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Research Article

Evaluation of the Anti-Cancer Effect of TRAF2 and NCK Interacting Protein Kinase (TNIK) Inhibition in Breast Cancer Cells

Selin Zeynep Özçelik[1], Kaan Furkan Hamarat[1], Gamze Güney Eskiler[2], Süleyman Kaleli[2*]

[1] Sakarya University, Faculty of Medicine, Sakarya, Türkiye
selinzeynep.ozcelik@gmail.com
hamaratkaanfurkan@gmail.com

[2] Sakarya University, Faculty of Medicine, Department of Medical Biology, Sakarya, Türkiye
gamzeguney@sakarya.edu.tr
skaleli@sakarya.edu.tr

* Corresponding Author

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Objective: This study aimed to evaluate the anticancer effect of NCB-0846, a TNIK inhibitor, in MCF-7 cells and to assess its impact on the expression levels of NF-κB and TNFA at the gene level.

Materials and Methods: The MCF-7 cell line was cultured at 37°C in a 5% CO2 atmosphere using Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics (50 IU/mL penicillin and 50 mg/mL streptomycin). Cell viability was analyzed using the CCK-8 assay to determine the cytotoxic effect of NCB-0846. Acridine Orange/Propidium Iodide (AO/PI) staining was performed to evaluate the effect of NCB-0846 on cellular morphology in the MCF-7 cell line. Total RNA was isolated from cells treated with NCB-0846. Data analysis was performed using the SPSS 22.0 statistical program.

Results: The data indicated that NCB-0846 significantly decreased the viability rates of MCF-7 cells in a dose-dependent manner (1-3 μM, $p<0.01$). RT-PCR analysis revealed that the expression level of NFKB1 increased 5.4-fold compared to the control group in MCF-7 cells treated with NCB-0846 at the effective dose and duration ($p<0.01$). In contrast, the expression level of TNFA decreased to 0.4-fold compared to the control group ($p<0.01$).

Conclusion: The results demonstrate that NCB-0846 induces changes in the mRNA levels of the NFKB1 and TNFA genes, which are associated with inflammatory signalling pathways in MCF-7 cells. However, further molecular analyses are necessary to clarify the effect of NCB-0846 on inflammation in breast cancer and other cancer types.

Keywords: Breast cancer, Inflammation, NCB-0846, TNIK

1. INTRODUCTION

Breast cancer is one of the most common cancers among women and is a leading cause of death.[1] The Luminal A breast cancer subtype is positive for estrogen receptors (ER) and progesterone receptors (PR), and negative for human epidermal growth factor receptor 2 (HER2).[2] The Luminal A breast cancer subtype, which represents over half of all breast cancer cases, is characterized by a better prognosis and less aggressive features compared to other subtypes. However, challenges such as resistance to hormonal treatment, the formation of cancer stem cells, and systemic toxicity can limit the effectiveness of current treatment options.[3-6] Therefore, there is a pressing need to develop innovative treatment strategies.

TNIK, or TRAF2 Nck-interacting protein kinase, is a serine/threonine kinase that belongs to the germinal center kinase (GCK) family. Members of the GCK family are subgroups within the STE20 kinase family, and they interact with two key proteins: tumour necrosis factor (TNF) receptor-associated factor 2 (TRAF2) and NCK adaptor protein 1 (NCK1). The TNIK molecule plays a crucial role in regulating the nuclear factor-κB

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(NF-κB) signaling pathway through its N-terminal kinase domain, as well as the c-JUN N-terminal kinase (JNK) pathway via its C-terminus.[7-9] The TNIK protein is a key molecular regulator in the Wnt signaling pathway, which is known to have varying activity levels in many cancers, including breast cancer. This pathway plays a crucial role in regulating various cellular processes, such as cell growth and differentiation.[10] The TNIK molecule plays a crucial role in the transcription of Wnt target genes by facilitating the interaction between T cell factor 4 (TCF-4), a member of the TCF/LEF transcription factor family, and β-catenin.[11] Due to its significant properties, TNIK presents a potential therapeutic target for various types of cancer, particularly breast cancer. The compound NCB-0846, known as cis-4-(2-(3H-benzo[d]imidazol-5-ylamino)quinazolin-8-yloxy) cyclohexanol, is a small molecule TNIK inhibitor that demonstrates inhibitory activity against TNIK, with a half-maximum inhibitory concentration (IC50) of 21 nM.[12,13] Studies have reported the efficacy of NCB-0846 across various cancer types, including colorectal, lung, breast, and prostate, demonstrating promising effects in suppressing Wnt-mediated tumor formation, reducing cancer stem cells, and inhibiting epithelial-mesenchymal transition (EMT).[14-22] The interaction of TNIK with TRAF2 also contributes to the activation of specific cytokine receptors, particularly tumor necrosis factor alpha (TNF-α). TNF-α is a critical cytokine that regulates apoptosis, cell survival, and inflammatory responses. It binds to two types of receptors: TNFR1 and TNFR2, triggering different molecular signaling pathways. When TNF-α interacts with TNFR1, the TRAF2 molecule acts as a mediator in its interaction with TNF receptor-associated death domain protein (TRADD). While TRADD can induce apoptosis through FADD, it simultaneously activates the NF-κB pathway, which has various effects, including inflammation and cell survival, by stimulating IκB kinases (IKK).[23-25] The NF-κB family comprises multiple transcription factors, including RelA, RelB, c-Rel, NF-κB1/p50, and NF-κB2/p52. These transcription factors remain inactive in the absence of signals and are located in the cytoplasm, bound by IκB proteins. Upon receiving a signal, the degradation of IκB facilitates the translocation of NF-κB transcription factors into the nucleus, leading to the production of pro-inflammatory molecules such as cytokines, chemokines, Bcl-XL, vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMP)-2, and MMP-9 in tumor cells. NF-κB plays a crucial role in cancer progression due to its dual function in regulating both apoptosis and cell survival.[23,26,27]

This study investigates, for the first time, the anticancer effects of NCB-0846, a TNIK inhibitor, in MCF-7 breast cancer cells. Additionally, it examines the impact of NCB-0846 on the TNF-α and NF-κB signaling pathways, which play crucial roles in inflammation and cell survival, at the gene level.

2. METHODOLOGY

2.1. Ethics committee approval

This study utilized commercially available cell cultures. Approval from an ethics committee is not required.

2.2. Cell culture

The MCF-7 cell line was grown at 37°C in an atmosphere with 5% CO2. The culture medium used was Dulbecco's Modified Eagle Medium (DMEM), which was supplemented with 10% fetal bovine serum (FBS) and contained 50 IU/mL of penicillin, along with 50 mg/mL of streptomycin.

2.3. Cell viability analysis

Cell viability was assessed using the CCK-8 assay to determine the cytotoxic effect of NCB-0846. MCF-7 cells were seeded in 96-well plates at a density of 20,000 cells per mL. NCB-0846 was

added at varying concentrations ranging from 1 to 10 μM, and the cells were incubated for either 24 or 48 hours. After the incubation period, 10 μL of CCK-8 dye was added to each well, and the plates were incubated for an additional hour at 37°C. The absorbance was then measured at 450 nm using an ELISA reader.

2.4. Acridine Orange/Propidium Iodide (AO/PI) staining

AO/PI staining was conducted to assess the impact of NCB-0846 on cellular morphology in the MCF-7 cell line. Cells were seeded at a density of 100,000 cells per mL in each well of a 6-well plate and incubated with 3 μM of NCB-0846 for 24 hours. After incubation, the cells were fixed with 4% paraformaldehyde, and acridine orange/propidium iodide (AO/PI) dye was added. The cells were then incubated in the dark for 30 minutes before imaging using the EVOS FL Cell Imaging System (Thermo Fisher Scientific).

2.5. RT-PCR analysis

To evaluate the changes in the expression levels of the NF-κB and TNF-α genes, cells were seeded at a concentration of 1,000,000 cells per mL in flasks. Total RNA was isolated from cells treated with 3 μM NCB-0846 for 24 hours. The quantity and purity of the isolated RNA were assessed using a Qubit device (Invitrogen). cDNA synthesis was performed on the obtained RNA, followed by RT-PCR analysis. Beta-actin was used as a control gene.

2.6. Statistical analysis

Data analysis was performed using the SPSS 22.0 statistical software, with a significance level set at $p < 0.05$. One-way ANOVA (Post-hoch Tukey) was employed to evaluate differences in cell viability percentages. Additionally, the CT values obtained from the RT-PCR analysis were presented as fold changes using the "REST (2009 V2.0.13)" software.

3. RESULTS

3.1. Evaluation of the anti-cancer effect of TNIK inhibition

A CCK-8 cell viability analysis was conducted to evaluate the cytotoxic effects of the TNIK inhibitor NCB-0846 on MCF-7 breast cancer cells. The data presented in Figure 1 indicate that cell viability in MCF-7 cells treated with 1, 2, 3, 4, 6, 8, and 10 μM of NCB-0846 for 24 hours was measured at 63.9±0.1%, 57±2.2%, 54.4±0.2%, 56.5±1.5%, 52.9±0.5%, 59.9±0.2%, and 63.7±0.2%, respectively ($p<0.01$). Following treatment with the same concentrations for 48 hours, the viability of MCF-7 cells was assessed at 33.3±2.9%, 26.1±1.7%, 24.7±2.3%, 26.6±2.6%, 25.5±1.6%, 24.9±1%, and 24.1±0.8%, respectively. This demonstrates an increased cytotoxic effect of TNIK inhibition over the 24-hour treatment period ($p<0.01$). Based on the results from the CCK-8 analysis, a 3 μM dose of NCB-0846 was subsequently applied to the cells for 24 hours in further molecular analyses (Figure 1).

3.2. Evaluation of the effect of TNIK inhibition on cell morphology

The morphological changes in MCF-7 cells after TNIK inhibition were evaluated using AO/PI staining. It was observed that breast cancer cells treated with 3 μM NCB-0846 for 24 hours showed signs of late apoptotic death when compared to the control cells (see Figure 2).

Furthermore, TNIK inhibition resulted in the formation of numerous vacuoles within the MCF-7 cells (Figure 2).

3.3. Effect of TNIK inhibition on gene expression levels

RT-PCR analysis was conducted to quantitatively evaluate the effects of NCB-0846 on the mRNA levels of NFKB1 and TNFA in MCF-7 cells. The results are presented in Figure 3. The analysis

revealed that TNFA expression decreased by 0.4-fold, while the mRNA level of NFKB1 increased by 5.4-fold. Both changes were statistically significant in breast cancer cells treated with 3 µM of NCB-0846 for 24 hours, compared to control cells ($p < 0.01$) (Figure 3).

Figure 1.

*Dose-dependent viability analysis results in MCF-7 cells treated with NCB-0846 (p<0.01**)*

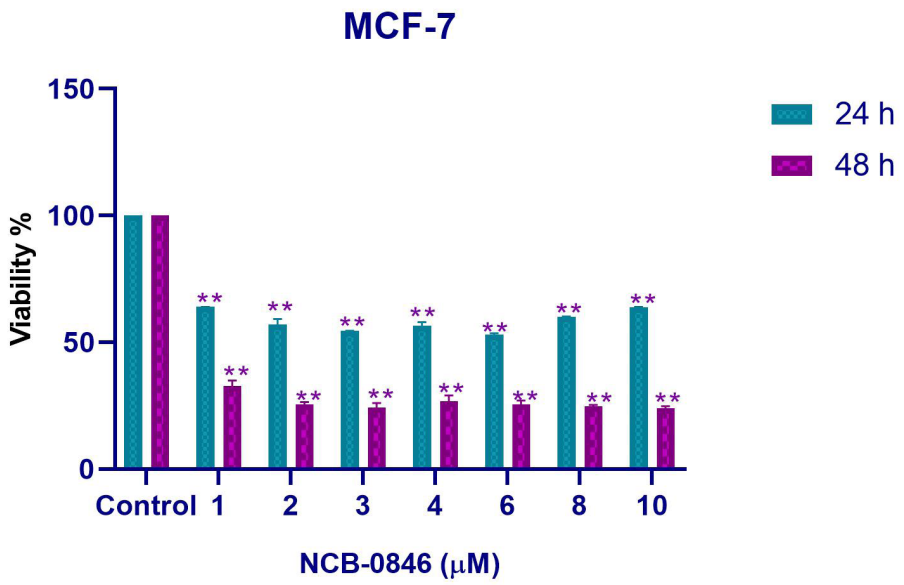


Figure 2.

AO/PI images in MCF-7 cells after NCB-0846 application. (A) Control (B) Cells treated with 3 µM NCB-0846 for 24 hours

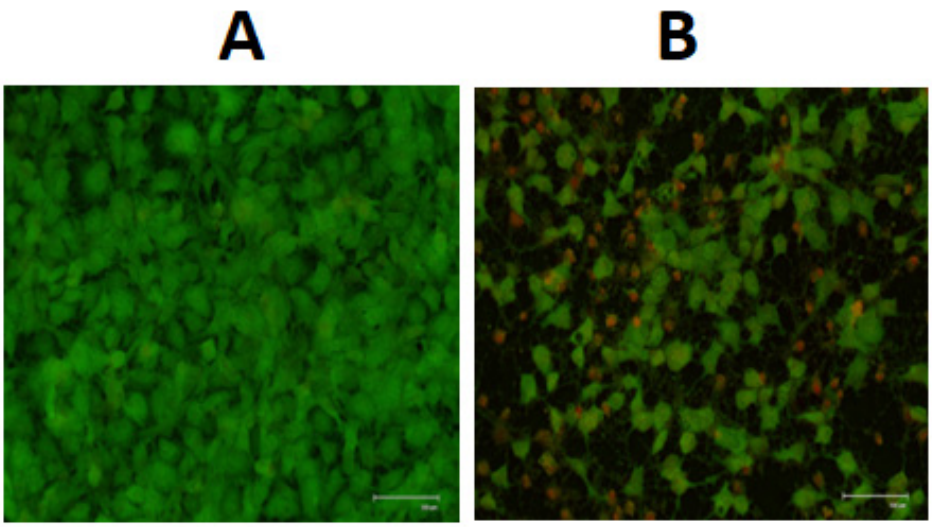
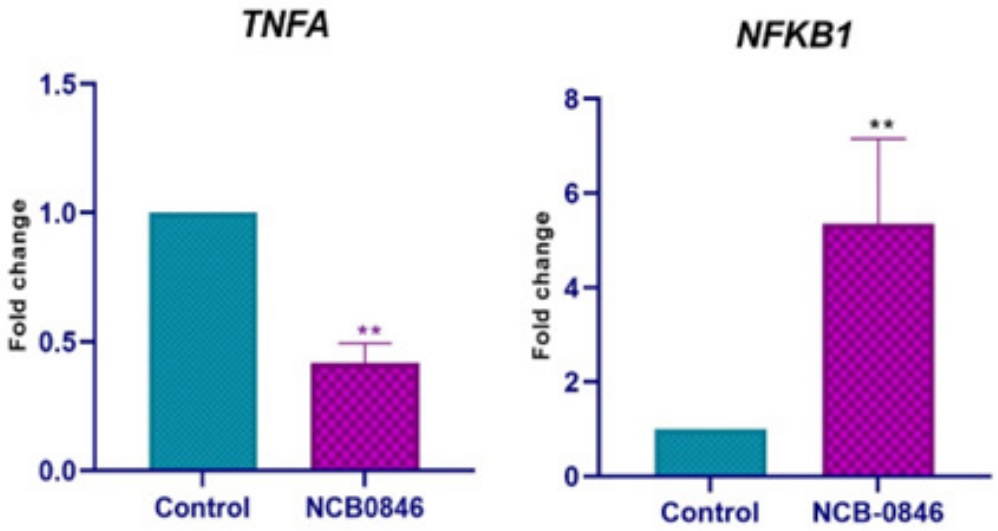


Figure 3.

*RT-PCR analysis results of NFKB1 and TNFA expression levels in MCF-7 cells treated with NCB-0846 (p<0.01**)*



4. DISCUSSION

This study is the first to investigate the anti-cancer effects of TNIK inhibition mediated by NCB-0846 in MCF-7 breast cancer cells. The data demonstrate that inhibiting TNIK results in a reduction of cell viability in MCF-7 cells, as well as the induction of apoptosis and the formation of multiple vacuoles. Furthermore, we observed an increase in the expression level of NFKB1 in MCF-7 cells treated with NCB-0846. These findings provide the first preclinical evidence suggesting that TNIK inhibition may be a potential therapeutic target for MCF-7 breast cancer cells.

Numerous studies in the literature have explored the therapeutic effects of TNIK inhibition as a potential treatment strategy for various types of cancer. [14-22] For example, a study conducted by Masuda et al. found that NCB-0846 exhibited anti-cancer effects both in vitro and in vivo by suppressing the Wnt signaling pathway in colorectal cancer (CRC). This compound demonstrated cytotoxic effects on CRC cells and stem cells, inducing apoptosis by increasing the sub-G1 cell population. Additionally, the study reported that NCB-0846 reduced tumor growth in animal models by inhibiting TCF/LEF activity in CRC. Alongside TNIK/Wnt inhibition, it also suppressed the expression of proteins such as CDK2/CycA2, JAK3, PDGFRα, and various mesenchymal biomarkers involved in different signaling pathways.[14] In a more recent study by Zhang et al., the anti-cancer efficacy of TNIK inhibition was investigated in papillary thyroid carcinoma (PTCa).[17] The findings demonstrated that NCB-0846, functioning as a TNIK inhibitor, significantly suppressed TNIK kinase activity in different PTC cell lines (TPC-1, KTC-1, and BCPAP). It induced apoptosis through the activation of apoptosis-related proteins and inhibited tumor growth in an in vivo PTC mouse model.[17] Additionally, several studies have shown that different TNIK inhibitors, particularly NCB-0846, exhibit anti-metastatic efficacy by suppressing the TGF-β1/SMAD-mediated epithelial-mesenchymal transition (EMT) in lung adenocarcinoma.[27-29] Sato et al. conducted a study on triple-negative breast cancer (TNBC) and revealed that 108600-mediated TNIK inhibition reduced colony formation and cell

growth in breast cancer stem cell-like cells. This inhibition induced apoptosis by causing cell cycle arrest in the G2/M phase and helped overcome chemotherapy resistance in an in vivo model.[29] Despite highlighting the therapeutic efficacy of TNIK inhibitors across various cancer types, research on the effectiveness of different TNIK inhibitors, particularly NCB-0846, in breast cancer remains limited. In the current study, NCB-0846 demonstrated cytotoxic and late apoptotic effects on MCF-7 Luminal A-type breast cancer cells. Its anti-cancer efficacy was confirmed; however, further studies are warranted to investigate the numerous vacuoles observed in cells treated with NCB-0846.

NF-κB is a critical transcription factor that regulates the expression of genes involved in various cellular processes, including inflammation, cell death, and proliferation. It is regulated by the N-terminal kinase domain of TNIK and the c-Jun N-terminal kinase (JNK) signalling pathway.[30] NF-κB significantly influences the expression of inflammatory genes, such as TNF-α, IL-1β, IL-6, IL-12p40, and COX2, particularly in cancer and immune cells. Notably, TNF-α and IL-1β are recognized activators of NF-κB, which shows a strong correlation with NF-κB expression.[31] Our study indicates that inhibiting TNIK leads to an increase in NFKB1 expression in MCF-7 cells while decreasing TNFA mRNA levels. Therefore, there is a need for advanced molecular analyses to explore other NF-κB-related inflammatory pathways and cytokine expressions as alternatives to the NF-κB/TNF-α-mediated signaling pathway associated with TNIK inhibition in breast cancer cells. The cytoskeleton is a dynamic system composed of actin filaments, intermediate filaments, and microtubules. These components can bind to one another or attach to various membrane compartments, forming organized structures. Modulating the cytoskeleton is essential for facilitating vacuole formation, as well as for processes like epithelial-mesenchymal transition (EMT) and metastasis in cancer cells.[32] Literature highlights the direct effects of TNIK on cytoskeleton remodelling, particularly regarding the actin cytoskeleton, as well as its indirect influence through the Wnt/β-catenin pathway.[33,34] Additionally, AO/PI staining results showed that TNIK inhibition with NCB-0846 in MCF-7 cells resulted in vacuole formation. To better understand the anti-cancer efficacy of TNIK inhibitors across different cancer types, especially breast cancer, further preclinical studies are needed to investigate the effects of TNIK inhibition on non-apoptotic cell death mechanisms associated with vacuole formation as an alternative to apoptosis.

In conclusion, this study demonstrates, for the first time, the anti-cancer activity of NCB-0846 as a TNIK inhibitor in MCF-7 breast cancer cells. We have also revealed, for the first time, the relationship between NCB-0846 and inflammation. However, further molecular analyses are required to explore the findings we obtained across different breast cancer cell lines. Additionally, future studies could investigate the NF-κB signalling pathway and related cytokines in MCF-7 cells treated with NCB-0846.

Ethics Committee Approval:
This study was conducted using commercially available cell cultures. Ethics committee approval is not required.

Author Contributions:
SZO and KFH conceptualized the study. SZO, KFH, and GGE designed the study. SZO, KFH, and GGE were responsible for data collection. GGT conducted the statistical analysis. SZO, KFH, and GGE prepared the draft manuscript, while GGE and SK finalized the manuscript.

Conflict of Interest:

All authors declare no conflict of interest.

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REFERENCES

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin. 2022 Jan;72(1):7-33. doi:10.3322/caac.21708
2. Baguley CB, Leung E. Heterogeneity of Phenotype in Breast Cancer Cell Lines. Breast Cancer - Carcinogenesis, Cell Growth and Signalling Pathways. 2011;245-256. InTech. Available at: http://dx.doi.org/10.5772/21984. Accessed on September 10, 2024
3. Giaquinto AN, Sung H, Miller KD, Kramer JL, Newman LA, Minihan A, et al. Breast Cancer Statistics, 2022. CA Cancer J Clin. 2022 Nov;72(6):524-541. doi:10.3322/caac.21754
4. Sweeney EE, McDaniel RE, Maximov PY, Fan P, Jordan VC. Models and Mechanisms of Acquired Antihormone Resistance in Breast Cancer: Significant Clinical Progress Despite Limitations. Horm Mol Biol Clin Investig. 2012 Feb;9(2):143-163. doi:10.1515/hmbci-2011-0004
5. Telang NT. The Divergent Effects of Ovarian Steroid Hormones in the MCF-7 Model for Luminal a Breast Cancer: Mechanistic Leads for Therapy. Int J Mol Sci. 2022 Apr 27;23(9):4800. doi:10.3390/ijms23094800
6. Dey P, Rathod M, De A. Targeting stem cells in the realm of drug-resistant breast cancer. Breast Cancer (Dove Med Press). 2019 Mar 7;11:115-135. doi:10.2147/BCTT.S189224
7. Shen RR, Zhou AY, Kim E, O'Connell JT, Hagerstrand D, Beroukhim R, et al. TRAF2 is an NF-κB-activating oncogene in epithelial cancers. Oncogene. 2015 Jan 8;34(2):209-16. doi:10.1038/onc.2013.543
8. Hayden MS, Ghosh S. NF-κB in immunobiology. Cell Res. 2011 Feb;21(2):223-44. doi: 10.1038/cr.2011.13
9. Bradley JR, Pober JS. Tumor necrosis factor receptor-associated factors (TRAFs). Oncogene. 2001 Oct 1;20(44):6482-91. doi:10.1038/sj.onc.1204788
10. Liu Y, Liu K, Huang Y, Sun M, Tian Q, Zhang S, et al. TRIM25 Promotes TNF-α-Induced NF-κB Activation through Potentiating the K63-Linked Ubiquitination of TRAF2. J Immunol. 2020 Mar 15;204(6):1499-1507. doi:10.4049/jimmunol.1900482
11. Koni M, Pinnarò V, Brizzi MF. The Wnt Signalling Pathway: A Tailored Target in Cancer. Int J Mol Sci. 2020 Oct 18;21(20):7697. doi: 10.3390/ijms21207697
12. Teng Y, Wu R, Bo W, Tang M, Wang T, Cui X, et al. Fragment growth-based discovery of novel TNIK inhibitors for the treatment of colorectal cancer. Eur J Med Chem. 2024 Mar 15;268:116240. doi: 10.1016/j.ejmech.2024.116240
13. Sugano T, Masuda M, Takeshita F, Motoi N, Hirozane T, Goto N, et al. Pharmacological blockage of transforming growth factor-β signalling by a Traf2- and Nck-interacting kinase inhibitor, NCB-0846. Br J Cancer. 2021 Jan;124(1):228-236.
14. Masuda M, Uno Y, Ohbayashi N, Ohata H, Mimata A, Kukimoto-Niino M, et al. TNIK inhibition abrogates colorectal cancer stemness. Nat Commun. 2016 Aug 26;7:12586. doi:10.1038/ncomms12586
15. Jung HR, Oh Y, Na D, Min S, Kang J, Jang D, et al. CRISPR screens identify a novel combination treatment targeting BCL-X_L and WNT signaling for KRAS/BRAF-mutated colorectal cancers. Oncogene. 2021 May;40(18):3287-3302.
16. Sekita T, Yamada T, Kobayashi E, Yoshida A, Hirozane T, Kawai A, et al. Feasibility of Targeting Traf2-and-Nck-Interacting Kinase in Synovial Sarcoma. Cancers (Basel). 2020 May 16;12(5):1258. doi:10.3390/cancers12051258
17. Zhang R, Yu Y, Yang Y, Zhang M, Zhang X, Chang Y, et al. Therapeutic targeting of TNIK in papillary thyroid carcinoma: A novel approach for tumor growth suppression. Med Oncol. 2024 May 20;41(6):160. doi:10.1007/s12032-024-02380-y
18. Nguyen T, Carrieri FA, Connis N, Lafargue A, Chang J, Chan A, et al. TNIK Inhibition Sensitizes TNIK-Overexpressing Lung Squamous Cell Carcinoma to Radiotherapy. Mol Cancer Ther. 2024 Jun 10:OF1-OF11. doi:10.1158/1535-7163.MCT-23-0412
19. Wang M, Gu Y, Li Q, Feng B, Lv X, Zhang H, et al. The Traf2 and NcK interacting kinase inhibitor NCB-0846 suppresses seizure activity involving the decrease of GRIA1. Genes Dis. 2023 Jun 24;11(3):100997. doi:10.1016/j.gendis.2023.03.036
20. Teng Y, Wu R, Bo W, Tang M, Wang T, Cui X, et al. Fragment growth-based discovery of novel TNIK inhibitors for the treatment of colorectal cancer. Eur J Med Chem. 2024 Mar 15;268:116240. doi:10.1016/j.ejmech.2024.116240
21. Sugano T, Masuda M, Takeshita F, Motoi N, Hirozane T, Goto N, et al. Pharmacological blockage of transforming

growth factor-β signalling by a Traf2- and Nck-interacting kinase inhibitor, NCB-0846. Br J Cancer. 2021 Jan;124(1):228-236.
22. Jiang N, Guo T, Wang M, Liao Y, Ma F, Gao J, et al. TNIK swaths AR to WNT pathway and drives Castration-Resistant Prostate Cancer. Research Square; 2020. doi:10.21203/rs.3.rs-18207/v1
23. Li Z, Lim SK, Liang X, Lim YP. The transcriptional coactivator WBP2 primes triple-negative breast cancer cells for responses to Wnt signaling via the JNK/Jun kinase pathway. The Journal of Biological Chemistry. 2018 Dec;293(52):20014-20028.
24. Balkwill F. TNF-alpha in promotion and progression of cancer. Cancer Metastasis Rev. 2006 Sep;25(3):409-16. doi:10.1007/s10555-006-9005-3
25. Cruceriu D, Baldasici O, Balacescu O, Berindan-Neagoe I. The dual role of tumor necrosis factor-alpha (TNF-α) in breast cancer: Molecular insights and therapeutic approaches. Cell Oncol (Dordr). 2020 Feb;43(1):1-18.
26. Shi JH, Sun SC. Tumor Necrosis Factor Receptor-Associated Factor Regulation of Nuclear Factor κB and Mitogen-Activated Protein Kinase Pathways. Front Immunol. 2018 Aug 9;9:1849.
27. Sokolova O, Naumann M. NF-κB Signaling in Gastric Cancer. Toxins (Basel). 2017 Mar 28;9(4):119. doi: 10.3390/toxins9040119
28. Kim J, Moon SH, Kim BT, Chae CH, Lee JY, Kim SH. A novel aminothiazole KY-05009 with potential to inhibit Traf2- and Nck-interacting kinase (TNIK) attenuates TGF-β1-mediated epithelial-to-mesenchymal transition in human lung adenocarcinoma A549 cells. PLoS One. 2014 Oct 22;9(10):e110180. doi:10.1371/journal.pone.0110180
29. Sato K, Padgaonkar AA, Baker SJ, Cosenza SC, Rechkoblit O, Subbaiah DRCV, et al. Simultaneous CK2/TNIK/DYRK1 inhibition by 108600 suppresses triple negative breast cancer stem cells and chemotherapy-resistant disease. Nat Commun. 2021 Aug 3;12(1):4671. doi: 10.1038/s41467-021-24878-z
30. Zinatizadeh MR, Schock B, Chalbatani GM, Zarandi PK, Jalali SA, Miri SR. The Nuclear Factor Kappa B (NF-kB) signaling in cancer development and immune diseases. Genes Dis. 2020 Jul 18;8(3):287-297.
31. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017;2:17023–. doi:10.1038/sigtrans.2017.23
32. Umekawa M, Klionsky DJ. The Cytoplasm-to-Vacuole Targeting Pathway: A Historical Perspective. Int J Cell Biol. 2012;2012:142634. doi:10.1155/2012/142634
33. Hu G, Huang N, Zhang J, Zhang D, Wang S, Zhang Y, et al. LKB1 loss promotes colorectal cancer cell metastasis through regulating TNIK expression and actin cytoskeleton remodeling. Mol Carcinog. 2023 Nov;62(11):1659-1672.
34. May-Simera HL, Kelley MW. Cilia, Wnt signaling, and the cytoskeleton. Cilia. 2012 May 2;1(1):7. doi: 10.1186/2046-2530-1-7