NKILA Regulates HSP90α, NF-κB subunits and β-Catenin Expressions in the MCF-7 Cells

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Abstract

Background: Non-coding RNAs are increasingly investigated and have great potential for diagnose, prognosis and treatment of cancer. Thus, we investigated the possible relation between NF-κB suppressor-NKILA and HSP90, NF-κB and β-catenin molecules in the MCF-7. HSP90 is a compelling stress protein and together with β-catenin and NF-κB molecules it can be responsible for the cancer cell development. However, NKILA is a novel molecule and there is not comprehensive data about it unlike HSP90, β-catenin and NF-κB alone. Therefore, we suggest that there might be a correlation between NKILA and these proteins.

Methods and Results: To investigate the NKILA role on these proteins we inhibited the NKILA by using transfection. MCF-7 cells transfected with NKILA-siRNA and incubated for 5 hours. Then, cells were collected and proteins were extracted to be separated in SDS-PAGE. Aforementioned proteins of siRNA transfected group were evaluated by comparing their band intensities with the control group protein bands by immunoblotting. According to this, HSP90 and NF-κB/p105, NF-κB/p65 and NF-κB/p50 subunits significantly increased while β-catenin significantly decreased after NKILA inhibition.

Conclusion:, For the first time we demonstrate that HSP90 and beta-catenin is associated with NKILA levels and this may highly related with canonical NF-κB pathway in MCF-7 cells. These novel findings may have important implications in cancer cells development and might present important hints for the future studies about the cancer cell targeted therapy.

Highlights

- First time we showed that NKILA is associated with a stres protein and β-catenin.
- NF-κB increase after NKILA inhibition is a supportive finding for NKILA effects on NF-κB
- These findings may present useful and novel data for the NF-κB targeted cancer therapies

Future Aspects

- We need to show other related stress proteins and Wnt/β-catenin pathway proteins to clarify the NKILA roles in cancer development in addition to NF-κB negative regulation.
- We should further investigate possible NKILA association with NF-κB and Wnt pathway cross-talk molecules to test whether NKILA based agents can be developed in the manner of cancer treatments.
- There is no any other study showed the HSP90α and β-catenin relation with NKILA which might involve rudimental information, thus Stress Response-Wnt signaling and NKILA association is needed to be investigated in more detail.

Introduction

Long non-coding RNAs (LncRNAs) are longer than 200 bp transcripts and lack of coding ability but have multiple roles in many pathways and epigenetic regulations (1). As transcripts, lncRNAs might involve chromosomal regulations, protein interactions and molecular signaling (2).

Normally, NF-kB is a key component in the cancer cells to provide survival to the cancer cells. In breast cancer patients NF-κB was found to be significantly activated and associated with oncogenesis (3). NF-κB is directly associated with HSP90 (heat shock protein 90) (5), which is another important anti-apoptotic molecule (6). NF-κB can bind to HSP90 promoter in cancer cells (7), or HSP90 may involve the IKK (IκB kinase) activation (8) thus HSP90 and NF-κB molecules are expected to be correlated and provide cancer cell survival. Moreover, beta-catenin is another important molecule that influence the metastasis of breast cancer cells (9), and it may negatively correlated with NF-κB because of cross-talk between Wnt and canonical NF-κB pathways. Like the NF-κB pathway catenin is also involves survival and proliferation progress in cancer cells but β-catenin and NF-κB may intertwined (4).

There are plenty of studies that show lncRNAs involve multiple pathways and included in cancer prognosis (10, 11, 12). NF-κB interacting long non-coding RNA (NKILA) is a novel lncRNA and negative regulator of NF-κB signaling pathway. Directly binding to NF-κB and inhibits the metastatic and prognostic features of the cancerous cells (13, 14). First it has been introduced to literature by Liu et al in 2015. Since then, NKILA has been determined as NF-κB regulator or suppressor in some cancer types such as;, tongue squamous cell carcinoma (14), lung cancer (13), malignant glioma (15), malignat melanoma (16) and breast cancer (17). NKILA associates with also NF-κB/Snail Pathway (13). All these studies indicate the NKILA as suppressor of cancer development.

However, today there are limited numbers of study shed light on this transcript and only few studies investigated its role within the cytoplasm. Accordingly, NKILA association with other signal pathways and proteins are needed to be comprehensively elaborated. Therefore, we investigated its association with stress protein HSP90α, β-catenin and NF-κB/p105-p50-p65 protein molecules and tried to enlighten molecular mechanism of NKILA in this study.

Material And Method

Experimentl Reagents, siRNA and Antibodies

Custom desgined NKILA-siRNA was purchased from SISEL custom siRNA 5 nM, Ambion^{TM} . The sense strand was 5’-GGACUGCGAGAAUUAAAUAtt-3’ and antisense strand 5’-UAUUUAAUUCUCGCAGUCtg-3’. Lipofectamine 2000^{TM} transfection reagent was obtained from ThermoScientific. Opti-MEM transfection medium were purchased from Invitrogen. Other tissue culture reagents were obtained from Gibco. RNA isolation kit was obtained from GeneBioMark. High Capacity cDNA Reverse transcription kit was purchased from Applied Biosystems. In this study different antibodies were used: anti-HSP90α (Thermo-Fisher), anti-beta-catenin (Thermo-Fisher), anti-NF-κB (P105/P50) (Santa Cruz), anti-NF-κB (P65) (Boster), anti-GAPDH (Boster), HRP-conjugated mouse anti-rabbit IgG (Thermo), HRP-conjugated goat anti-rabbit

IgG (Thermo) and HRP-conjugated goat anti-mouse IgG (Thermo). Other Chemicals were obtained from Sigma.

Cell Culture Conditions

The human breast cancer MCF-7 cells were obtained from Istanbul University Cell Culture Collections. MCF-7 cell line was cultured in DMEM-F12 (Dulbecco's Modified Ham's F-12), medium supplemented with 10% FBS (Fetal Bovine Serum) and 1% antibiotics (100 U/ml penicilin and 100 mg/ml streptomycin). The cell line was maintained in humidified air atmosphere with 5% CO_2 at 37^oC.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

We performed to MTT assay on the MCF-7 cell line in order to detect cytotoxic activities of the preferred NKILA-siRNA and transfection reagent concentrations. 1x10^5 cells/ml MCF-7 cells were seeded in a 96 well tissue culture plate. After 24 hours, determined cytotoxic effect of cell transfection, the treatments were performed as described in the title “siRNA transfection” and two different doses of siRNA (25 and 50 nM) were used with two different transfection reagent concentrations (12 and 15 µl). After siRNA exposure at defined concentrations, cells were incubated for 48 hours. Following the incubation period, cell media were removed and 30 µl MTT were added to each well. After 4 hours of MTT incubation formazan crystals were observed under inverted microscope. At the end of this period, 150 µL DMSO were added to each well to solve the formazan crystals and then optical density (540 nm) of the cells were measured to determine the cell viability by using Eon^{TM} Microplate Reader (PowerWave HT™). All experiments were performed in triplicate. Cell viability percentages were calculated according to following equation:

$$Cell\ viability\ percentage = \frac{A\ sample}{A\ control} x100$$

siRNA Transfection

Cells were transfected with NKILA-siRNA to knock-down the NKILA gene expression. Tranfection reagent (TR) and NKILA-siRNA concentrations were determined according to the MTT results. For transfection, MCF-7 cells were incubated in 60x15 mm Petri dishes at 2x10^5 cells/ml concentration by grouping as control, NKILA-siRNA, mock and scrambled. TR and NKILA-siRNA diluted with Opti-MEM transfection medium for the final concentration of the reagents. Then, cells were exposed to 25 nM siRNA (in 12 µl TR) for 5 hours then siRNA containing cell medium refreshed by normal DMEM-Ham's F12 medium at the end of this transfection period. By following the medium refreshment, cells were incubated in normal medium for 48 hours at 37^oC humidified atmosphere with 5% CO_2.

RNA extraction and reverse-transcription polymerase chain reaction (RT-PCR)

Control and transfected cells were collected and RNA isolated from these cells by using RNA Lysis buffer according to manufacturer's instruction. Total RNA concentration was measured by NanoDrop 2000^{TM} Spectrophotometer (ThermoFisher). Before the quantitative analyses of gene amplification by RT-qPCR, RNA samples were separated and 18S and 28S ribosomal-RNA bands visualized in denaturing 1.5% agarose gel to check the RNA integrity. cDNA was obtained from these RNA samples by using cDNA Reverse Transcription Kit . Primers for PCR were prepared for cDNA by using gmPFU buffer and DNA polymerase.

After producing the related cDNA from total RNA samples, NKILA transcript levels were determined by RT-qPCR (BioRad-CFX96 Touch^{TM}) using Power SYBER® Green Master Mix. First, reaction mix was prepared which contains nuclease-free water, SYBER® and reverse and forward primers added to each well of plate. And then cDNA added to each well (20 µl of total volume in each well) and no template control (NTC) also prepared with the three technical replicates. Then, plate were placed to sample block and qPCR process was started with appropriate (two-step RT-qPCR) thermal-cycling conditions. Beta-actin was used as the housekeeping gene to normalize the data. Data were analyzed by using the $2^{-\Delta\Delta Ct}$ method to calculate fold change of the NKILA gene expression.

Cycling conditions were used as follow in BioRad-CFX real-time qPCR system 95˚C for 30 sec followed by 40 cycles of denaturing at 95˚C for 5 sec and annealing and extension at 55˚C for 45 sec. The following primers (ThermoFisher) were used:

Gene specific primer sequences for NKILA transcript were as follows:

Forward: 5'ACCTACCCACAACGCTTGAC3'

Reverse: 5'GCGCCAGAGACCACTAAGTC3'

Gene specific primer sequences for beta-actin (as housekeeping gene) were as follows:

Forward: 5'TGCGCAGAAAACAAGATGAG3'

Reverse: 5'GTCACCTTCACCGTTCCAGT3'

Immunoblotting

Control and transfected cells were analyzed by Western blotting as previously described (18). Total proteins were extracted from the control and transfected cells using RIPA-lysis buffer. BCA protein assay kit was used to determine total protein concentrations and then proteins were loaded to SDS-PAGE (10%) gels equally (30µg/well) and separated by their molecular weight. After SDS-PAGE, proteins transferred to PVDF membrane (Sigma) by using wet-transfer system. The membranes were blocked in non-fat skimmed milk (5%) for 1h and then incubated overnight with the HSP90α (1:1000), β-catenin (1:1000) and NF-κB/p105-p50 (1:1000), NF-κB/p65 (1:1000) primary antibodies at 4^{o}C temperature. After primary antibody incubation membranes were washed 5 times for 5 min. each time and then incubated with

related HRP conjugated secondary antibodies (1:2500) for 2 hours at room temperature. All experiment results were gathered from at least triplicate results.

Statistical Analysis

All the statistical analyses were performed using GraphPad Prism (ver.7.0). Data were presented as mean ± standard deviation. One-Way ANOVA test were used for the comparison of the multiple groups with the control group. Student-t test were used to compare experimented group with the control. P values <0.0001 (****), <0.001 (***), <0.01 (*) levels were accepted as significantly different.

Results

Toxicity of NKILA-siRNA

Different concentrations of siRNA and TR were applied to the MCF-7 cells for determining of the cytotoxic effects. After MTT assay, we determined the proper NKILA-siRNA and TR concentrations. According to this analysis, MCF-7 cell line showed minimum 90% cell viability at the concentration of 25 nM NKILA-siRNA in 12 µl TR. According to the statistical results, cell viability was significantly decreased in the 25 nM siRNA (in 15 µl TR) and 50 nM siRNA in (12 and 15 µl TR) treated groups ($p<0.0001$), (Fig.1).

Knockdown efficiency

25 nM of NKILA-siRNA caused inhibition of NKILA transcript expression levels according to our RT-qPCR results (Fig 2). As expected, mock (no-siRNA) and scrambled (non-target siRNA) groups were not effected the NKILA levels and showed no significant difference in their expression levels comparing to the control group. NKILA transcription levels were decreased approximately 67% in NKILA-siRNA treated group (Fig.2).

NKILA knockdown effected the HSP90, NF-κB and β-catenin levels

After transfection, NKILA transcription levels significantly decreased by 33% of the control group (67% inhibition efficiency, $p<0.0001$, Fig. 2). This inhibition caused significant change of HSP90α ($p<0.001$), p105-p50 ($p<0.001$), p65 ($p<0.01$) and β-catenin ($p<0.001$) expression levels. Thereafter, according to our results expression of HSP90α, NF-κB/p50, NF-κB/p105 and NF-κB/p65 increased by 54.2±5.9%, 44.3±14.3 % 33±9.7%, 33±10.4% respectively (Fig 3) but β-catenin levels decreased by 36.4±2.9% (Fig.3) ($p<0.001$). NF-κB/p105, NF-κB/p50 and NF-κB/p65 subunits levels changed as expected.

Discussion

In our study there was a negative association between p105-p50-p65 and NKILA transcript, and NF-κB subunits were significantly ($p<0.001$) increased in the NKILA-siRNA group. Inhibition of the NKILA transcript resulted in NF-κB increase. In this case, our result is expected if we considered the fact that NKILA is the natural suppressor of the NF-κB and binds to p65 subunits to inhibit the NF-κB in the cancer

cells (17, 19, 20). We showed the negative association between p105-p50-p65 and NKILA. Moreover, p105 is a precursor protein which means it might degraded by proteosome to form p50 (21). p105 involves to various cellular mechanisms through the NF-κB signaling (22). Besides, p105 may have different role aside from forming the p50 such as involment in MAP kinase regulation (23) and it is expressed constituvely (24, 25). Normally, NF-κB is a complex transcription factor which directly binds to DNA, associated with tumor development, proliferation and metastatic properties of the cancer cells (25). p105 subunit of NF-κB protein is known to be precursor of the p50 subunit (NFK1 homodimer). Additionally, p52 and its precursor p100 and RelA (p65), RelB and c-Rel are the other parts of this complicated transcription factor. Nevertheless, p105 has crucial role for the p50 function within the nucleus of the cancer cells (26, 27). Rather than NF-κB subunits p65, p50 and p52 homodimers, p105 has independed roles from the NF-κB complex and related with more signaling pathways within the cancer cells (28). Lin et al., found that NF-κB subunits p65, p105 and p50 showed discrepant expression profile in the non-small cell lung cancer (NSCLC). However, p65 and p105 was marker for the poor prognosis in the NSCLC (26).

Therefore, our findings provides supportive data for the literature and showed the NF-κB levels can be attenuated in case of NKILA inhibition which is pivotal molecule for the cancer cell prognosis as shown by several studies.

Moreover, we approve that HSP90α levels increase their expression levels in the siRNA treated group which means HSP90 and NKILA levels can be negatively correlated as with the NF-κB subunits. HSP90α is known as stress protein and provides necessary response against stress conditions but also attenuate cancer progression (19). In this case after inhibiting the NKILA HSP90 levels may increased its activation as response to inhibition of an inhibitory molecule such as NKILA. Because, NKILA transcript normally suppress the activity of NF-κB as mentioned above. HSP90 is responsible for the drug resistance, is also inflammatory response in cancer mainly and these kind of conditions are closely related with the NF-κB protein levels which known as poor prognosis marker in the cancer patients (5). Under these circumstances our findings suggest that HSP90 levels might increase as response to NKILA inhibition because of close relation with the NF-κB subunits. This brings the question such as NKILA alone can trigger or block the stress response in the breast cancer.

Beta-catenin was significantly decreased ($p<0.001$) in the NKILA-siRNA treated group which can be sign to the relation between NF-κB and β-catenin. Because, in some studies showed that catenin and NF-κB (32) found as negatively correlated to each other because catenin molecules within the cytoplasm can bind to the NF-κB promoter regions and that cause antagonistic effect, but also there are other studies to show positive correlation between β-catenin and NF-κB subunits (29). However in our study we only showed that β-catenin and NF-κB protein expression levels altered oppositely under the effect of NKILA inhibition. In a study, they detected an association between HSP90 and β-catenin levels as related with GSK3-β signaling pathway (29) in the MCF-7 cells. Another study indicated negative correlation between these two proteins. Deng et al., showed that β-catenin can interact and inhibit the NF-κB transcription factor in colon and breast cancer cells. Additionally, high levels of β-catenin within the cytoplasm declines

the NF-κB activation (32). In our study HSP90 levels increased as catenin levels declined in the NKILA-siRNA group. We suggest that this may caused by possible negative correlation between HSP90 and β-catenin in the cytoplasm but this suggestion requires another study which focused on catenin and HSP90 relation. Nevertheless, after inhibition of NKILA increase of HSP90 with β-catenin decreasing suggests the idea of negative correlation between these two molecules. We suggest that NKILA inhibition indirectly upregulated the HSP90α in the MCF-7 cells (Fig. 8).

In our study, p50, p65 and p105 levels increased as with decrease of β-catenin. Most probably, NF-κB increased its expression in the cytoplasm as a result of after inhibition of NKILA which is a negative regulator of the NF-κB by silencing then showing its antagonistic effect. Further studies are needed to approve this implication but decline of catenin levels while increasing of NF-κB can be suggested in this way. However, association between β-catenin and NKILA transcript has never been shown before and there are no other data to compare our results recently (Fig. 8). Thus, future studies again are needed to clarify the relation between these molecules in the manner of relevant signaling pathways.

We can suggest that this opposite direction of β-catenin and NF-κB expression levels after NKILA inhibition may resulted due to Wnt/β-catenin signaling pathway which normally shows cross-talk with canonical NF-κB signaling pathway. β-catenin and p50 might show negative correlation (29) but in another study it was positively associated with β-catenin in glioblastoma cells (31). Generally, NF-κB cross-talking and competes with the catenin signaling pathway in breast cancer cells (29). Therefore we can suggest that increase of NF-κB as with decline of β-catenin levels might be related with cross-talk of these proteins.

These results suggest that NKILA expressions in MCF-7 cells suppress the NF-κB subunits and down-regulates catenins. However HSP90 levels were also increase which suggested as HSP90 might associate with NF-κB transcription levels under the effect NKILA transcript. Due to blocking effect of NKILA on NF-κB transcription complex, it seems HSP90 levels were effected by this non-coding protein which normally associates with NF-κB molecule as indicated by literature.

Consequently, we first time found that HSP90 and NF-κB subunits expression levels increased but β-catenin levels decreased after NKILA inhibition in MCF-7 breast cancer cells. Thus, we suggest that these molecules might in a vanishing point as associated with NKILA because this transcript has direct effect on the NF-κB protein subunits which is one of the main cross-talk molecules in the cancer cells. Today, increasing number of study focusing on the noncoding RNA-protein association to enlighten and reveal mechanism of cancer development in molecular biology and clinic science (32-35). Thus, more detailed studies about non-coding RNA molecule involvement in various signaling pathways should be investigated. However, there are no other information in the literature about HSP90α and β-catenin association with the NKILA transcript. Therefore, further studies are needed to clarify and/or approve the role of this transcript molecule on these protein molecules which are on the cross-talk points between various signaling pathways.

Besides, regulatory effects of NKILA transcript on the other signaling pathways different than NF-κB pathway might reveal interesting key points to explain dual role of NF-κB subunits. With all these aspects, these aforementioned future studies might be pivotal for better understanding of cancer cell development and targeted therapies.

Abbreviations

lncRNA, long non-coding RNA; NKILA, NF-κB interacting long non-coding RNA; NF-κB, nuclear factor κB; HSP90 heat shock protein 90, MCF-7; breast cancer cell line, siRNA; small interfering RNA

Declarations

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Competing Interest

The authors declare that they have no competing interest

Availability of data and materials

The related detailed data from this study is available from the corresponding author.

Code availability

Not applicable

Author's contributions

MAT designed the study. MAT and EOU performed the study and MAT analyzed the results. MAT wrote and EOU drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicaple

Patient consent for publication

Not applicaple

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References

1. Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E, Chang HY, 2007, "Functional Demarcation of Active and Silent Chromatin Domains in Human Hox Loci by Noncoding Rnas." Cell 129, 7: 1311–23. http://dx.doi.org/10.1016/j.cell.2007.05.022
2. Peng F, Li TT, Wang KL, Xiao GQ, Wang JH, Zhao HD, Kang ZJ, Fan WJ, Zhu LL, Li M, Cui B, Zheng FM, Wang HJ, Lam EW, Wang B, Xu J, Liu Q (2017) "H19/Let-7/Lin28 Reciprocal Negative Regulatory Circuit Promotes Breast Cancer Stem Cell Maintenance. "Cell Death Dis 8:1. http://dx.doi.org/10.1038/cddis.2016.438. e2569.
3. Oeckinghaus A, Hayden MS, Ghosh S (2011) "Crosstalk in Nf-Kappab Signaling Pathways. " Nat Immunol 12:8: 695–708. http://dx.doi.org/10.1038/ni.2065
4. Du Q, Geller DA. 2010, "Cross-Regulation between Wnt and Nf-Kappab Signaling Pathways." For Immunopathol Dis Therap, 1, 3: 155 – 81. http://dx.doi.org/10.1615/ForumImmunDisTher.v1.i3
5. Thangjam GS, Joshi AD, Snead C, Wallace CL, Catravas JD (2012) "Hsp90 Inhibition Suppresses Lps-Mediated Nfkb Target Gene Expression without Affecting Nfkb Nuclear Translocation in Human Lung Microvascular Endothelial Cells". FASEB J 26:S1: 1058–1014. https://doi.org/10.1152/ajplung.00054.2016
6. Zagouri F, Bournakis E, Koutsoukos K, Papadimitriou CA (2012) "Heat Shock Protein 90 (Hsp90) Expression and Breast Cancer." Pharmaceuticals (Basel) 5, 9: 1008-20. http://dx.doi.org/10.3390/ph5091008
7. Ammirante M, Rosati A, Gentilella A, Festa M, Petrella A, Marzullo L, Pascale M, Belisario MA, Leone A, and Turco M. C (2008) "The Activity of Hsp90 Alpha Promoter Is Regulated by Nf-Kappa B Transcription Factors. " Oncogene 27:8: 1175–1178. http://dx.doi.org/10.1038/sj.onc.1210716
8. Broemer M, Krappmann D, Scheidereit C (2004) "Requirement of Hsp90 Activity for Ikappab Kinase (Ikk) Biosynthesis and for Constitutive and Inducible Ikk and Nf-Kappab Activation. " Oncogene 23:31: 5378–5386. http://dx.doi.org/10.1038/sj.onc.1207705
9. Jang GB, Kim JY, Cho SD, Park KS, Jung JY, Lee HY, Hong IS, Nam JS, 2015"Blockade of Wnt/Beta-Catenin Signaling Suppresses Breast Cancer Metastasis by Inhibiting Csc-Like Phenotype." Sci Rep, 5: 12465. http://dx.doi.org/10.1038/srep12465
10. Zhu D, Lv W, Zhou X, He Y, Yao H, Yu Y, Zhang G, Zhang Q (2021) "Long Non-Coding Rna Tmpo-As1 Promotes Tumor Progression Via Sponging Mir-140-5p in Breast Cancer". Exp Ther Med 21:1: 17. http://dx.doi.org/10.3892/etm.2020.9449

11. Park JY, Lee JE, Park JB, Yoo H, Lee SH, Kim JH (2014) Roles of Long Non-Coding Rnas on Tumorigenesis and Glioma Development. Brain Tumor Res Treat 2:1: 1–6. http://dx.doi.org/10.14791/btrt.2014.2.1.1
12. Prensner JR, Chinnaiyan AM (2011) "The Emergence of Lncrnas in Cancer Biology." Cancer Discov 1, 5: 391–407. http://dx.doi.org/10.1158/2159-8290.CD-11-0209
13. Lu Z, i Y, Wang J, Che Y, Sun S, Huang J, Chen Z, He J (2017) Long Non-Coding Rna Nkila Inhibits Migration and Invasion of Non-Small Cell Lung Cancer Via Nf-Kappab/Snail Pathway. J Exp Clin Cancer Res 36:1: 54. http://dx.doi.org/10.1186/s13046-017-0518-0
14. Huang WX, Chen Cui J, Feng Y, Song E, Li J, Liu Y (2016) "Long Non-Coding Rna Nkila Inhibits Migration and Invasion of Tongue Squamous Cell Carcinoma Cells Via Suppressing Epithelial-Mesenchymal Transition. " Oncotarget 7:38: 62520–62532. http://dx.doi.org/10.18632/oncotarget.11528
15. Chen Z, Li S, Shen L, Wei X, Zhu H, Wang X, Yang M, Zheng X (2020) Nf-Kappa B Interacting Long Noncoding Rna Enhances the Warburg Effect and Angiogenesis and Is Associated with Decreased Survival of Patients with Gliomas. Cell Death Dis 11:5: 323. http://dx.doi.org/10.1038/s41419-020-2520-2
16. Bian D, Gao C, Bao K, Song G (2017) "The Long Non-Coding Rna Nkila Inhibits the Invasion-Metastasis Cascade of Malignant Melanoma Via the Regulation of Nf-Kb". Am J Cancer Res 7:1: 28–40. http://www.ncbi.nlm.nih.gov/pubmed/28123845
17. Liu B, Sun L, Liu Q, Gong C, Yao Y, Lv X, Lin L, Yao H, Su F, Li D, Zeng M, Song E (2015) "A Cytoplasmic Nf-Kappab Interacting Long Noncoding Rna Blocks Ikappab Phosphorylation and Suppresses Breast Cancer Metastasis. " Cancer Cell 27(3 (Mar 9):370–381. http://dx.doi.org/10.1016/j.ccell.2015.02.004
18. Sengelen A, Onay-Ucar E (2018) "Rosmarinic Acid and Sirna Combined Therapy Represses Hsp27 (Hspb1) Expression and Induces Apoptosis in Human Glioma Cells". Cell Stress Chaperones 23:5: 885–896. http://dx.doi.org/10.1007/s12192-018-0896-z
19. Calderwood SK (2010) "Heat Shock Proteins in Breast Cancer Progression–a Suitable Case for Treatment?". Int J Hyperthermia 26:7: 681–685. http://dx.doi.org/10.3109/02656736.2010.490254
20. Wu W, Chen F, Cui X, Yang L, Chen J, Zhao J, Huang D, Liu J, Yang L, Zeng J, Zeng Z, Pan Y, Su F, Cai J, Ying Z, Zhao Q, Song E, Su S (2018) "Lncrna Nkila Suppresses Tgf-Beta-Induced Epithelial-Mesenchymal Transition by Blocking Nf-Kappab Signaling in Breast Cancer". Int J Cancer 143:9: 2213–2224. http://dx.doi.org/10.1002/ijc.31605
21. Fan CM, Maniatis T (1991) "Generation of P50 Subunit of Nf-Kappa B by Processing of P105 through an Atp-Dependent Pathway. " Nature 354:6352: 395–398. http://dx.doi.org/10.1038/354395a0
22. Moorthy AK, Savinova OV, Ho JQ, Wang VY, Vu D, Ghosh G (2006) The 20s Proteasome Processes Nf-Kappab1 P105 into P50 in a Translation-Independent Manner. EMBO J 25:9: 1945–1956. http://dx.doi.org/10.1038/sj.emboj.7601081

23. Waterfield MR, Zhang M, Norman LP, Sun SC (2003) "Nf-Kappab1/P105 Regulates Lipopolysaccharide-Stimulated Map Kinase Signaling by Governing the Stability and Function of the Tpl2 Kinase. " Mol Cell 11:3: 685–694. http://dx.doi.org/10.1016/s1097-2765(03)00070-4
24. Tan X, Sun X, Gonzalez-Crussi FX, Gonzalez-Crussi F, Hsueh W (1994) "Paf and Tnf Increase the Precursor of Nf-Kappa B P50 Mrna in Mouse Intestine: Quantitative Analysis by Competitive Pcr". Biochim Biophys Acta 1215(1–2):157–162. http://dx.doi.org/10.1016/0005-2760(94)90105-8
25. Zheng C, Yin Q, Wu H (2011) "Structural Studies of Nf-Kappab Signaling. " Cell Res 21:1: 183–195. http://dx.doi.org/10.1038/cr.2010.171
26. Lin G, Li C, Huang C, Zhuang W, Huang Y, Xu H, Miao Q, Hu D, 2018 "Co-Expression of Nf-Kappab-P65 and Phosphorylated Nf-Kappab-P105 Is Associated with Poor Prognosis in Surgically Resectable Non-Small Cell Lung Cancer." J Cell Mol Med 22, 3: 1923–30. http://dx.doi.org/10.1111/jcmm.13476
27. Cartwright T, Perkins ND, Wilson LC (2016) "Nfkb1: A Suppressor of Inflammation, Ageing and Cancer". FEBS J 283:10: 1812–1822. http://dx.doi.org/10.1111/febs.13627
28. Ma B, Hottiger MO (2016) "Crosstalk between Wnt/Beta-Catenin and Nf-Kappab Signaling Pathway During Inflammation. " Front Immunol 7:378. http://dx.doi.org/10.3389/fimmu.2016.00378
29. Cooper LC, Prinsloo E, Edkins AL, Blatch GL (2011))"Hsp90α/B Associates with the Gsk3β/Axin1/Phospho-B-Catenin Complex in the Human Mcf-7 Epithelial Breast Cancer Model. Biochem Biophys Res Commun 413:4: 550–554. http://dx.doi.org/10.1016/j.bbrc.2011.08.136
30. Cho HH, Song JS, Yu JM, Yu SS, Choi SJ, Kim DH, Jung JS, (2008), "Differential Effect of Nf-Kappab Activity on Beta-Catenin/Tcf Pathway in Various Cancer Cells." FEBS Lett 582, no. 5: 616 – 22. http://dx.doi.org/10.1016/j.febslet.2008.01.029
31. Schmitt AM, Chang HY (2016) "Long Noncoding Rnas in Cancer Pathways. " Cancer Cell 29:4: 452–463. http://dx.doi.org/10.1016/j.ccell.2016.03.010
32. Singh D, Khan MA, Siddique HR, 2020"Emerging Role of Long Non-Coding Rnas in Cancer Chemoresistance: Unravelling the Multifaceted Role and Prospective Therapeutic Targeting." Mol Biol Rep 47, 7: 5569–85. http://dx.doi.org/10.1007/s11033-020-05609-x
33. Guo Q, Lv S, Wang B, Li Y, Cha N, Zhao R, Bao W, Jia B, 2019 "Long Non-Coding Rna Prncr1 Has an Oncogenic Role in Breast Cancer." Exp Ther Med 18, 6: 4547–54. http://dx.doi.org/10.3892/etm.2019.8152
34. Deng J, Miller SA,. Wang HY, Xia W, Wen Y, Zhou BP,. Li Y, Lin SY, Hung MC (2002) "Beta-Catenin Interacts with and Inhibits Nf-Kappa B in Human Colon and Breast Cancer. " Cancer Cell 2:4: 323–334. http://dx.doi.org/10.1016/s1535-6108(02)00154-x
35. Tang F, Xu Y, Wang H, Bian E, Zhao B, "Lncrna-Atb in Cancers: What Do We Know So Far?" 2020 Mol Biol Rep 47, 5: 4077–86. http://dx.doi.org/10.1007/s11033-020-05415-5

Figures

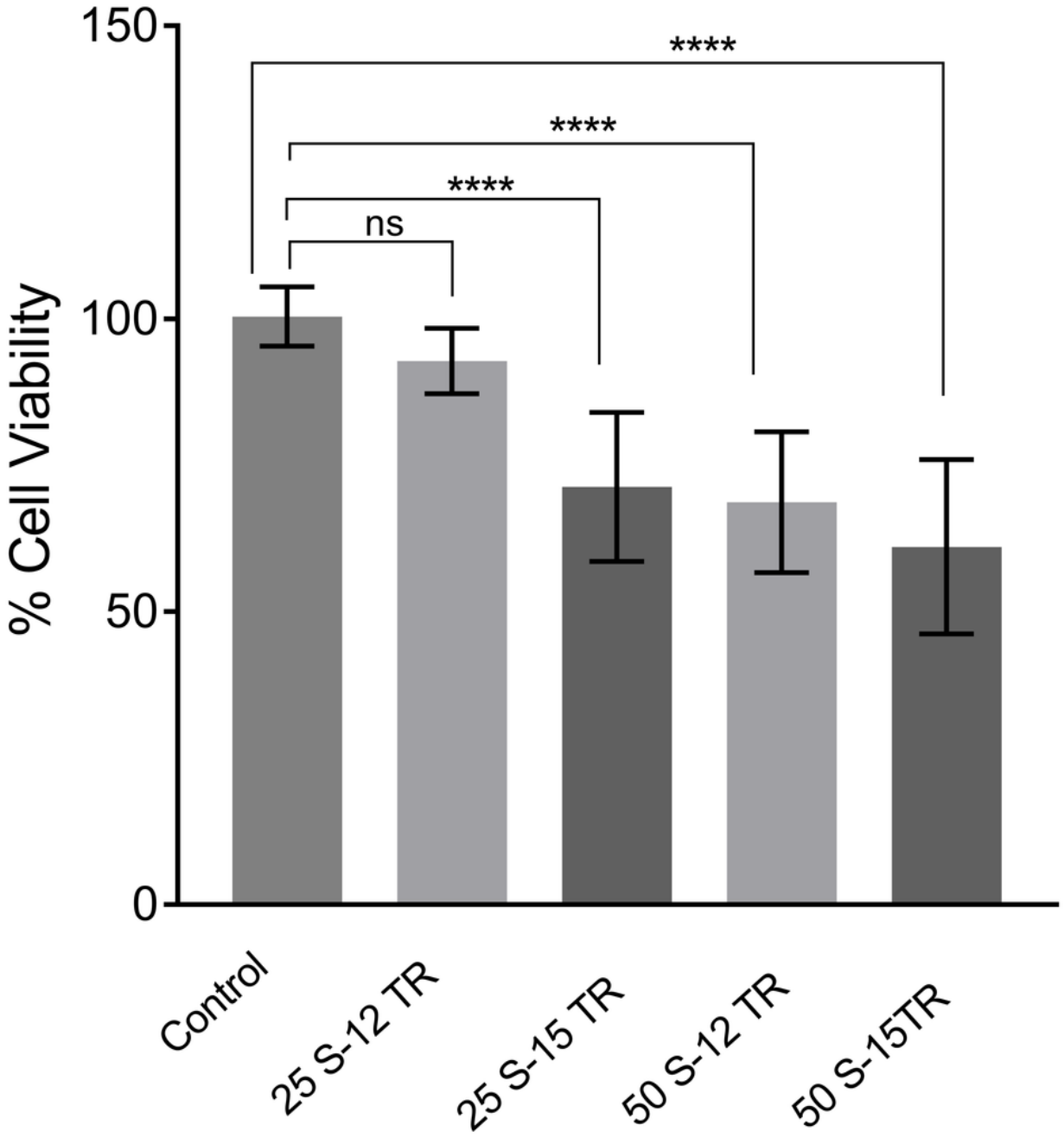

Figure 1

Determination of siRNA transfection effect on MCF-7 cell viability [S=siRNA (nM), TR=Transfection Reagent (µl/ml)]. Data represented mean ± S.D. Stars indicate significance level ($p<0.0001$), ns=not significant. 12 TR= 12µl Transfection Reagent, 15 TR= 15 µl transfection reagent, 25 S= 25 nM siRNA, 50 S= 50 nM siRNA.

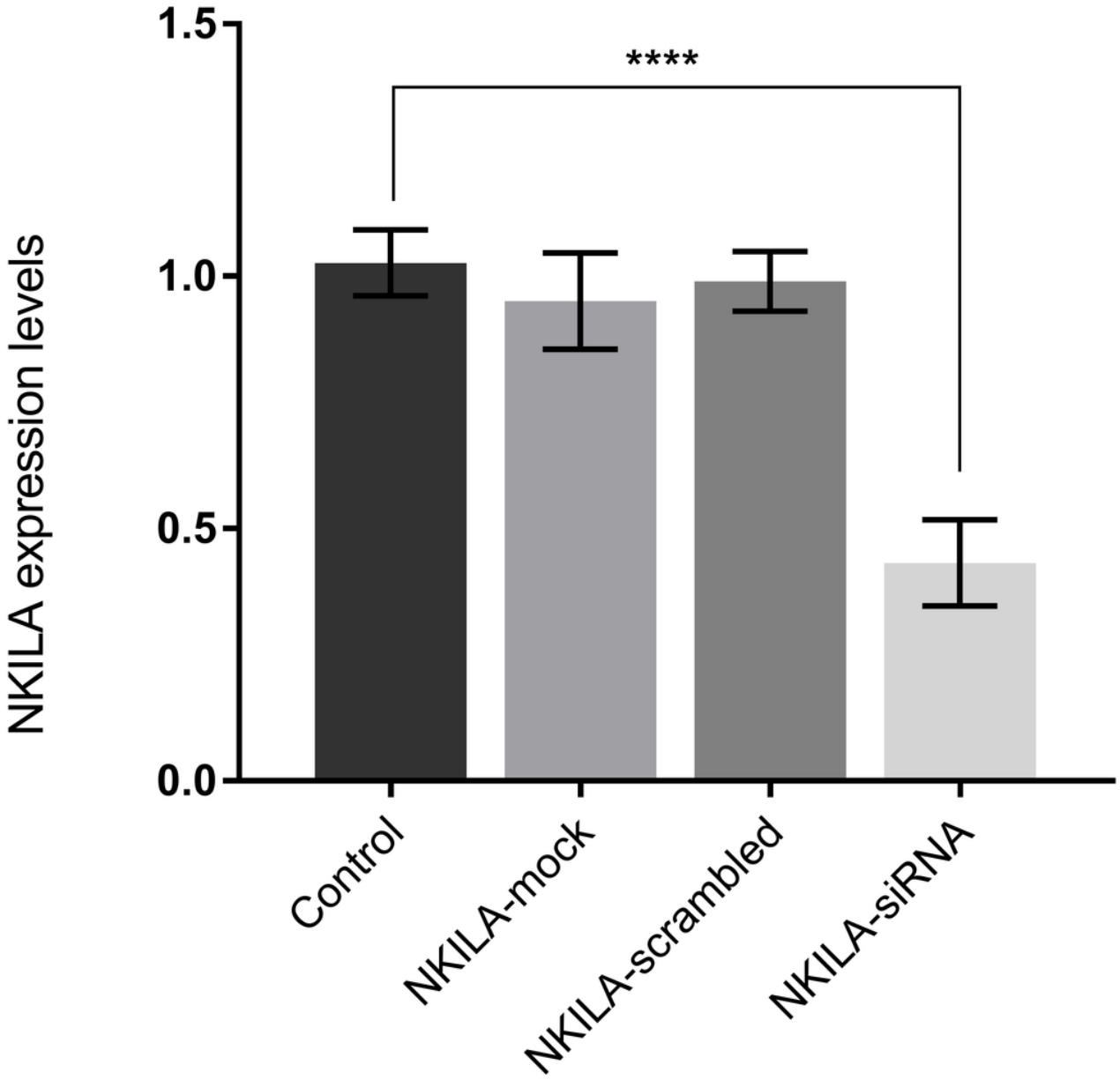

Figure 2

NKILA knockdown efficiency of the NKILA-siRNA, Mock (no-siRNA) and Scrambled (non-target siRNA as negative control) groups in the MCF-7 cells, ($p<0.0001$). After siRNA transfection, NKILA expression levels were decreased to 33% of the control. Mock and scrambled groups showed no significant difference in comparing to the control group as expected. Stars indicates significancy degree ($p<0.0001$).

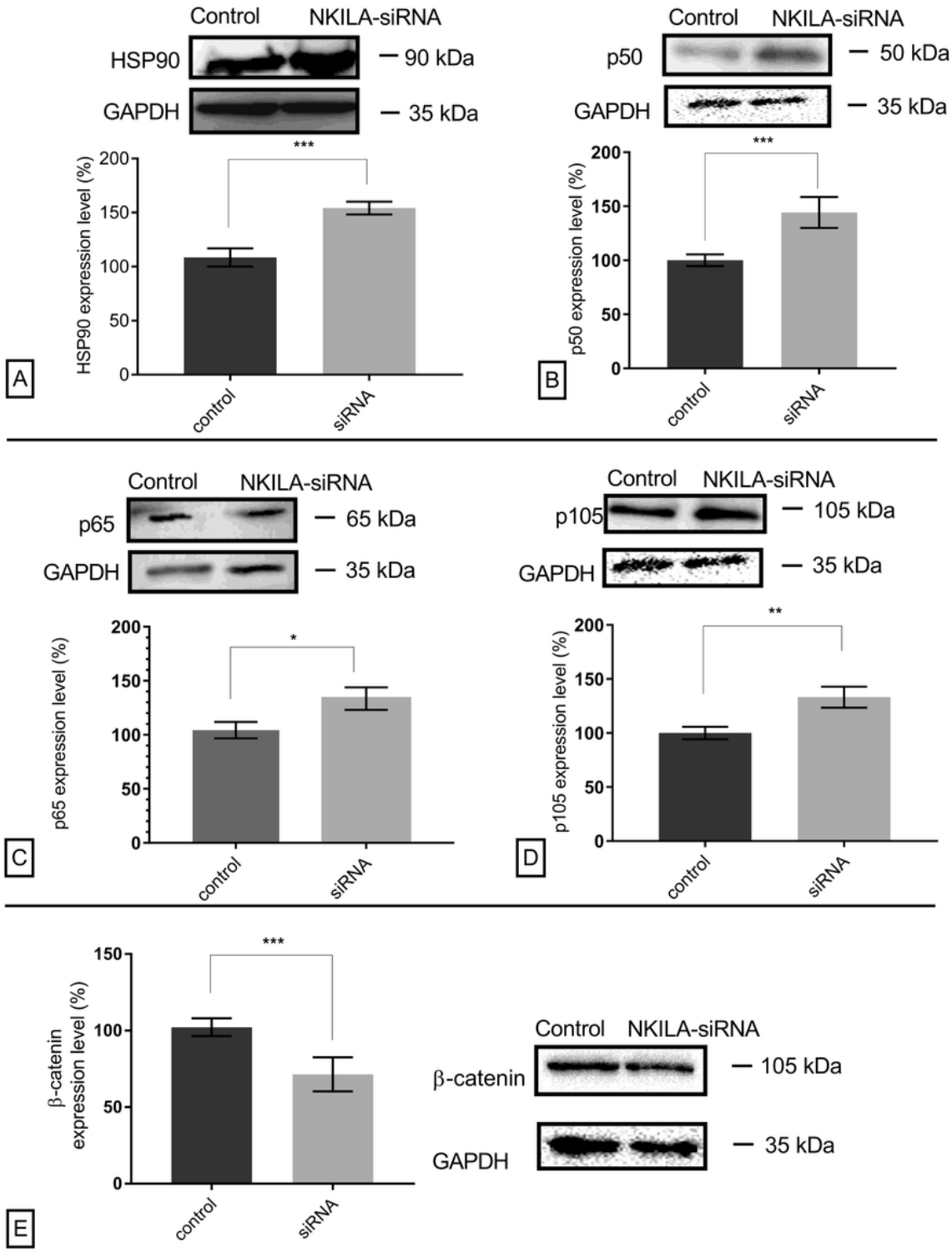

Figure 3

A) Relative expression levels of Hsp90 protein in NKILA-siRNA group of MCF-7 cell. B) Relative expression levels of NF-κB/p50 protein in NKILA-siRNA group of MCF-7 cells and band intensities of the groups. C) Relative expression levels of NF-κB/p65 protein in NKILA-siRNA group of MCF-7 cells and band intensities of the groups. D) Relative expression levels of NF-κB/p105 protein in NKILA-siRNA group of MCF-7 cells and band intensities of the groups. E) Relative expression levels of β-catenin protein in NKILA-siRNA

group of MCF-7 cell and band intensities of the groups. Stars indicate significancy. P values <0.0001 (****), <0.001 (***), <0.01 (*) levels were accepted as significantly different.

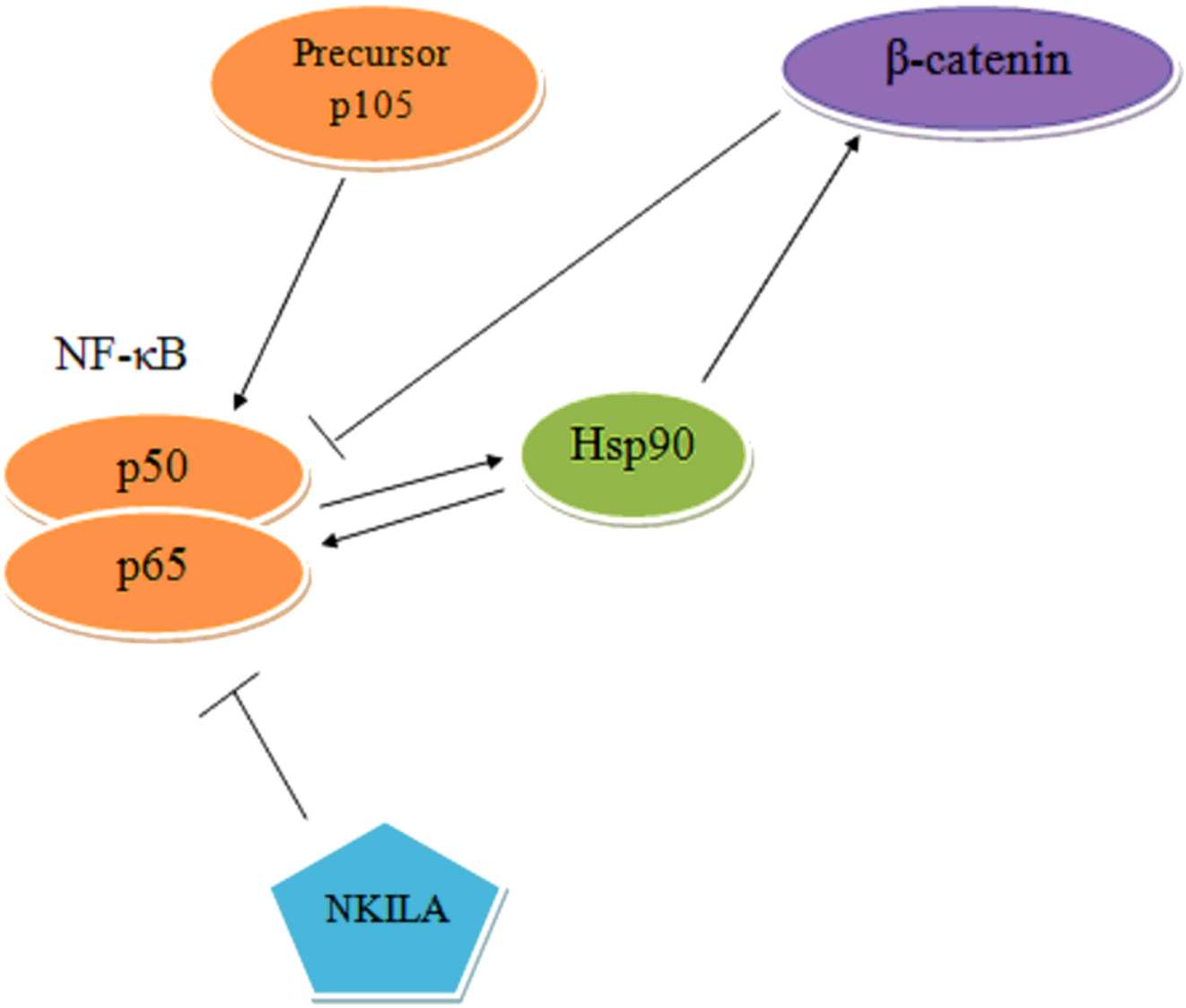

Figure 4

Possible association between NKILA, HSP90α and NF-κB subunits. Our findings suggest the literature which indicates negative association between β-catenin and NF-κB due to cross-talks between their relevant signaling pathways. Plus, as a stress protein HSP90 might involve this association as response to NF-κB emerging after NKILA inhibition which is natural NF-κB suppressor. Moreover, HSP90 was shown to positively regulate by NF-κB in various signaling pathways or by directly binding to HSP90 promoter in literature. We suggest that NKILA inhibition is indirectly upregulates HSP90 in MCF-7 cells.