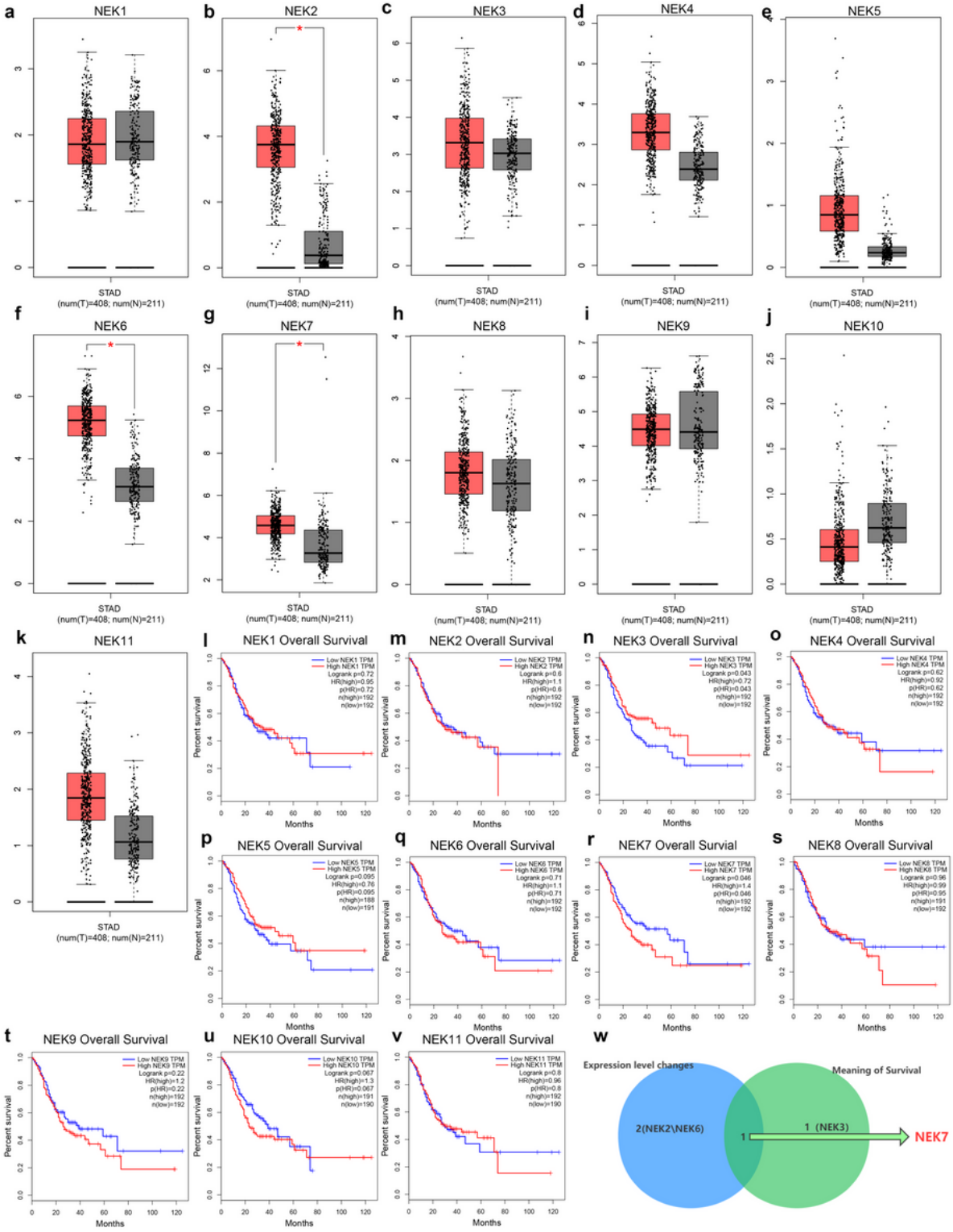

Figure 1

a-k. The expression of NEK1-NEK11 in tumor and normal tissue (the red boxes: tumor, the gray boxes: normal tissue). l-v. The correlation between the expression of NEK1-NEK11 and OS, PPS, and FP. w. The venn diagram shows the intersection between the expression level changes and meaning of survival.

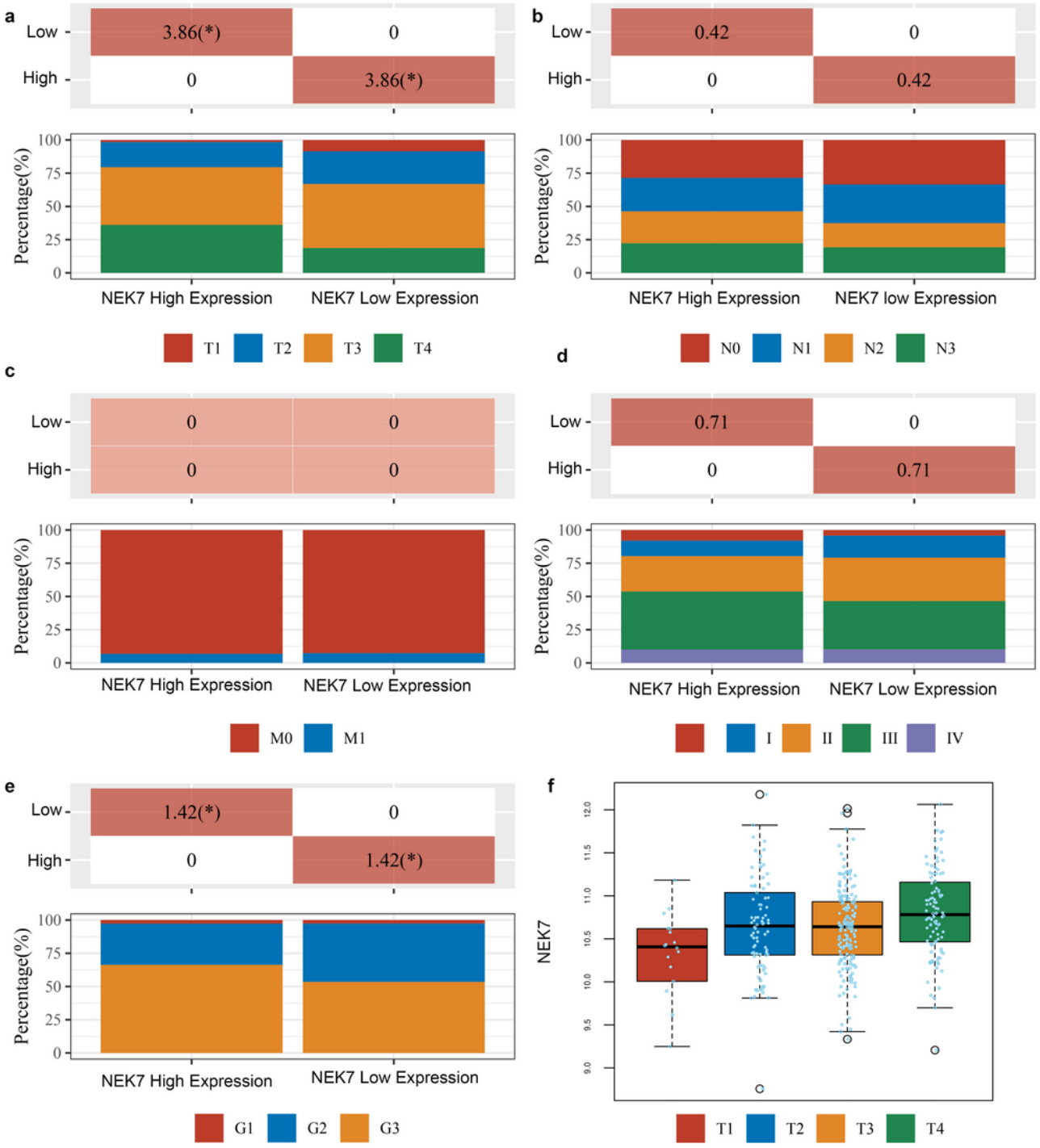

Figure 2

a-d. The stacked diagram shows the percentage of each stage of the TNM staging at different NEK7 expression level. The horizontal axis represents the samples with high or low NEK7 expression and the vertical axis represents clinical information contained in the corresponding grouped samples. analyzed p value by chi-square test. And analyze the significance where the value is -log10 (p value), if marked with *, it means that there is a significant difference ($p < 0.05$). a The percentage of pT staging. b The percentage

of pN staging. c The percentage of pM staging. d The percentage of pTNM staging. e. The stacked diagram shows the percentage of pathological grading at different NEK7 expression level. f. The box plot shows NEK7 expression level in samples of different pT stage staging.

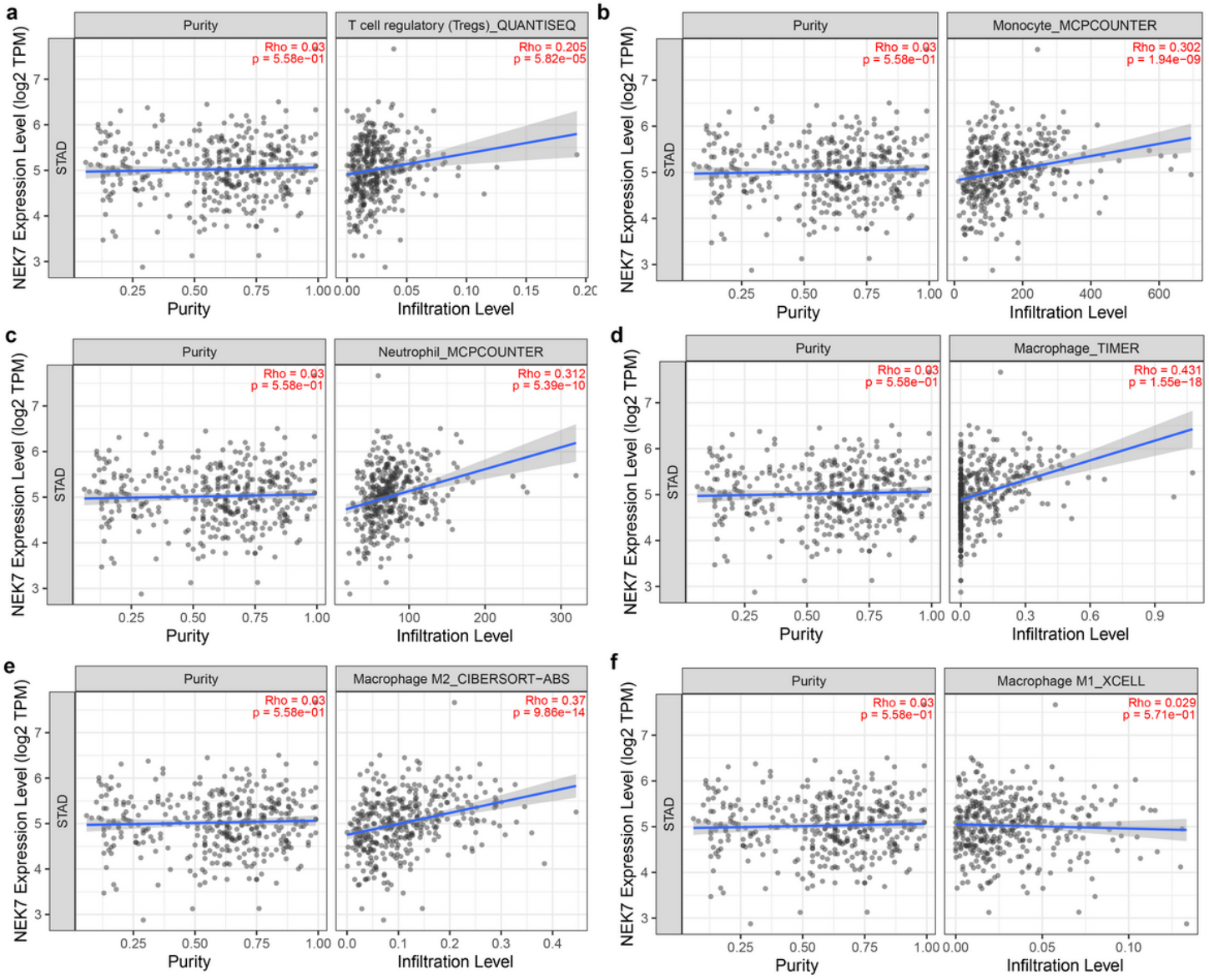

Figure 3

a. The spot plot shows that Tregs infiltration is positively related to NEK7 expression level. b. The spot plot shows that Monocyte infiltration is positively related to NEK7 expression level. c. The spot plot shows that Neutrophil infiltration is positively related to NEK7 expression level. d. The spot plot shows that Macrophage infiltration is positively related to NEK7 expression level. e. The spot plot shows that Macrophage M2 infiltration is positively related to NEK7 expression level. f. The spot plot shows that Macrophage M1 infiltration is negetively related to NEK7 expression level.

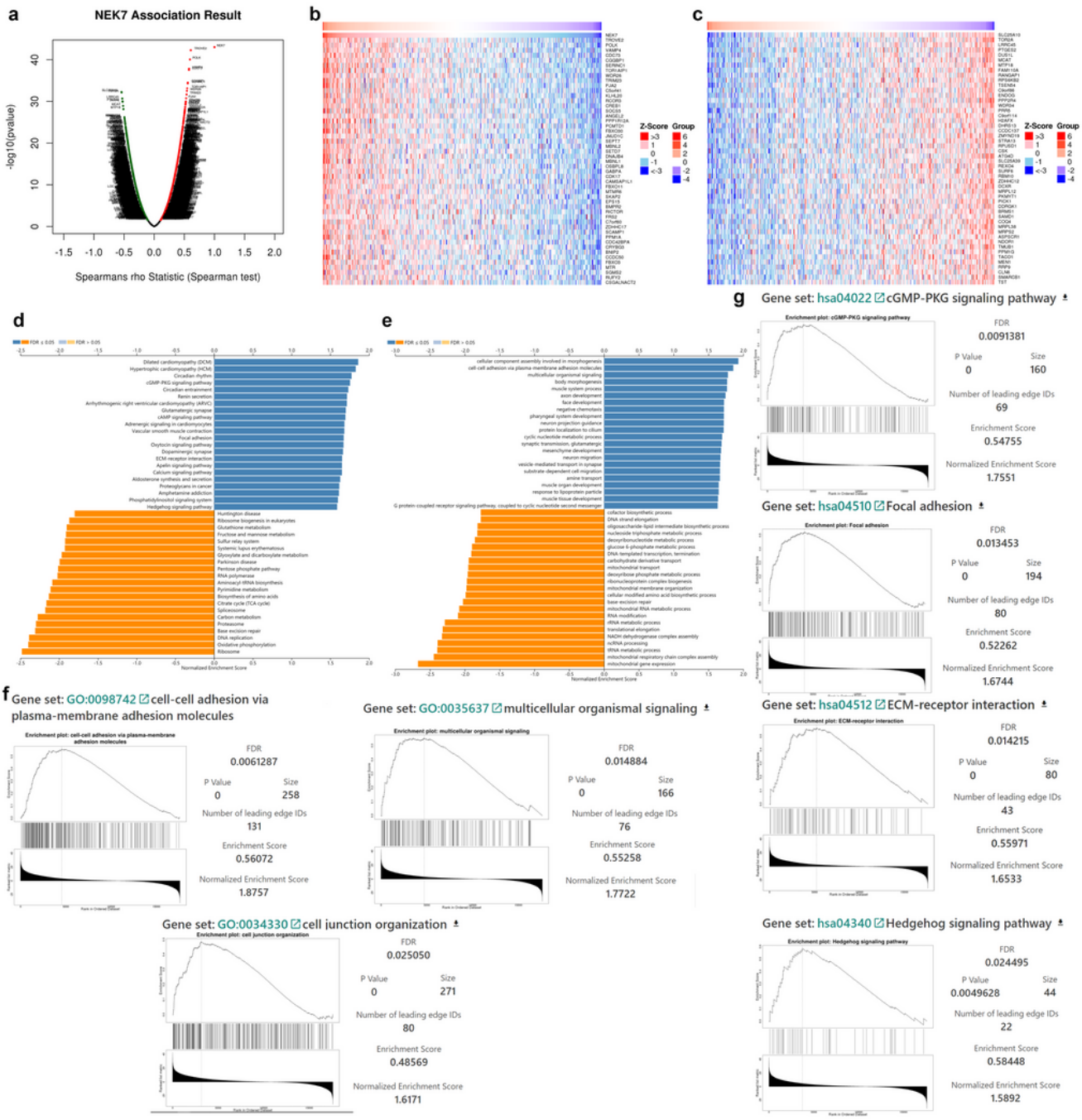

Figure 4

a. The volcano plot shows the genes associated with NEK7. b. The heat map shows first 50 pathways positively relate to NEK7. c. The heat map shows the first 50 pathways negatively relate to NEK7. d. KEGG analysis of gene pathways associated with NEK7. e. GO analysis of gene pathways associated with NEK7. f. GO-BP analysis shows pathways associated with NEK7. g. GSEA analysis shows the relationship

between NEK7 and cGMP-PKG signaling pathway, focal adhesion, ECM-receptor interaction and Hedgehog signaling pathway.

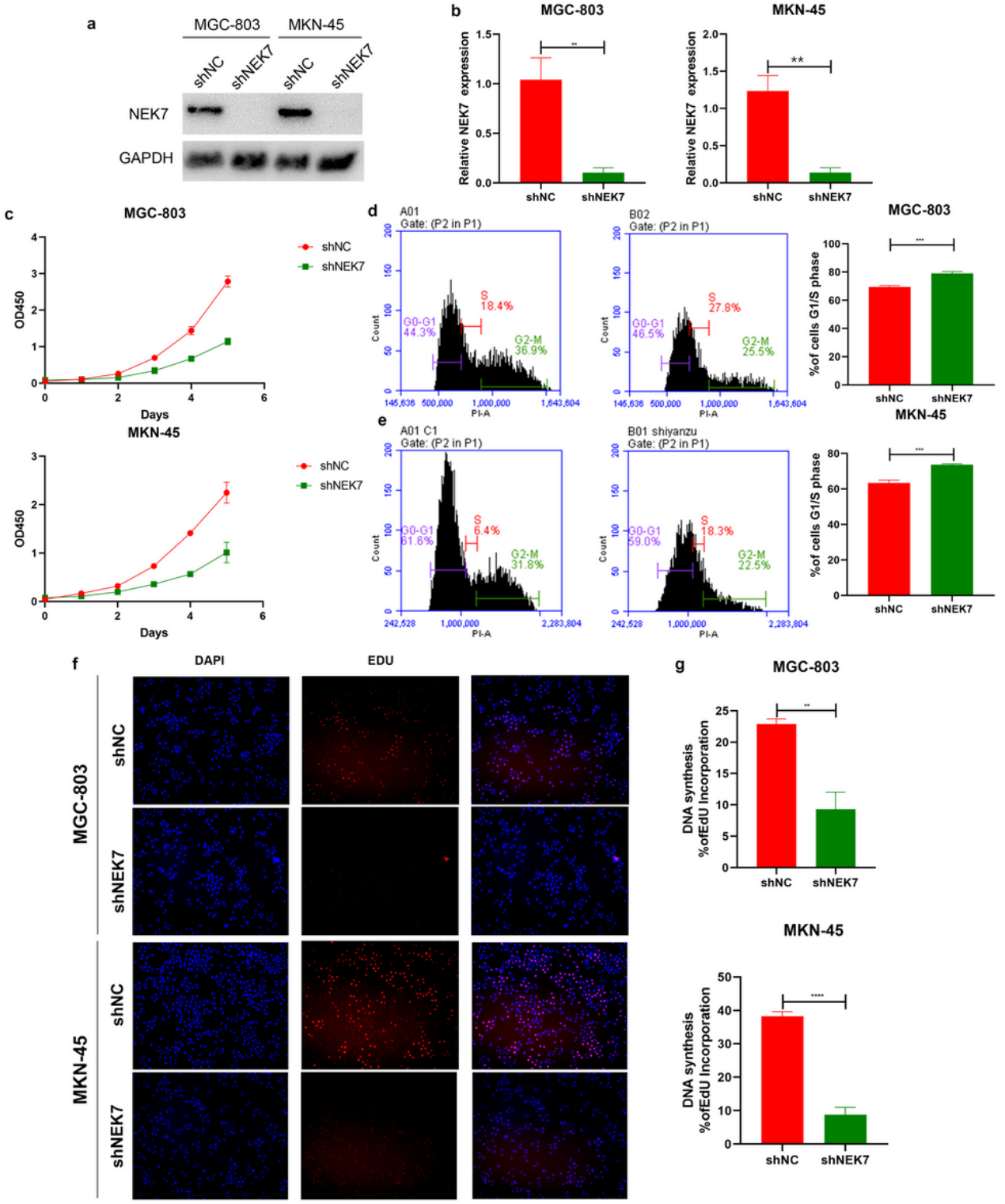

Figure 5

a-b The shRNA-mediated NEK7 repression and NEK7 overexpression confirmed by western blotting assay and qRT-PCR after lentivirus infection in the MGC-803 and MKN-45 cells. c. CCK-8 assay compares the OD450 values of shNEK7 cells and negative control over time. d-e. The result of flow cytometry shows the

effect of NEK7 on cell cycle. f-g. EdU assay and its statistical result show the effect of NEK7 on cell proliferation.

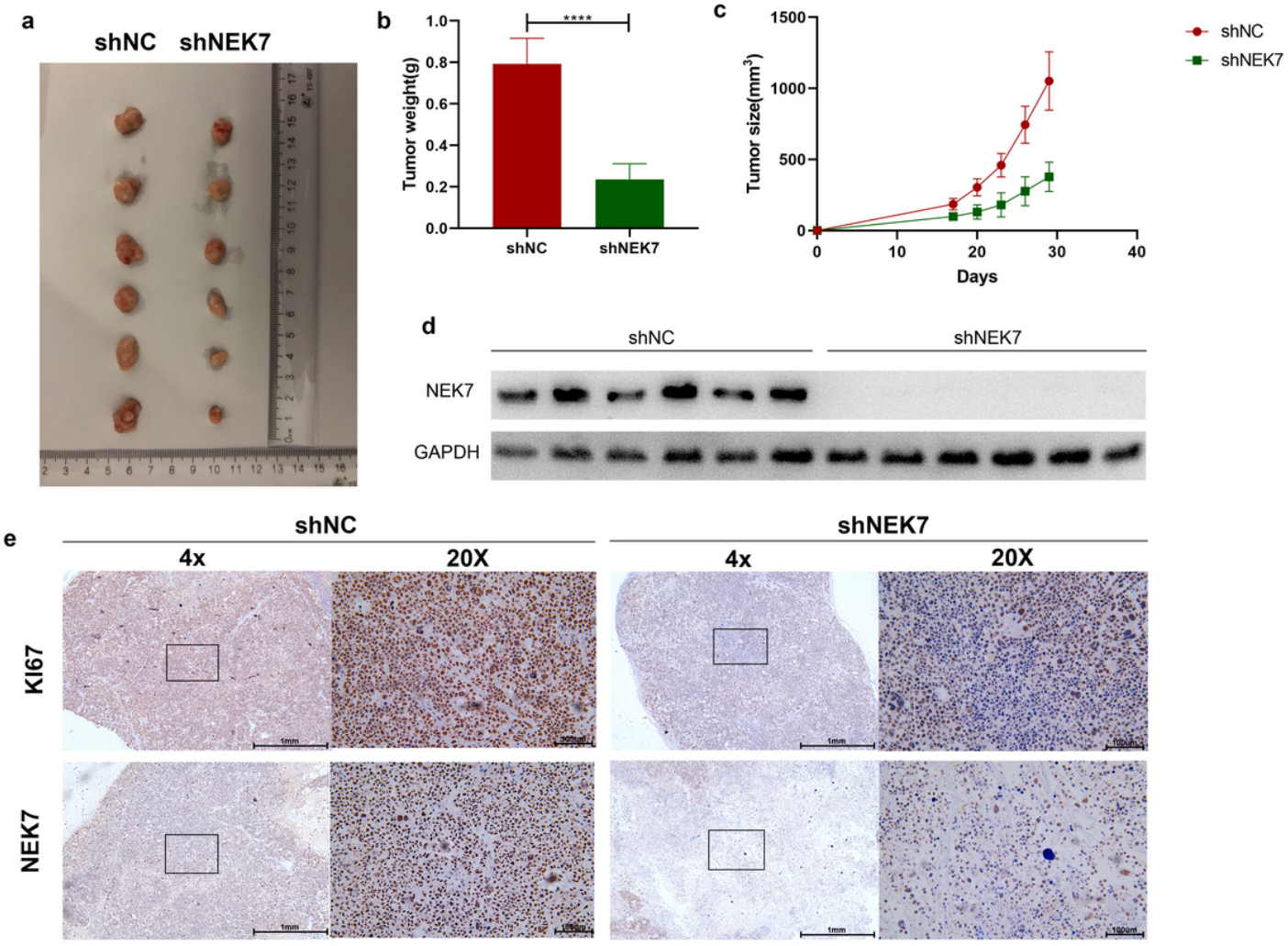

Figure 6

a-c. Knockdown of NEK7 effectively suppressed GC subcutaneous tumor growth in nude mice, and the tumor weight as well as tumor size was quantitatively analyzed. d. Western blotting confirmed the expression of NEK7 in the subcutaneous tumor. e. IHC (NEK7)-stained and IHC(KI67) paraffin-embedded sections obtained from the MKN45-shNC and MKN45-shNEK7 subcutaneous tumors.

Supplementary Files

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- table.1.xlsx