Original Article
NEK2 promotes proliferation, migration and tumor growth of gastric cancer cells via regulating KDM5B/H3K4me3

Yiwei Li[1*], Lijuan Chen[1*], Lixing Feng[1], Mengli Zhu[1], Qiang Shen[2], Yanfen Fang[1,3], Xuan Liu[2], Xiongwen Zhang[1]

[1]Shanghai Engineering Research Center of Molecular Therapeutics and New Drug Development, School of Chemistry and Molecular Engineering, East China Normal University, Shanghai 200062, China; [2]Institute of Interdisciplinary Integrative Biomedical Research, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China; [3]Division of Anti-Tumor Pharmacology, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China. *Equal contributors.

Abstract: The mechanisms of how Never in Mitosis (NIMA) Related Kinase 2 (NEK2) coordinates altered signaling to malignant gastric cancer (GC) transformation remain unclear. Overexpression of NEK2 and KDM5B were observed in GC cell lines with high sensitivity to NEK2 inhibitors. Here we investigated the biological behaviors of NEK2 and the possible mechanisms of regulative effects of NEK2 on KDM5B in GC cell lines both *in vitro* and *in vivo*. The results showed that NEK2 and KDM5B were highly expressed in most of the 10 GC cell lines. NEK2 knockdown in MGC-803 cells led to suppression of cell proliferation and migration *in vitro* and tumor growth *in vivo*, while NEK2 overexpression in BGC-823 cells exhibited the reverse biological effect. When NEK2 was inhibited by NEK2 inhibitors or shNEK2, cellular KDM5B level decreased and H3K4me3 level increased, while overexpression of NEK2 resulted in enhanced KDM5B expression and decreased H3K4me3 level. Though direct interaction between NEK2 and KDM5B was excluded, NEK2 could regulate KDM5B/H3K4me3 expression through β-catenin/Myc both *in vitro* and *in vivo,* which was double confirmed by c-myc and KDM5B inhibitor experiments. Taken together, our study showed that NEK2 was highly expressed in GC cell lines and related to promoting cell proliferation, migration and tumor growth. A NEK2/β-catenin/Myc/KDM5B/H3K4me3 signaling pathway may contribute to the important carcinogenic role of NEK2-mediated malignant behaviors in GC.

Keywords: Gastric cancer, NEK2, KDM5B, β-catenin, c-Myc

Introduction

GC is the fifth most common cancer and ranks as the third leading cause of cancer-related death worldwide, along with an increasing rate in recent years [1, 2]. Surgery, radiation therapy, and chemotherapy are the main therapies for GC and can improve patient survival, but the 5-year survival rate of GC patients is still less than 30% [3]. Exploring the mechanisms of malignant proliferation of GC cells is important for the treatment and improvement of patient survival.

Never in Mitosis (NIMA) Related Kinase 2 (NEK2) is a serine/threonine kinase localizes to the centrosome/centriole and helps to establish a bipolar spindle by initiating the separation of centrosome [4-6]. Overexpression of NEK2 had been found in various human malignant cancers, including breast cancer, prostate cancer, ovarian cancer [7, 8], as well as GC [9, 10]. NEK2 has been implicated in various aspects of malignant transformation, including tumorigenesis, drug resistance and tumor progression. The close relationship between NEK2 and cancer has made it an attractive target for anticancer therapeutic development [11]. Our previous studies had shown that NEK2 inhibitors effectively inhibited the proliferation of cancer cells by inducing cell cycle arrest and apoptosis and significantly suppressed tumor growth in vivo [12, 13]. In a panel of 19 cancer cell lines including gastric, leukemia, colorectal, prostate, breast and hepatoma cancer cells, GC cell lines were relatively more sensitive to NEK2 inhibitor MBM-5 than other cancer cell lines [13]. These results suggested that NEK2 plays

critical roles in the development of GC and GC cancer cells might be specifically useful in clarifying the pro-tumor mechanisms of NEK2. Exploration of mechanisms of NEK2 in promoting malignant GC may shed light on the cancer therapeutics of GC patients and development of NEK2 inhibitors.

KDM5B (also known as JARID1B or PLU1) is a histone demethylase and belongs to the family of JmjC domain-containing proteins that regulate chromatin structure or gene expression by specifically removing methyl residues from tri-, di, and monomethylated lysine 4 on histone H3 lysine 4 (H3K4) [14-16]. Histone methylation is a kind of posttranslational modification that affects biochemical processes such as chromatin formation and translational regulation while broad H3K4me3 at tumor suppressor genes leads to tumor suppression [17, 18]. Accordingly, decreased H3K4me3 was observed in esophageal squamous cell carcinoma tumor growth [19] and increased level of H3K4me3 was inversely associated with lymph node and liver metastasis and predicted an improved prognosis in colorectal cancer [20]. Elevated expression levels of KDM5B has been observed in various primary cancers including GC [21], breast cancer [22], cervical cancer [23], ovarian cancer [24], NSCLC [25] and promotes tumor initiation, invasion and metastasis [26]. Overexpression of KDM5B in GC cells could promote cell growth and metastasis *in vitro* and xenograft formation, growth and metastasis *in vivo* [21]. Bao *et al*. showed that miR-194 inhibited GC cell proliferation and tumorigenesis by targeting KDM5B [27]. Our preliminary study results showed that cancer cell lines sensitive to treatment of NEK2 inhibitors exhibited high expression levels of both NEK2 and KDM5B. When NEK2 was inhibited by NEK2 inhibitors, cellular KDM5B level would decrease which then caused increase in H3K4me3. Despite the relationship between NEK2 and cancer has been frequently reported, the detailed mechanisms of how NEK2 coordinates altered signaling to malignant transformation remain unclear [11]. Our results suggested possible relationship between NEK2 and KDM5B, which have never been reported and might be one of the mechanisms of pro-tumor activities of NEK2.

Hence, this study commits to observe the influence of NEK2 knockdown or overexpression on the cell proliferation and migration of GC cells *in vitro* and tumor growth *in vivo* to confirm the role of NEK2 in malignant GC. Furthermore, we tried to investigate the new relationship between NEK2 and KDM5B, the possible mechanisms of regulative effects of NEK2 on KDM5B expression in GC cells both *in vitro* and *in vivo*. Now, the experimental procedures and findings are reported as follows.

Materials and methods

Cell lines, cell culture and reagents

Human GC cell lines used in this study included AGS (F-12K), MKN-45 (RPMI-1640), MGC-803 (RPMI-1640), BGC-823 (RPMI-1640), SNU-1 (RPMI-1640), Hs736t (DMEM), IM95M (DMEM), SGC-7901 (RPMI-1640), NCI-N87 (RPMI-1640), KATOIII (IMDM), normal gastric cell GES-1 (RPMI-1640), and 293T (DMEM) were from Cell Bank of China Science Academy (Shanghai, China). All cells were cultured in medium supplemented with 10% FBS and 1% Penicillin-Streptomycin. pLKO-Tet-on vector and NEK2 shRNA were from ADDGENE (Watertown, MA, USA). The NEK2 shRNA sequences were as follows: CCGGCGTTACTCTGATGAATTGAATCTCGA-GATTCAATTCATCAGAGTAACGTTTTT (sense), AA-TTAAAACGTTACTCTGATGAATTGAATCTCGAGA-TTCAATTCATCAGAGTAACG (anti-sense). pLVX-Tight-Puro vector and pLVX-Tet-off Advanced vector were from Clontech (Mountain View, CA, USA). Primer sequences were designed by Sigma-Aldrich (St. Louis, MO, USA) based on the published NEK2 gene sequence (Accession No. NM002497) and synthesized chemically. Doxycycline (Dox) was from Rainbio (Shanghai, China). CPI-455 and 10058-F4 were from Selleck Chemicals (Houston, TX, USA).

Transfection to establish stable MGC-803-NEK2-KD and BGC-823-NEK2-OE cell lines

NEK2 shRNA coding sequence was cloned into pLKO-Tet-On vector, and the recombinant sh-NEK2 plasmid was transfected into 293T cells along with Δ8.9 and VSV-G via GENE 9 to generate lentiviral supernatants containing NEK2 shRNA coding sequence. The lentivirus was used to transduce MGC-803 cells and puromycin (2 ng/ml) was used to select for a stable Tet-regulated NEK2 shRNA expression cells named MGC-803-NEK2-KD stable cell line. Dox (100 ng/ml) was administrated to knock down the NEK2 expression of MGC-803-NEK2-KD cells.

Gene of NEK2 oligonucleotides were cloned into pLVX-Tight-Puro vector, and the recombinant pLVX-Tight-Puro plasmid containing gene of NEK2 was transfected into 293T cells along with VSV-G to generate lentiviral supernatants. pLVX-Tet-off Advanced vector was transfected into 293T cells in the same way to generate lentivirus. On this basis, these two kinds of lentiviruses were simultaneously co-transduced into BGC-823 and puromycin (3 ng/ml) or G418 (500 ng/ml) was used to select for a stable Tet-regulated NEK2 over-expression cells named BGC-823-NEK2-OE stable cell line. The stable cells were continuously administrated with Dox (100 ng/ml) to inhibit the expression of NEK2. The efficacy of transfection was identified by western blot analysis.

Cell proliferation assay

Proliferation of cells was checked using CCK8 assay. Briefly, MGC-803 and MGC-803-NEK2-KD cells (1000 cells/well) or BGC-823 and BGC-823-NEK2-OE cells (500 cells/well) were plated in 96-well plates and CCK8 reagent was added to wells after 2, 4, 6, or 8 days of culture. After incubation for 1 h, the absorbance at 450 nm was measured.

Colony formation assay

Cells were plated into 12-well plates at a density of 500 cells/well and cultured for 8 days. Cells were fixed in methanol for 15 min and then stained with crystal violet (0.1%, w/v) for 15 min. The colonies were observed under inverted microscope.

Wound healing assay

Cells were plated into 12-well plates at a density of 1×10^5 cells/well and then allowed to grow until confluent. Cells was made a straight scratch to stimulate a wound and the culture medium was changed from essential RPMI-1640 medium to RPMI-1640 medium with 1% FBS after PBS washes. At time points of 0, 24 and 48 h, the wound was observed under inverted microscope.

Migration assay

Cell migration was examined with 24-well transwell chambers with 8 μm pores. The 5×10^4 cells/well were suspended in RPMI-1640 medium with 1% FBS and seeded into upper chambers, and RPMI-1640 medium containing 10%

FBS was added into the lower chamber. After cultured for 24 h, cells migrated to the reverse side of the transwell membrane were fixed in methanol for 15 min and stained with crystal violet for 15 min. After PBS washes, migrated cells were counted under inverted microscope for image acquisition as well.

Cell cycle analysis

Cells were fixed with 70% ethanol overnight and incubated with RNase A (10 mg/mL) and propidium iodide (50 mg/mL), step by step. The cellular DNA content was analyzed on a flow cytometer and Flowjo V10 software was used to measure the percentage of cells at each cell cycle phase.

Xenograft animal models

Female BALB/c nu/nu mice (5-6 weeks, 16-18 g) were obtained from Shanghai SLAC Laboratory Animal, Co., Ltd. (Shanghai, China). Cancer cells (6×10^6 cells/mouse for MGC-803-NEK2-KD and 5×10^6 cells/mouse for BGC-823-NEK2-OE) were inoculated subcutaneously in the right flank of each mouse. Tumors were allowed to reach 80-120 mm^3 in size before randomly divided into two groups based on tumor volumes. For MGC-803-NEK2-KD tumor model (n = 6), NEK2-KD (off) control group was orally administrated water daily while NEK2-KD (on) group was orally administrated 1 mg/ml Dox water daily. For BGC-823NEK2-OE tumor model (n = 8), NEK2-OE (off) control group was orally administrated 1 mg/ml Dox water daily while NEK2-OE (on) group was orally administrated water daily. Body weight and tumor size of mice were measured every other day from inoculation to completion of the study. Tumor volume was calculated using the formula length × width^2 × 0.5 (mm^3). At the end of experiments the mice were euthanized by CO_2 inhalation and the tumors were harvested and stored for further analysis.

Western blot analysis

Cells were homogenized in RIPA buffer containing phosphatase protease inhibitor cocktail (Thermo scientific) on the ice and tumor tissue were homogenized by a tissue lyser (QIAGEN) in RIPA buffer. The lysates were prepared and 20 μg of proteins was subjected to Western blot analysis in 8% or 12% SDS-PAGE gels electrophoresis under denaturing and reducing con-
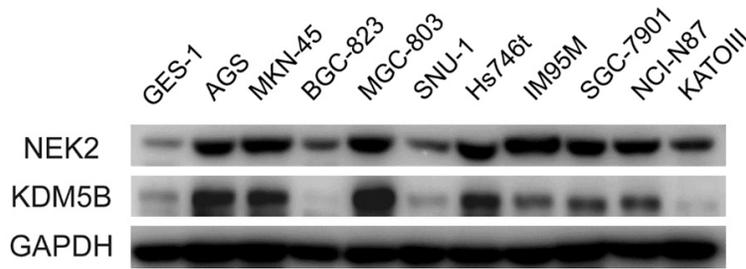
Figure 1. NEK2 and KDM5B overexpression occurred in multiple GC cells. The expression levels of NEK2 and KDM5B in normal gastric mucosa GES-1 cell line and 10 kinds of GC cell lines detected by western blot analysis.

ditions. Primary antibodies for NEK2, KDM5B, H3, H3K4me3, c-Myc, and β-catenin were purchased from Cell Signaling Technology and antibody against GAPDH-HRP was from Santa Cruz Biotechnology.

Co-immunoprecipitation (Co-IP) analysis

Co-IP analysis was performed as described previously [28] with some modifications. Briefly, total protein from BGC-823-NEK2-OE cells was extracted in RIPA buffer on the ice. Protein A/G agarose beads and the total protein were gently mixed at low speed at 4°C for 1 h to remove un-specific binding proteins and centrifuged at 1000 rpm for 3 min. The supernatant was then incubated with anti-NEK2 antibody at 4°C overnight (rabbit IgG used as negative control). Then protein A/G agarose beads were added and the antigen-antibody mixture was shaken slowly at 4°C for 1 h. After washing three times with PBST buffer, precipitates were analyzed by western blot using the indicated antibodies.

Statistical analysis

Statistical analyses were performed with GraphPad Prism software and reported data results were expressed as means ± SEM. Experiments were repeated three times independently. Student's t-test was used to compare paired samples while one-way ANOVA was used for comparisons among more than two groups. $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.001$ (***) was considered significant.

Results

High expression level of NEK2 and KDM5B was observed in GC

Western blot analysis results of NEK2 and KDM5B expression levels in 10 kinds of GC cell lines and one normal gastric cell GES-1 were shown in **Figure 1**. In general, GC cells showed higher expression of NEK2 and KDM5B compared with GES-1 cells. Among these 10 kinds of GC cells, 8 kinds of GC cells showed higher expression of NEK2 and 7 kinds of GC cells showed higher KDM5B expression. MGC-803 cells exhibit higher level of NEK2 and KDM5B expression while BGC-823 cells exhibit lower level of NEK2 and KDM5B expression. Therefore, MGC-803 cells were used to check influence of NEK2 knockdown on cell characteristics while BGC-823 cells were used to check influence of NEK2 overexpression on cell characteristics.

NEK2 knockdown leads to suppression of proliferation, migration and cell cycle arrest in MGC-803 cells in vitro

As shown in **Figure 2A**, MGC-803-NEK2-KD cells with tet-on was successfully established. After addition of Dox, NEK2 expression level was significantly decreased in MGC-803-NEK2-KD (on) cells. Compared with MGC-803-NEK2-KD (off) cells without Dox, proliferation inhibition (**Figure 2B**), colony formation inhibition (**Figure 2C, 2D**) and cell cycle arrest at G2/M phase (**Figure 2E, 2F**) were observed in MGC-803-NEK2-KD (on) cells with 100 ng/ml Dox. NEK2 knockdown also caused decrease in migration capacity of cells as shown in transwell assay (**Figure 2G, 2H**) as well as wound healing assay (**Figure 2I, 2J**).

NEK2 overexpression promotes cancer cell proliferation and migration in BGC-823 cells in vitro

As shown in **Figure 3A**, BGC-823-NEK2-OE cells with tet-off was successfully established. After removal of Dox pressing, NEK2 expression level was significantly increased in BGC-823-NEK2-OE (on) cells. Compared with BGC-823-NEK2-OE (off) cells under Dox (100 ng/ml) pressing, increase in proliferation (**Figure 3B**) and colony formation capability (**Figure 3C, 3D**) were observed in BGC-823-NEK2-OE (on) cells without Dox. There was no significant difference in cell cycle distribution between BGC-823-NEK2-OE (off) and BGC-823-NEK2-OE (on) cells (**Figure 3E, 3F**). NEK2 overexpression also
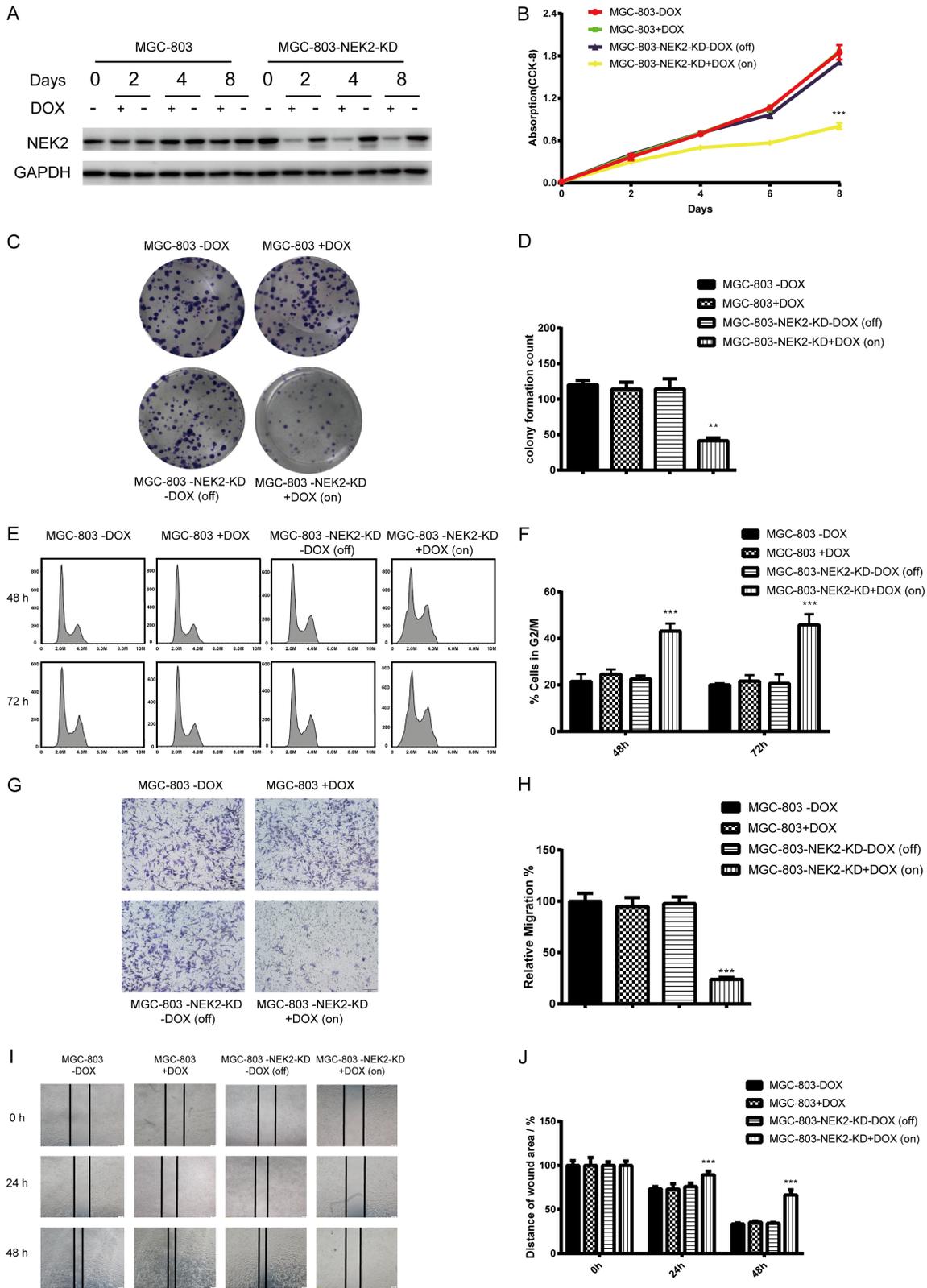
Figure 2. NEK2 knockdown led to suppression of cell proliferation, migration and cell cycle arrest in MGC-803 cells. (A) Silencing of NEK2 expression in MGC-803-NEK2-KD cells after addition of 100 ng/ml Dox confirmed by western blot assay. (B) Cell proliferation of MGC-803 cells and MGC-803-NEK2-KD cells with or without 100 ng/ml Dox treatment. (C) Colony formation ability of MGC-803 cells and MGC-803-NEK2-KD cells with or without 100 ng/ml Dox treatment for 8 days. (D) Quantitative analysis results of (C). (E) DNA histograms of cells treated with or without

100 ng/ml Dox were obtained by flow cytometry analysis. Accumulation in G2/M phase was observed in MGC-803-NEK2-KD with 100 ng/ml Dox treatment. (F) Quantitative analysis results of (E). (G) Transwell migration assay results of cells treated with or without 100 ng/ml Dox for 24 h. (H) Quantitative analysis results of (G). (I) Wound healing assay results of cells treated with or without 100 ng/ml Dox. (J) Quantitative analysis results of (I). Data are expressed as means ± SEM of three independent experiments. Statistical significance was analyzed using one-way ANOVA, **P < 0.01, ***P < 0.001 versus MGC-803-NEK2-KD (without Dox, off) cells.
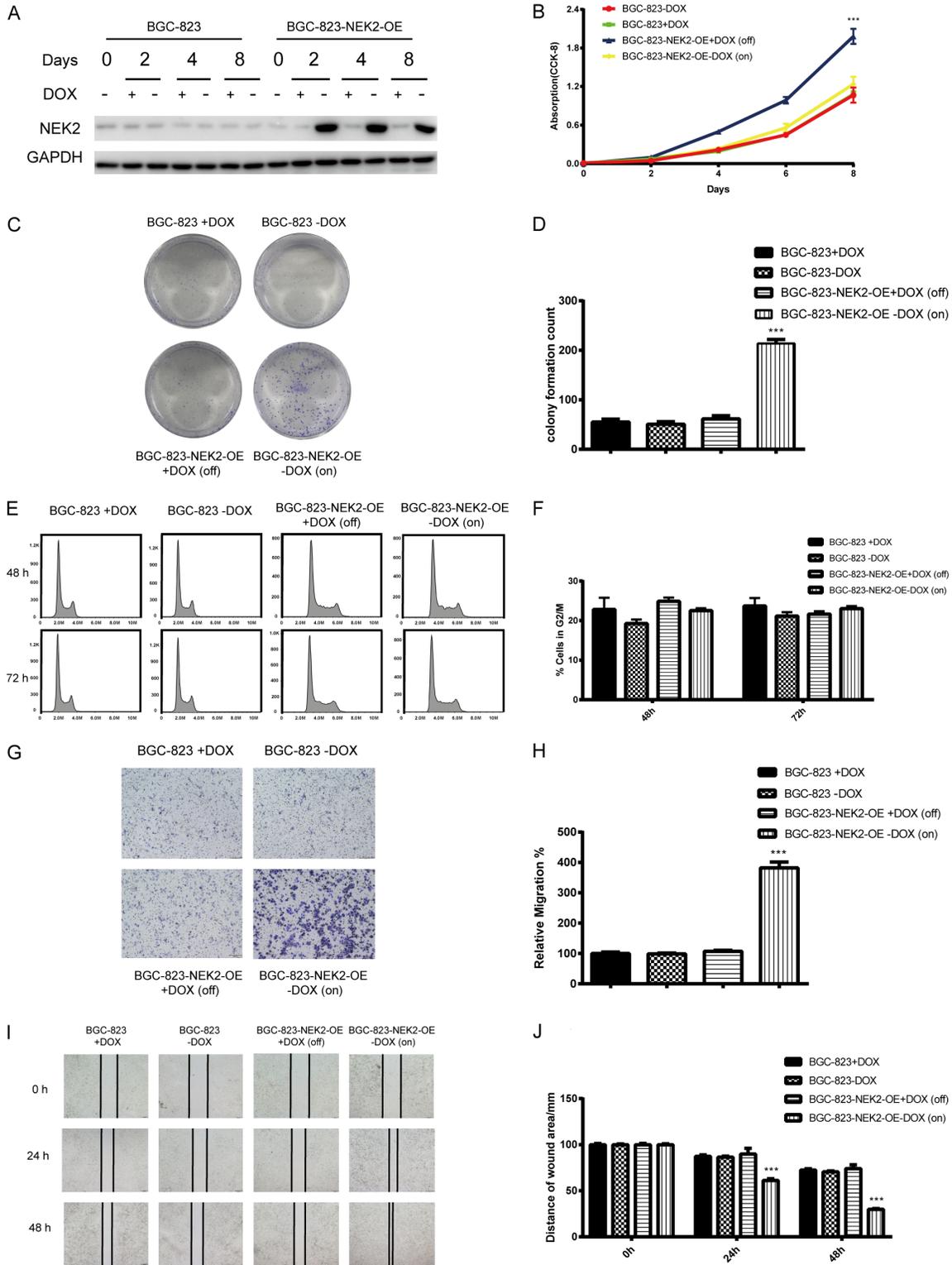
Figure 3. NEK2 overexpression led to enhanced cell proliferation, migration and cell cycle arrest in BGC-823 cells. (A) Overexpression of NEK2 in BGC-823-NEK2-OE cells after removing 100 ng/ml Dox confirmed by western blot assay. (B) Cell proliferation of BGC-823 cells and BGC-823-NEK2-OE cells with or without 100 ng/ml Dox treatment. (C) Colony formation ability of BGC-823 cells and BGC-823-NEK2-OE cells with or without 100 ng/ml Dox treatment for 8 days. (D) Quantitative analysis results of (C). (E) DNA histograms of cells treated with or without 100 ng/ml Dox were obtained by flow cytometry analysis. No significant change was observed between BGC-823NEK2-OE cells with or without 100 ng/ml Dox treatment. (F) Quantitative analysis results of (E). (G) Transwell migration assay results of cells treated with or without 100 ng/ml Dox for 24 h. (H) Quantitative analysis results of (G). (I) Wound healing assay results of cells treated with or without 100 ng/ml Dox. (J) Quantitative analysis results of (I). Data are expressed as means ± SEM of three independent experiments. Statistical significance was analyzed using one-way ANOVA, **P < 0.01, ***P < 0.001 versus BGC-823-NEK2-OE (with Dox, off) cells.
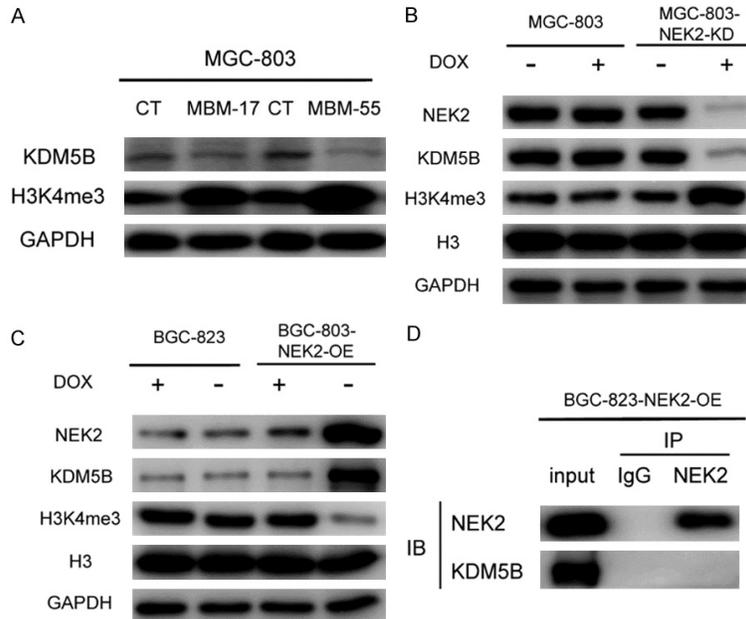
Figure 4. Possible relationship between NEK2 and KDM5B/H3K4me3 in GC. (A) Treatment of NEK2 inhibitors on the levels of KDM5B and H3K4me3. (B, C) The results of the regulatory effects of NEK2 on KDM5B in MGC-803-NEK2-KD cells (B) and BGC-823-NEK2-OE cells (C) treated with or without 100 ng/ml Dox for 48 h. (D) Results of co-IP experiment showed no direct interaction between NEK2 and KDM5B. Immunoprecipitation in BGC-823-NEK2-OE cells was performed with anti-NEK2 antibody, and the precipitates were subjected to western blot probed with anti-KDM5B and anti-NEK2 antibody. Mouse IgG was used as an antibody control for immunoprecipitation.

cells (**Figure 4A**). These results suggested possible relationship between NEK2 and KDM5B. So, we tried to confirm the regulative effects of NEK2 on KDM5B using MGC-803-NEK2-KD cells and BGC-823-NEK2-OE cells. As shown in **Figure 4B**, when NEK2 was knocked down in MGC-803-NEK2-KD cells, decrease in expression level of KDM5B and increase in expression level of H3K4me3 were observed. Accordingly, when NEK2 was overexpressed in BGC-823-NEK2-OE cells, expression level of KDM5B increased and H3K4me3 level decreased (**Figure 4C**). Then we used co-IP assay to check whether there was a direct interaction between NEK2 and KDM5B. As shown in **Figure 4D**, results of Co-IP assay revealed that there was no direct interaction between NEK2 and KDM5B. The regulative effects of NEK2 on KDM5B expression might be indirectly.

caused increase in migration capacity of cells as shown in transwell assay (**Figure 3G, 3H**) as well as in wound healing assay (**Figure 3I, 3J**).

Possible relationship between NEK2 and KDM5B/H3K4me3 in GC

MBM-55 and MBM-17 were novel selective NEK2 inhibitors which showed selective NEK2 inhibitory activity and *in vitro* and *in vivo* anticancer activities in our previous study [12]. Interestingly, MBM-55 and MBM-17 also induced down-regulation of KDM5B expression and up-regulation of H3K4me3 level in MGC-803

NEK2 might regulate KDM5B/H3K4me3 expression through β-catenin/Myc

The possible involvement of β-catenin or c-Myc in the regulation effects of NEK2 on KDM5B was checked. As shown in **Figure 5A**, when NEK2 was knockdown in MGC-803-NEK2-KD cells, decrease in expression levels of β-catenin and c-Myc was also observed besides of the decrease in KDM5B and increase in H3K4me3. Accordingly, when NEK2 was overexpressed in BGC-823-NEK2-OE cells, expression levels of β-catenin and c-Myc increased besides of the increase in KDM5B and decrease in H3K4me3
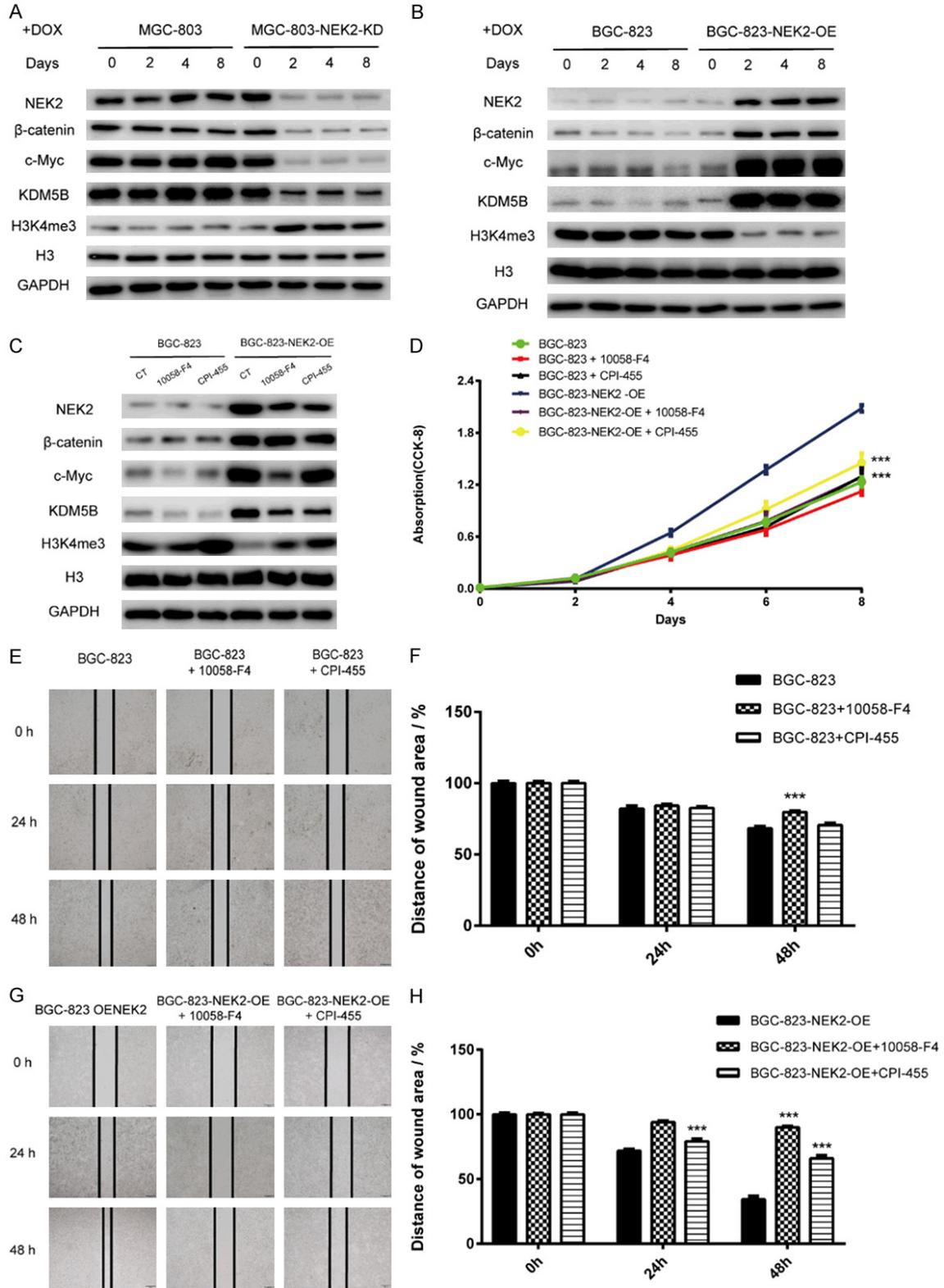
Figure 5. NEK2 might regulate KDM5B expression through β-catenin/Myc pathway. (A) Influence of NEK2 knock-down on β-catenin, c-Myc, KDM5B and H3K4me3 levels in MGC-803-NEK2-KD cells. (B) Influence of NEK2 over-expression on β-catenin, c-Myc, KDM5B and H3K4me3 levels in BGC-823-NEK2-OE cells. (C) Influence of c-Myc inhibitor 10058-F4 (10 μM) or KDM5B inhibitor CPI-455 (10 μM) on NEK2, β-catenin, c-Myc, KDM5B and H3K4me3 levels in both BGC-823 and BGC-823-NEK2-OE cells. (D) Influence of c-Myc inhibitor 10058-F4 (10 μM) or KDM5B

inhibitor CPI-455 (10 µM) on cell proliferation of BGC-823 and BGC-823-NEK2-OE cells. (E) Influence of c-Myc inhibitor 10058-F4 (10 µM) or KDM5B inhibitor CPI-455(10 µM) on cell migation capacity of BGC-823 cells in wound healing assay. (F) Quantitative analysis results of (E). (G) Influence of c-Myc inhibitor 10058-F4 (10 µM) or KDM5B inhibitor CPI-455 (10 µM) on cell migation capacity of BGC-823-NEK2-OE cells in wound healing assay. (H) Quantitative analysis results of (G). Data are expressed as means ± SEM of three independent experiments. Statistical significance was analyzed using one-way ANOVA, ***P < 0.001 versus BGC-823 cells or BGC-823-NEK2-OE cells.

(**Figure 5B**). These results suggested that β-catenin and c-Myc might be involved in the regulative effects of NEK2 on KDM5B/H3K4me3 expression. Then, the selected c-Myc inhibitor 10058-F4 [29-31] and selected KDM5B inhibitor CPI-455 [32-36], at a non-cytotoxic dose of 10 mM, were used to treat BGC-823 and BGC-823-NEK2-OE cells, respectively. As shown in **Figure 5C**, c-Myc inhibitor 10058-F4 induced decrease in c-Myc and KDM5B and increase in H3K4me3 levels but did not cause significant change in β-catenin level in BGC-823-NEK2-OE cells. As to KDM5B inhibitor CPI-455, it could increase H3K4me3 level but did not cause significant change in c-Myc or β-catenin levels in both BGC-823 cells and BGC-823-NEK2-OE cells. These results suggested there might be a NEK2/β-catenin/Myc/KDM5B pathway. Interestingly, both KDM5B inhibitor and c-Myc inhibitor caused decrease in NEK2 level in BGC-823-NEK2-OE cells (**Figure 5C**). The results indicated that a feedback system might also work in the control of NEK2 expression.

As shown in **Figure 5D**, both KDM5B inhibitor and c-Myc inhibitor dramatically inhibited proliferation capability of BGC-823-NEK2-OE cells, while exhibited no inhibitory effects on BGC-823 cells. The migration capability of BGC-823-NEK2-OE cells was significantly inhibited by KDM5B inhibitor and c-Myc inhibitor (**Figure 5E, 5F**). The migration capability of BGC-823 cells was slightly inhibited by c-Myc inhibitor (**Figure 5G, 5H**).

Role of NEK2/β-catenin/Myc/KDM5B/ H3K4me3 pathway in in vivo tumor growth

To study the effect of NEK2 on tumor growth of GC cells *in vivo*, the tumor growth of GC cells with knockdown or overexpression of NEK2 were observed. The nude mice inoculated with MGC-803-NEK2-KD cells were divided into two groups. The mice of MGC-803-NEK2-KD (off) group were treated with water and the mice of MGC-803-NEK2-KD (on) group were treated with 1.0 mg/kg Dox to induce knockdown of NEK2 expression. Tumor growth was inhibited in MGC-803-NEK2-KD (on) group compared

with that of MGC-803-NEK2-KD (off) group (**Figure 6A**) while there was no difference in the body weight of the two groups of mice (**Figure 6B**). The photo of tumor tissues (**Figure 6C**) and results of tumor weight (**Figure 6D**) also confirmed that knockdown of NEK2 suppressed tumor growth.

The nude mice inoculated with BGC-823-NEK2-OE cells were divided into two groups. The mice of BGC-823-NEK2-OE (off) group were treated with 1.0 mg/kg Dox and the mice of BGC-823-NEK2-OE (on) group were treated with water to induce overexpression of NEK2 expression. Tumor growth was increased in BGC-823-NEK2-OE (on) group compared with that of BGC-823-NEK2-OE (off) group (**Figure 6E**) while there was no difference in the body weight of the two groups of mice (**Figure 6F**). The photo of tumor tissues (**Figure 6G**) and results of tumor weight (**Figure 6H**) also confirmed that overexpression of NEK2 increased tumor growth.

To validate the role of NEK2/β-catenin/Myc/KDM5B/H3K4me3 pathway *in vivo*, the expression levels of the signal proteins in tumor tissues were checked. Results of western blot analysis of MGC-803-NEK2-KD (on) tumor tissues showed that NEK2 down-regulation led to suppression of β-catenin, inhibition of KDM5B and enhanced H3K4me3 (**Figure 6I**). Accordingly, NEK2 overexpression in BGC-823-NEK2-OE (on) tumor tissues led to activation of β-catenin, enhanced KDM5B expression and inhibition of H3K4me3 (**Figure 6J**). These results suggested that NEK2 might mediate *in vivo* tumor growth by regulating β-catenin/Myc/KDM5B/H3K4me3 pathway.

Discussion

NEK2 has been reported to be involved in caner development and NEK2 inhibitors were designed and developed as candidates for anticancer therapy [12, 13, 37-39]. In the present study, we focused on studying the roles of NEK2 in the malignant behaviors of GC and the possible mechanisms of regulative effects of NEK2 on KDM5B expression in GC cell lines
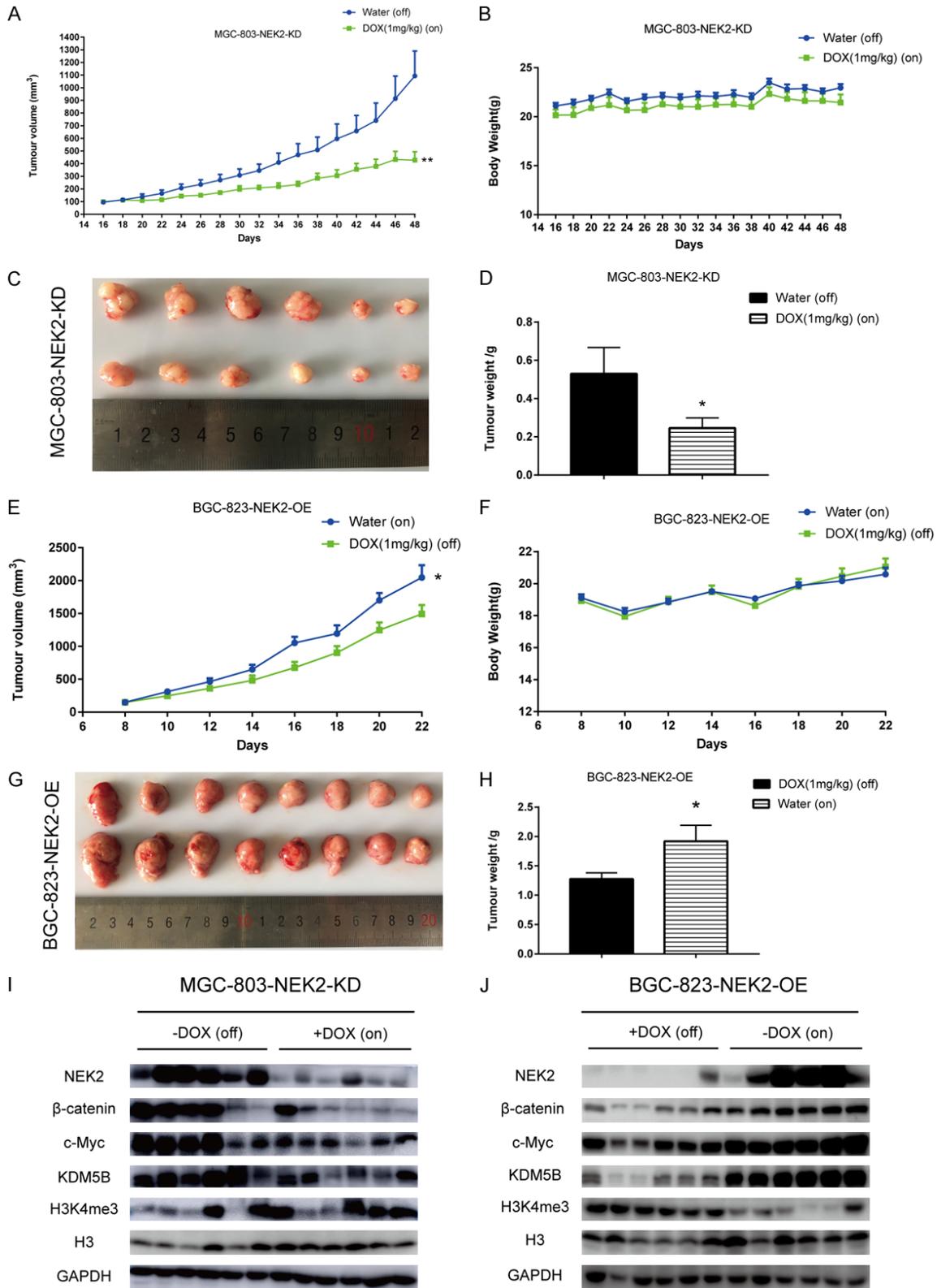
Figure 6. Involvement of NEK2/β-catenin/Myc/KDM5B/H3K4me3 pathway in *in vivo* tumor growth. A. Tumor growth of MGC-803-NEK2-KD cells in nude mice treated with 1.0 mg/kg Dox to induce NEK2 knockdown. B. Body weight of nude mice inoculated with MGC-803-NEK2-KD cells with or without 1.0 mg/kg Dox. C. Photo of tumor tissues isolated from the nude mice inoculated with MGC-803-NEK2-KD cells with or without 1.0 mg/kg Dox. D. Weight of tumor tissues isolated from nude mice inoculated with MGC-803-NEK2-KD cells with or without 1.0 mg/kg Dox. E. Tumor growth of BGC-823-NEK2-OE cells in nude mice treated only with water to induce NEK2 overexpression. F.

Body weight of nude mice inoculated with BGC-823-NEK2-OE cells with or without 1.0 mg/kg. G. Photo of tumor tissues isolated from nude mice inoculated with BGC-823-NEK2-OE cells with or without 1.0 mg/kg Dox. H. Weight of tumor tissues isolated from nude mice inoculated with BGC-823-NEK2-OE cells with or without 1.0 mg/kg Dox. I. Levels of NEK2, β-catenin, c-Myc, KDM5B and H3K4me3 in tumor tissues isolated from nude mice inoculated with MGC-803-NEK2-KD cells with or without 1.0 mg/kg Dox. J. Levels of NEK2, β-catenin, c-Myc, KDM5B and H3K4me3 in tumor tissues isolated from nude mice inoculated with BGC-823-NEK2-OE cells with or without 1.0 mg/kg Dox. Data are expressed as means ± SEM. Statistical significance was analyzed using Student's t-test, *P < 0.05, **P < 0.01 versus MGC-803-NEK2-KD water (off) group or BGC-823-NEK2-OE Dox (off) group.

both *in vitro* and *in vivo*. Our results showed that, knockdown of NEK2 in MGC-803 cells inhibited cell proliferation and migration while overexpression of NEK2 in BGC-823 cells resulted in enhanced cell proliferation and migration *in vitro*. At the same time, in *in vivo* study, knockdown of NEK2 in MGC-803 cells inhibited tumor growth while overexpression of NEK2 in BGC-823 cells resulted in enhanced tumor growth. Results of the present study suggested that NEK2 did play an important role in malignant GC, both *in vitro* and *in vivo*.

Furthermore, a NEK2/β-catenin/Myc/KDM5B pathway which might be a new mechanism of NEK2 in GC was found in our study. High levels of NEK2 and KDM5B were both correlated with GC in clinic [9, 10, 21]. In our study, we found that NEK2 high expressed cancer cells were accompanied by high expression level of KDM5B. NEK2 inhibitors could induce decrease in level of KDM5B and increase in level of H3K4me3. Knockdown of NEK2 in MGC-803 cells also led to KDM5B inhibition while overexpression of NEK2 in BGC-823 cell lines resulted in enhanced KDM5B expression. These results suggested a possible relationship between NEK2 and KDM5B which has never been reported before. Results of co-IP assay excluded direct interaction between NEK2 and KDM5B, which suggested that other signal proteins might be involved in the regulative effects of NEK2 on the expression of KDM5B.

It was reported that NEK2 could bind and participate phosphorylation of β-catenin, stabilize and recruit β-catenin to regulate centrosome disjunction/splitting [40-42]. Overexpression of NEK2 in multiple myeloma ARP1 cells and lung cancer H1299 cells resulted in nuclear accumulation of β-catenin, while NEK2 knockdown decreased the level of nuclear β-catenin [43]. Moreover, overexpression of NEK2 in resected colorectal cancer tissues was associated with lower tumor membranous β-catenin expression and higher cytoplasmic and nuclear β-catenin accumulation [40]. β-catenin could translocate into the nucleus and form a complex with T-cell factor (TCF)/lymphocyte enhancer factor (LEF) to activate transcription of target genes such as c-Myc [44].

NEK2 depletion in hepatocellular carcinoma HepG2 cells reduced β-catenin expression and diminished expression of c-Myc as well [45, 46]. At the same time, c-Myc and KDM5B could interact each other and form complex in regulating the level of H3K4me3 at the endogenous promoter and expression of CDKN1A [47]. Therefore, we predicted that there might be a NEK2/β-catenin/Myc/KDM5B/H3K4me3 pathway in GC cells.

Results of our further studies showed that, NEK2 knockdown led to down-regulation of β-catenin and c-Myc, accompanied with decrease in KDM5B and increase in H3K4me3 level. On the other hand, when NEK2 was overexpressed, β-catenin and c-Myc were enhanced, accompanied with KDM5B up-regulation and H3K4me3 depletion. These findings confirmed that the involvement of the β-catenin/Myc pathway in the regulative effects of NEK2 on KDM5B/H3K4me3 expression. To further confirm the results, the selected c-Myc inhibitor (10058-F4) and KDM5B inhibitor (CPI-455) were used to verify the upstream and downstream relationship of NEK2/KDM5B and β-catenin/Myc pathways. 10058-F4 could disrupt the association between c-Myc and Max [29] and dramatically inhibited the transcriptional activity of c-Myc, and thus resulted in down-regulated expression of downstream targets of c-Myc [30, 31]. CPI-455, a specific KDM5 inhibitor, could inhibit the demethylase activity of KDM5A and increased global levels of H3K4 trimethylation (H3K4me3) in multiple cancer cell lines [32, 36]. Our results showed that, c-Myc inhibitor (10058-F4) induced decrease in c-Myc and KDM5B and increase in H3K4me3 levels but did not cause significant change in β-catenin level in BGC-823-NEK2-OE cells. As to KDM5B inhibitor CPI-455, it only decreased KDM5B level and increased H3K4me3 level, but did not
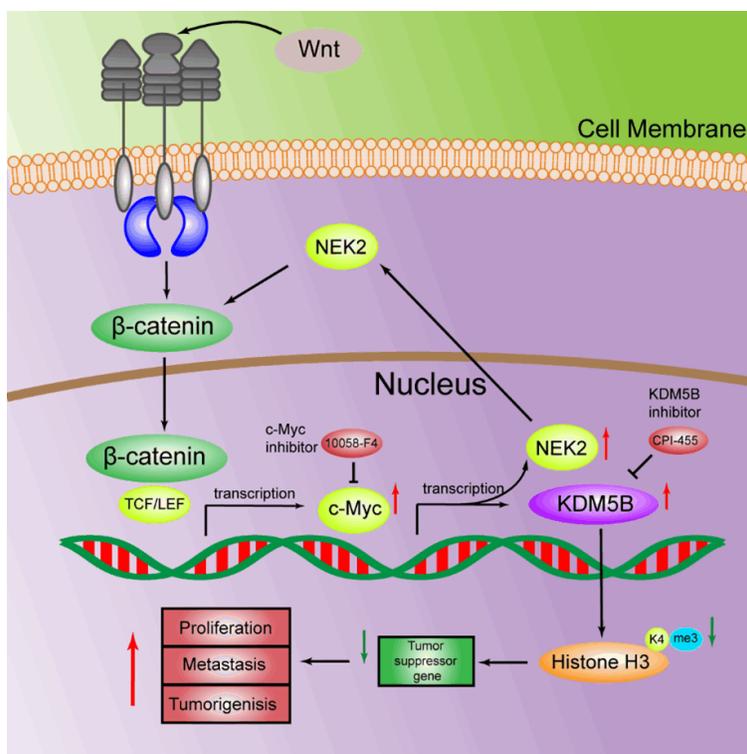
Figure 7. Illustration of the NEK2/β-catenin/Myc/KDM5B/H3K4me3 pathway. NEK2 binds to and stabilized β-catenin [40-42], promotes β-catenin translocation into nucleus [40, 43]. β-catenin forms a complex with TCF/LEF to activate the transcription of c-Myc gene [44]. As a transcriptional factor, c-Myc may transcriptionally regulate the expression of NEK2 and KDM5B. KDM5B demethylates H3K4me3 to a transcriptionally inactive state and decreases the transcription of tumor suppressor genes, then promotes cancer cell proliferation, metastasis and tumorigenesis.

cause significant change in c-Myc or β-catenin levels in BGC-823-NEK2-OE cells. Both KDM5B inhibitor and c-Myc inhibitor dramatically inhibited proliferation and migration capability of BGC-823-NEK2-OE cells. These results confirmed the finding of NEK2/β-catenin/Myc/KDM5B/H3K4me3 pathway. While, c-Myc and KDM5B inhibition were also accompanied with down-regulation of NEK2. The results suggested possible feedback system in control of NEK2 expression which was in accordance with the previous report that NEK2 was transcriptionally regulated by c-Myc in myeloma cells. NEK2 expression was significantly decreased at both transcription and protein levels following inhibition of c-Myc [48]. The pathway of NEK2/β-catenin/Myc/KDM5B/H3K4me3 was also confirmed in *in vivo* study. In mice inoculated with GC cells, NEK2 down-regulation led to suppression of tumor growth, inhibition of β-catenin, KDM5B and enhanced H3K4me3. And, NEK2 overexpression led to increased tumor growth, activation of β-catenin, KDM5B and inhibition of H3K4me3.

In summary, our results indicate that NEK2 plays an important role in the proliferation, migration and tumor growth in GC. Also, based on our findings, we predicted the signal cascades related to the pathway of NEK2/β-catenin/Myc/KDM5B/H3K4me3 (**Figure 7**). NEK2 binds to and stabilized β-catenin [40-42], promotes β-catenin translocation into nucleus [40, 43]. β-catenin forms a complex with TCF/LEF to activate the transcription of c-Myc gene [44]. As a transcriptional factor, c-Myc may transcriptionally regulate NEK2 and KDM5B expression. KDM5B demethylates H3K4me3 to a transcriptionally inactive state and decreases the transcription of tumor suppressor genes, then promotes cancer cell proliferation, metastasis and tumorigenesis.

Our study demonstrates that NEK2 was highly expressed in GC cell lines and related to enhance cell proliferation, migration and tumor growth. NEK2 could regulate KDM5B/H3K4me3 expression through β-catenin/Myc both in vitro and in vivo. There might be a NEK2/β-catenin/Myc/KDM5B/H3K4me3 signaling pathway which could contribute to the important carcinogenic role of NEK2-mediated malignant behaviors in GC. This finding reveals a new mechanism of NEK2 causing the malignant transformation of GC and can help drive the design of more effective therapies in NEK2-targeted treatment of GC.

Acknowledgements

Disclosure of conflict of interest

None.

Abbreviations

BGC-823-NEK2-OE, BGC-823 cells with NEK2 overexpression; Co-IP, co-immunoprecipitation; Dox, doxycycline; GC, gastric cancer; H3K4me3, tri-methylated histone H3 lysine 4; MGC-803-NEK2-KD, MGC-803 cells with NEK2 knockdown; NEK2, Never in Mitosis (NIMA) Related Kinase 2; shRNA, short hairpin RNA.

Address correspondence to: Xiongwen Zhang, Shanghai Engineering Research Center of Molecular Therapeutics and New Drug Development, School of Chemistry and Molecular Engineering, East China Normal University, Shanghai 200062, China. E-mail: xwzhang@sat.ecnu.edu.cn; Xuan Liu, Institute of Interdisciplinary Integrative Biomedical Research, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China. E-mail: xuanliu@shutcm.edu.cn; Yanfen Fang, Division of Anti-Tumor Pharmacology, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China. Email: yffang@simm.ac.cn.

References

[1] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017; 67: 7-30.

[2] Huang KK, Ramnarayanan K, Zhu F, Srivastava S, Xu C, Tan ALK, Lee M, Tay S, Das K, Xing M, Fatehullah A, Alkaff SMF, Lim TKH, Lee J, Ho KY, Rozen SG, Teh BT, Barker N, Chia CK, Khor C, Ooi CJ, Fock KM, So J, Lim WC, Ling KL, Ang TL, Wong A, Rao J, Rajnakova A, Lim LG, Yap WM, Teh M, Yeoh KG and Tan P. Genomic and epigenomic profiling of high-risk intestinal metaplasia reveals molecular determinants of progression to gastric cancer. Cancer Cell 2018; 33: 137-150, e135.

[3] Rugge M, Genta RM, Di Mario F, El-Omar EM, El-Serag HB, Fassan M, Hunt RH, Kuipers EJ, Malfertheiner P, Sugano K and Graham DY. Gastric cancer as preventable disease. Clin Gastroenterol Hepatol 2017; 15: 1833-1843.

[4] Fry AM, Meraldi P and Nigg EA. A centrosomal function for the human Nek2 protein kinase, a member of the NIMA family of cell cycle regulators. EMBO J 1998; 17: 470-481.

[5] Ha Kim Y, Yeol Choi J, Jeong Y, Wolgemuth DJ and Rhee K. Nek2 localizes to multiple sites in mitotic cells, suggesting its involvement in multiple cellular functions during the cell cycle. Biochem Biophys Res Commun 2002; 290: 730-736.

[6] Fry AM. The Nek2 protein kinase: a novel regulator of centrosome structure. Oncogene 2002; 21: 6184-6194.

[7] Hayward DG and Fry AM. Nek2 kinase in chromosome instability and cancer. Cancer Lett 2006; 237: 155-166.

[8] Bowers AJ and Boylan JF. Nek8, a NIMA family kinase member, is overexpressed in primary human breast tumors. Gene 2004; 328: 135-142.

[9] Tong H, Wang J, Chen H, Wang Z, Fan H and Ni Z. Transcriptomic analysis of gene expression profiles of stomach carcinoma reveal abnormal expression of mitotic components. Life Sci 2017; 170: 41-49.

[10] Ouyang Y, Li Y, Huang Y, Li X, Zhu Y, Long Y, Wang Y, Guo X and Gong K. CircRNA circPDSS1 promotes the gastric cancer progression by sponging miR-186-5p and modulating NEK2. J Cell Physiol 2019; 234: 10458-10469.

[11] Fang Y and Zhang X. Targeting NEK2 as a promising therapeutic approach for cancer treatment. Cell Cycle 2016; 15: 895-907.

[12] Xi JB, Fang YF, Frett B, Zhu ML, Zhu T, Kong YN, Guan FJ, Zhao Y, Zhang XW, Li HY, Ma ML and Hu W. Structure-based design and synthesis of imidazo[1,2-a]pyridine derivatives as novel and potent Nek2 inhibitors with in vitro and in vivo antitumor activities. Eur J Med Chem 2017; 126: 1083-1106.

[13] Fang Y, Kong Y, Xi J, Zhu M, Zhu T, Jiang T, Hu W, Ma M and Zhang X. Preclinical activity of MBM-5 in gastrointestinal cancer by inhibiting NEK2 kinase activity. Oncotarget 2016; 7: 79327-79341.

[14] Xiang Y, Zhu Z, Han G, Ye X, Xu B, Peng Z, Ma Y, Yu Y, Lin H, Chen AP and Chen CD. JARID1B is a histone H3 lysine 4 demethylase up-regulated in prostate cancer. Proc Natl Acad Sci U S A 2007; 104: 19226-19231.

[15] Kidder BL, Hu G and Zhao K. KDM5B focuses H3K4 methylation near promoters and enhancers during embryonic stem cell self-renewal and differentiation. Genome Biol 2014; 15: R32.

[16] Benevolenskaya EV. Histone H3K4 demethylases are essential in development and differentiation. Biochem Cell Biol 2007; 85: 435-443.

[17] Wang W, Zheng K, Pei Y and Zhang X. Histone demethylase JARID1B is overexpressed in osteosarcoma and upregulates cyclin D1 expression via demethylation of H3K27me3. Oncol Res 2018; 26: 373-384.

[18] Dhar SS, Zhao DY, Lin T, Gu BN, Pal K, Wu SJ, Alam H, Lv J, Yun K, Gopalakrishnan V, Flores ER, Northcott PA, Rajaram V, Li W, Shilatifard A, Sillitoe RV, Chen KF and Lee MG. MLL4 is required to maintain broad H3K4me3 peaks and super-enhancers at tumor suppressor genes. Mol Cell 2018; 70: 825-841.

[19] Liu SN, Liu F, Huang WN, Gu LN, Meng LJ, Ju YC, Wu YY, Li J, Liu LH and Sang MX. MAGE-A11 is activated through TFCP2/ZEB1 binding sites de-methylation as well as histone modification and facilitates ESCC tumor growth. Oncotarget 2018; 9: 3365-3378.

[20] Liu H, Li YM, Li JW, Liu YL and Cui BB. H3K4me3 and Wdr82 are associated with tumor progression and a favorable prognosis in human colorectal cancer. Oncol Lett 2018; 16: 2125-2134.

[21] Wang Z, Tang F, Qi G, Yuan S, Zhang G, Tang B and He S. KDM5B is overexpressed in gastric cancer and is required for gastric cancer cell proliferation and metastasis. Am J Cancer Res 2015; 5: 87-100.

[22] Cappello P, Blaser H, Gorrini C, Lin DC, Elia AJ, Wakeham A, Haider S, Boutros PC, Mason JM, Miller NA, Youngson B, Done SJ and Mak TW. Role of Nek2 on centrosome duplication and aneuploidy in breast cancer cells. Oncogene 2014; 33: 2375-2384.

[23] Koch M and Wiese M. Gene expression signatures of angiocidin and darapladib treatment connect to therapy options in cervical cancer. J Cancer Res Clin Oncol 2013; 139: 259-267.

[24] Hayward DG, Clarke RB, Faragher AJ, Pillai MR, Hagan IM and Fry AM. The centrosomal kinase Nek2 displays elevated levels of protein expression in human breast cancer. Cancer Res 2004; 64: 7370-7376.

[25] Zhong X, Guan X, Dong Q, Yang S, Liu W and Zhang L. Examining Nek2 as a better proliferation marker in non-small cell lung cancer prognosis. Tumour Biol 2014; 35: 7155-7162.

[26] Han M, Xu W, Cheng P, Jin H and Wang X. Histone demethylase lysine demethylase 5B in development and cancer. Oncotarget 2017; 8: 8980-8991.

[27] Bao J, Zou JH, Li CY and Zheng GQ. miR-194 inhibits gastric cancer cell proliferation and tumorigenesis by targeting KDM5B. Eur Rev Med Pharmacol Sci 2016; 20: 4487-4493.

[28] Wen S, Liu Y, Yang M, Yang K, Huang J and Feng D. Increased NEK2 in hepatocellular carcinoma promotes cancer progression and drug resistance by promoting PP1/Akt and Wnt activation. Oncol Rep 2016; 36: 2193-2199.

[29] Yin X, Giap C, Lazo JS and Prochownik EV. Low molecular weight inhibitors of Myc-Max interaction and function. Oncogene 2003; 22: 6151-6159.

[30] Zhong Q, Liu ZH, Lin ZR, Hu ZD, Yuan L, Liu YM, Zhou AJ, Xu LH, Hu LJ, Wang ZF, Guan XY, Hao JJ, Lui VWY, Guo L, Mai HQ, Chen MY, Han F, Xia YF, Grandis JR, Zhang X and Zeng MS. The RARS-MAD1L1 fusion gene induces cancer stem cell-like properties and therapeutic resistance in nasopharyngeal carcinoma. Clin Cancer Res 2018; 24: 659-673.

[31] Qu Y, Yang Q, Liu J, Shi B, Ji M, Li G and Hou P. c-Myc is required for BRAF(V600E)-induced epigenetic silencing by H3K27me3 in tumorigenesis. Theranostics 2017; 7: 2092-2107.

[32] Vinogradova M, Gehling VS, Gustafson A, Arora S, Tindell CA, Wilson C, Williamson KE, Guler GD, Gangurde P, Manieri W, Busby J, Flynn EM, Lan F, Kim HJ, Odate S, Cochran AG, Liu Y, Wongchenko M, Yang Y, Cheung TK, Maile TM, Lau T, Costa M, Hegde GV, Jackson E, Pitti R, Arnott D, Bailey C, Bellon S, Cummings RT, Albrecht BK, Harmange JC, Kiefer JR, Trojer P and Classon M. An inhibitor of KDM5 demethylases reduces survival of drug-tolerant cancer cells. Nat Chem Biol 2016; 12: 531-538.

[33] Arifuzzaman S, Rahman MS and Pang MG. Research update and opportunity of non-hormonal male contraception: histone demethylase KDM5B-based targeting. Pharmacol Res 2019; 141: 1-20.

[34] Yang GJ, Ko CN, Zhong HJ, Leung CH and Ma DL. Structure-based discovery of a selective KDM5A inhibitor that exhibits anti-cancer activity via inducing cell cycle arrest and senescence in breast cancer cell lines. Cancers (Basel) 2019; 11.

[35] Banelli B, Daga A, Forlani A, Allemanni G, Marubbi D, Pistillo MP, Profumo A and Romani M. Small molecules targeting histone demethylase genes (KDMs) inhibit growth of temozolomide-resistant glioblastoma cells. Oncotarget 2017; 8: 34896-34910.

[36] Facompre ND, Harmeyer KM, Sahu V, Gimotty PA, Rustgi AK, Nakagawa H and Basu D. Targeting JARID1Bs demethylase activity blocks a subset of its functions in oral cancer. Oncotarget 2018; 9: 8985-8998.

[37] Henise JC and Taunton J. Irreversible Nek2 kinase inhibitors with cellular activity. J Med Chem 2011; 54: 4133-4146.

[38] Lebraud H, Coxon CR, Archard VS, Bawn CM, Carbain B, Matheson CJ, Turner DM, Cano C, Griffin RJ, Hardcastle IR, Baisch U, Harrington RW and Golding BT. Model system for irreversible inhibition of Nek2: thiol addition to ethynylpurines and related substituted heterocycles. Org Biomol Chem 2014; 12: 141-148.

[39] Carbain B, Bayliss R, Boxall K, Coxon C, Lebraud H, Matheson C, Turner D, Zhen WL and Griffin RJ. 118 2-arylamino-6-ethynylpurines as potent irreversible inhibitors of the mitotic kinase Nek2. Eur J Cancer 2012; 48: 37-37.

[40] Neal CP, Fry AM, Moreman C, McGregor A, Garcea G, Berry DP and Manson MM. Overexpression of the Nek2 kinase in colorectal cancer correlates with beta-catenin relocalization and shortened cancer-specific survival. J Surg Oncol 2014; 110: 828-838.

[41] Mbom BC, Siemers KA, Ostrowski MA, Nelson WJ and Barth AI. Nek2 phosphorylates and stabilizes beta-catenin at mitotic centrosomes downstream of Plk1. Mol Biol Cell 2014; 25: 977-991.

[42] Mbom BC, Nelson WJ and Barth A. beta-catenin at the centrosome: discrete pools of beta-catenin communicate during mitosis and may co-ordinate centrosome functions and cell cycle progression. Bioessays 2013; 35: 804-809.

[43] Zhou W, Yang Y, Xia J, Wang H, Salama ME, Xiong W, Xu H, Shetty S, Chen T, Zeng Z, Shi L, Zangari M, Miles R, Bearss D, Tricot G and Zhan F. NEK2 induces drug resistance mainly through activation of efflux drug pumps and is associated with poor prognosis in myeloma and other cancers. Cancer Cell 2013; 23: 48-62.

[44] Ashihara E, Takada T and Maekawa T. Targeting the canonical Wnt/beta-catenin pathway in hematological malignancies. Cancer Sci 2015; 106: 665-671.

[45] Lai XB, Nie YQ, Huang HL, Li YF, Cao CY, Yang H, Shen B and Feng ZQ. NIMA-related kinase 2 regulates hepatocellular carcinoma cell growth and proliferation. Oncol Lett 2017; 13: 1587-1594.

[46] Zhang MX, Xu XM, Zhang P, Han NN, Deng JJ, Yu TT, Gan YY, He XQ and Long ZX. Effect of silencing NEK2 on biological behaviors of HepG2 in human hepatoma cells and MAPK signal pathway. Tumour Biol 2016; 37: 2023-2035.

[47] Wong PP, Miranda F, Chan KV, Berlato C, Hurst HC and Scibetta AG. Histone demethylase KDM5B collaborates with TFAP2C and Myc to repress the cell cycle inhibitor p21(cip) (CDK-N1A). Mol Cell Biol 2012; 32: 1633-1644.

[48] Gu ZM, Xia JL, Xu HW, Frech I, Tricot G and Zhan FH. NEK2 promotes aerobic glycolysis in multiple myeloma through regulating splicing of pyruvate kinase. Haematologica 2017; 10: 17.