Original Article

Postexercise downregulation of *NUP155* in regulating non-small cell lung cancer progression via the PTEN/AKT signaling pathway

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Background: Research interest into regulation of gene expression by physical activity and its effect on cancer prognosis has intensified. This study investigated the role of an exercise-related gene, *NUP155*, in the progression of non-small cell lung cancer (NSCLC) and its potential as therapy target.

Methods: Using the GSE41914 dataset, which includes data related to exercise, and the Cancer Genome Atlas (TCGA)-NSCLC dataset, we identified differentially expressed genes (DEGs) and selected *NUP155* as a hub gene for further analysis. *NUP155* expression levels were measured in NSCLC cell lines and normal lung cells using *in vitro* assays. The functional roles of *NUP155* were investigated through small interfering RNA (siRNA) knockdown experiments, assessing effects on migration, cell proliferation, invasion, and apoptosis. The involvement of the PTEN/AKT signaling pathway was examined using the PTEN inhibitor SF1670.

Results: *NUP155* was downregulated in postexercise samples and upregulated in NSCLC samples, indicating its association with poor prognosis in NSCLC. Knockdown of *NUP155* in NSCLC cell lines resulted in reduced cell viability, migration, and invasion, alongside increased apoptosis. Western blotting revealed that *NUP155* knockdown upregulated PTEN levels and downregulated phosphorylated AKT (p-AKT), without altering total AKT levels. The addition of SF1670 partially reversed the effects of *NUP155* knockdown, indicating the involvement of the signaling pathway PTEN/AKT in *NUP155*-mediated tumorigenesis.

Conclusions: *NUP155* is upregulated in NSCLC, which promotes cell invasion and migration via the PTEN/AKT signaling pathway. Targeting *NUP155*, potentially influenced by exercise, could be a promising therapy. Combining exercise with targeted treatments may enhance patient outcomes.

Keywords: Exercise; non-small cell lung cancer (NSCLC); *NUP155*; PTEN/AKT signaling pathway; tumor progression

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Introduction

Lung cancer (LC) continues to be the leading cause of cancer-related death worldwide, with non-small cell lung cancer (NSCLC) being particularly linked to well-established risk factors such as smoking, radon gas exposure, asbestos exposure, environmental contaminant exposure, and genetic predispositions (1,2). Despite advancements in treatment options such as surgery, chemotherapy, radiotherapy, and targeted therapies, the prognosis of many patients with LC remains poor, highlighting the need for novel diagnostic biomarkers, therapeutic strategies, and prognostic indicators to improve patient outcomes (3). Recent studies have begun to explore the potential relationship between physical activity and the progression of NSCLC, suggesting that exercise may play a crucial role in disease management (4,5). Research indicates that physical activity may enhance overall survival (OS) rates and quality of life for patients with LC by improving immune function, reducing systemic inflammation, and enhancing physical fitness (6). These results imply that exercise may also reduce the risk of cancer recurrence and enhance tolerance to treatments such as chemotherapy and radiation therapy. Additionally, physical activity can mitigate treatment-related side effects, thereby improving patients' functional capacity and mental health (7). Despite these promising outcomes, the exact mechanisms by which exercise influences LC progression are not yet fully understood. Ongoing studies are investigating various pathways and biological processes that are affected by exercise, including immune response modulation, hormonal changes, and alterations in the tumor microenvironment (8,9). Among these pathways, the PI3K/AKT signaling pathway, regulated by the PTEN tumor suppressor, is essential to cell survival and proliferation, making it a key area of interest in cancer research (10). Given the significant burden and high mortality rate of LC, exploring exercise as an adjunct therapy may hold considerable clinical relevance.

NUP155 encodes a key protein component of the nuclear pore complex (NPC), which is necessary for regulating the movement of molecules from the cytoplasm to the nucleus (11). Recent research has highlighted a possible connection between cancer and *NUP155*. Disruptions in *NUP155* expression or function can lead to aberrant nucleocytoplasmic transport, resulting in altered cell cycle regulation and genomic instability hallmarks of cancer (12). For instance, research by Forest *et al.* revealed that *NUP155* expression is significantly higher in grade 3 lung adenocarcinoma (LUAD) compared to grade 2, suggesting its involvement in disease progression and severity, as well as its potential role in chemoresistance (13). Furthermore, a pan-cancer analysis by Wang *et al.* identified *NUP155* as an important contributor to the emergence and spread of tumors (14). The study noted *NUP155* upregulation in 26 different cancer types, with associations to advanced pathological stages and poor prognosis. Microsatellite instability, stemness score, tumor mutational load, and DNA methylation correlate with *NUP155* expression, implicating it in genomic instability and cancer stem cell characteristics. Additionally, *NUP155* influences the tumor immune microenvironment (TIME), affecting immune cells that invade tumors and the genes linked to immunoregulation (15). Moreover, the TIME is involved in immunomodulatory pathways, particularly in antigen processing and presentation. In breast cancer, *NUP155*

Highlight box

Key findings

- *NUP155*, an exercise-related gene, is noticeably elevated in non-small cell lung cancer (NSCLC) and is linked to worse clinical results.
- Knockdown of *NUP155* prevents NSCLC migration, cell proliferation, and invasion while promoting apoptosis.
- *NUP155* regulates tumor cell behavior through the PTEN/AKT signaling pathway.

What is known and what is new?

- *NUP155* is recognized for its association with the nuclear pore complex and has been identified as upregulated in multiple cancer types, correlating with a poor prognosis.
- Our study found that NUP155 is downregulated after exercise and upregulated in NSCLC, and it affects tumor cell behavior through the PTEN/AKT signaling pathway. The research suggests that combining exercise with targeted NUP155 therapy may be a promising treatment approach.

What is the implication, and what should change now?

- The identification of *NUP155* as a key factor in NSCLC progression opens up new avenues for therapeutic intervention, particularly that targeting the PTEN/AKT signaling pathway.
- Integrating lifestyle modifications, such as regular exercise, with molecular-targeted therapies could provide a comprehensive approach to improving patient outcomes in NSCLC.
- Future research should focus on the mechanisms by which exercise modulates *NUP155* expression and its clinical applications in cancer therapy.

promotes cell proliferation, migration, and resistance to apoptosis. Savci-Heijink *et al.* identified 15 signature genes, including *NUP155*, that were predictive of bone metastasis in breast carcinomas (16). This signature significantly correlated with metastatic behavior and survival outcomes, emphasizing the role of *NUP155* in the metastatic process and highlighting its potential as a therapeutic target and prognostic indicator for different types of cancer.

The interplay between physical activity and cancer biology has garnered increasing attention, with a study suggesting that exercise can influence gene expression and tumor progression (17). *NUP155*, a nucleoporin gene, has been implicated in various malignancies, including its association with LUAD (13,14). However, the relationship between exercise-related modulation of *NUP155* expression and its potential impact on cancer prognosis remains unexplored. The objective of this study was thus to examine the expression levels of *NUP155* in NSCLC and assess its role in the PTEN/AKT signaling pathway, particularly in the context of physical activity. By elucidating the mechanisms through which *NUP155* affects NSCLC cell behavior, we aimed to gain a novel understanding of the molecular causes of cancer progression and explore the potential therapeutic strategies that integrate lifestyle interventions, such as exercise, for improved cancer management. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1619/rc).

Methods

Ethical statement

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Differential expression analysis of relevant datasets

The GSE41914 dataset was obtained from the Gene Expression Omnibus (GEO) (accession no. GSE41914; https://www.ncbi.nlm.nih.gov/geo/) and comprises 12 samples of natural killer (NK) cells collected both before and after exercise. Additionally, 1,017 NSCLC samples and 108 control normal samples were downloaded from the Clinical Bioinformatics website (https://www.aclbi.com/static/index.html#/). These two datasets were employed to determine differentially expressed genes (DEGs) via the "Limma" package in version 3.42.2 (The R Foundation for Statistical Computing). DEGs were classified based on fold change (FC) values: genes with an FC greater than 1.3 were considered upregulated, while those with an FC less than 0.77 were considered downregulated. A P value <0.05 indicated that genes were both upregulated and downregulated.

Expression and prognostic analysis of NUP155

The Venn online graph tool (https://bioinformatics.psb.ugent.be/webtools/Venn/) was used to filter upregulated DEGs and downregulated DEGs in the GSE41914 dataset and the Cancer Genome Atlas (TCGA)-NSCLC dataset. We selected *NUP155* among the overlapping genes as a hub gene for further analysis. The expression levels of *NUP155* in the GSE41914 dataset and the TCGA-NSCLC dataset were analyzed via the Sangerbox 3.0 website (http://vip.sangerbox.com/home.html). Subsequently, the cohorts in the GSE41914 dataset and the TCGA-NSCLC dataset were divided into two risk categories (low risk and high risk) according to the expression patterns of the relevant genes. The Wilcoxon test was used to assess the variations in *NUP155* expression levels across the groups. The Kaplan-Meier (KM) analysis was employed to determine the OS and first progression probabilities (FPPs) of the two risk groups. In addition, the statistical significance of the survival difference between the two groups was assessed using the log-rank test. The hazard ratio (HR) of the high-risk group was also calculated to further clarify the relative risk.

Cell lines and culture

The National Collection of Authenticated Cell Cultures (Shanghai, China) provided human normal lung cells (BEAS-2B; cat. No. SCSP-5067) and NSCLC cells (A549, cat. No. SCSP-503; H1299, cat. No., SCSP-589; H1975, cat. No. SCSP-597), while the BeNa Culture Collection (Beijing, China) provided another NSCLC cell line, H157 (cat. No. BNCC359887). Their diet consisted of Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 2 mM of L-glutamine added as supplements. Cell cultures were maintained in an enhanced humidity environment with 5% CO_2 at 37 ℃. Mycoplasma contamination testing was conducted regularly, with negative results confirmed before each experimental setup. Cell lines were used at passages below 20 to minimize the risk of phenotypic drift, while primary cultures were used within the first 5 passages to preserve their native cellular characteristics. No genetic

modifications were introduced to the primary cultures during the experiments.

Cell treatment and transfection

SF1670 is a small molecule inhibitor specifically designed to inhibit PTEN activity (18). NSCLC cells received treatment of 10 μM of SF1670 for 24 hours. For transient transfection, NSCLC cells were transfected with two distinct small interfering RNAs (siRNAs) that target *NUP155* to attain knockdown of *NUP155* expression, and cells were cultured for a certain amount of time to effectively knockdown *NUP155*. Cells were transfected using Lipofectamine 3000 (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions.

Quantitative real-time polymerase chain reaction (qRT-PCR)

According to a previous study, qRT-PCR was performed (19). The data were examined using the $2^{-\Delta\Delta CT}$ approach, with *GAPDH* abundance serving as the standard. The amplification procedure made use of the primer sequences listed below: *NUP155* forward, 5'-CTTAGTGTCTACCTGGCTGCTTGG-3'; and *NUP155* reverse, 5'-TGATGCTGATGCTGATGCTTCTGG-3'. Similarly, the forward and reverse primers for *GAPDH*, which was used as the reference gene, were as follows: *GAPDH* forward, 5'-ATTCCACCCATGGCAAATT-3'; and *GAPDH* reverse, 5'-TGGGATTTCCATTGATGACAAG-3'.

Western blotting

Protease and phosphatase inhibitors (CoWin Biosciences, Nanjing, China) were added to a RIPA lysis buffer (Solarbio, Beijing, China) to facilitate the preparation of protein lysates from NSCLC cells. The protein content was determined using the BCA Protein Assay Kit (Beyotime, Shanghai, China). Proteins in equal quantities were divided using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then placed onto polyvinylidene fluoride membranes (Beyotime). The membranes were blocked with 5% skim milk and incubated with the following primary antibodies (each at a 1:1,000 dilution): NUP155 (cat. No. ab199528; Abcam, Cambridge, UK), Bax (cat. No. ab32503; Abcam), Bcl-2 (cat. No. ab32124; Abcam), caspase-9 (cat. No. ab32539, Abcam), PTEN (cat. No. ab267787; Abcam), p-AKT (cat. No. ab8933; Abcam), and AKT (cat. No. ab192623; Abcam). After a wash, the membranes were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin G (IgG) secondary antibody (cat. No. ab6721; Abcam) at a 1:5,000 dilution for 1 hour at room temperature. GAPDH (1:5,000; cat. No. KC-5G5; Kangcheng, Shanghai, China) was included for internal use only. An enhanced chemiluminescence (ECL) kit (Tiangen, Beijing, China) was used to visualize the protein bands, and ChemiDoc imaging equipment (Bio-Rad, Hercules, CA, USA) was used to obtain the images.

Cell Counting Kit 8 (CCK8) assay

CCK8 (KeyGEN, Nanjing, China) was used to determine the viability of the cells. NSCLC cells were planted in 96-well plates at a concentration of 5×10^3 per well. After a CCK8 reagent was added to each well after treatment, a microplate reader (Kehua Technologies, Inc., Shanghai, China) was used to measure the absorbance at 450 nanometers after 0, 24, 48, 72, 96, and 120 hours.

Flow cytometry

NSCLC cells were separated using 0.25% trypsin-EDTA (Life Technologies Inc., Carlsbad, CA, USA) and then cleaned with phosphate-buffered saline (PBS) in preparation for flow cytometry. To distinguish viable, apoptotic, and necrotic cells, the annexin V and propidium iodide (PI) staining procedures were completed in line with the manufacturer's instructions. A flow cytometer (Jiyuan Biotechnology Co., Ltd., Guangzhou, China) was used for the flow cytometry, and FlowJo software (FlowJo, BD Biosciences, Franklin Lakes, NJ, USA) was used for data analysis to calculate the cell apoptosis rate.

Cell invasion and migration assays

Transwell assay was used to measure migration and invasion of cells. Transfected NSCLC cells were suspended in serum-free media within the top chamber of the Transwell (Corning, Corning, NY, USA). Subsequently, 10% FBS was included in the medium in the lower chamber of the Transwell. After an incubation period, cells with moving cell membranes were stained with DAPI and fixed with 4% paraformaldehyde. Finally, to count the number of migrating cells in the field of vision, inverted microscopy was used. The aforementioned cell invasion assays were performed with Matrigel (BD Biosciences, Shanghai, China) covering the top chamber.

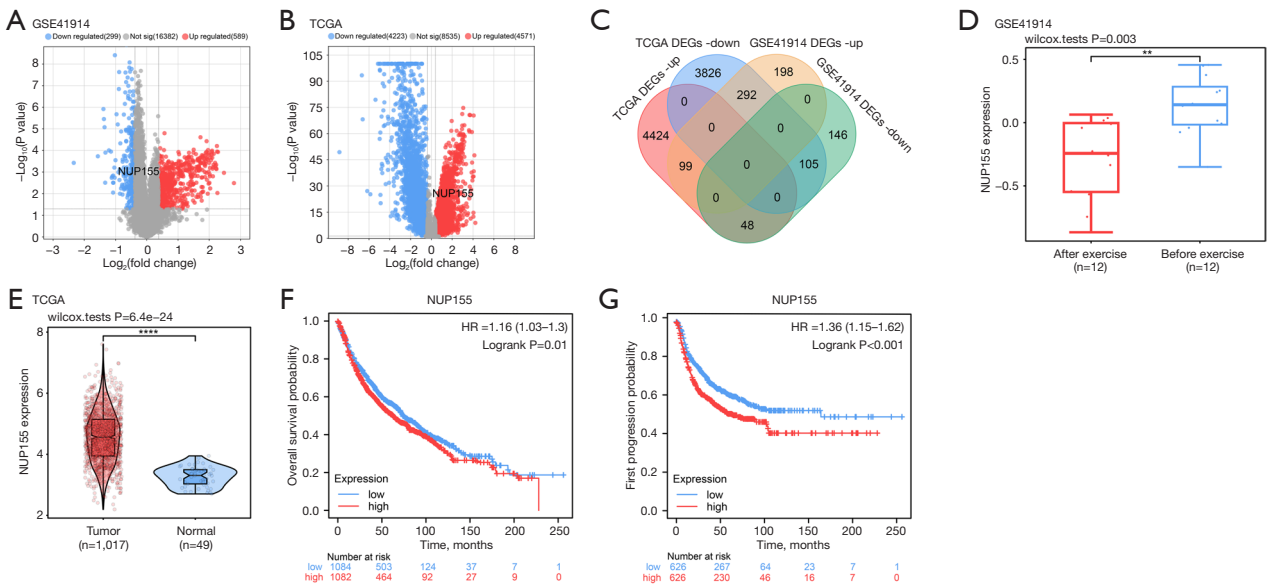

Figure 1 Differential expression and prognostic significance of *NUP155* in NSCLC-related datasets. (A,B) DEGs screening of the GSE41914 dataset and TCGA-NSCLC dataset. Blue represents the downregulated DEGs, and red represents the upregulated DEGs. (C) Cross-analysis of upregulated and downregulated DEGs in the GSE41914 and TCGA-NSCLC datasets for obtaining the overlapping genes. (D) Boxplot showing the expression level of *NUP155* in pre-exercise and postexercise samples in the GSE41914 dataset. (E) Boxplot showing the expression level of *NUP155* in NSCLC samples and normal samples in TCGA dataset. (F) Kaplan-Meier survival curve showing the patients' overall chance of survival from NSCLC. The red line represents high *NUP155* expression, and the blue line represents low *NUP155* expression. (G) Kaplan-Meier survival curve of first progression probability of patients with NSCLC. The red line represents high *NUP155* expression, and the blue line represents low *NUP155* expression. **, P<0.01; ****, P<0.0001. NSCLC, non-small cell lung cancer; DEG, differentially expressed gene; TCGA, The Cancer Genome Atlas.

Statistical analysis

R was used to carry out statistical analyses. Each experiment was performed with three technical replicates, and the mean ± standard deviation (SD) of the findings is given. In order to assess the importance of the variations, one-way analysis of variance was used, and the Tukey test was used for post hoc analysis. P<0.05 was established as the threshold for statistical significance.

Results

A high expression of exercise-related gene NUP155 in NSCLC was associated with poor prognosis

A total of 589 upregulated DEGs and 299 downregulated DEGs were identified between pre-exercise and postexercise samples from the GSE41914 dataset, alongside 4,571 upregulated DEGs and 4,223 downregulated DEGs between the TCGA-NSCLC dataset's tumor and normal samples (*Figure 1A,1B*). Additionally, 204 overlapping genes were identified from the upregulated and downregulated DEGs of the GSE41914 and TCGA-NSCLC datasets (*Figure 1C*). Notably, *NUP155* was selected as a hub gene for further analysis. In the GSE41914 dataset, *NUP155* expression levels were noticeably lower in postexercise samples than in pre-exercise samples, suggesting that this gene may be regulated by physical activity (*Figure 1D*). Furthermore, in TCGA dataset, *NUP155* expression was noticeably elevated in NSCLC tissues in contrast to normal tissues (*Figure 1E*). High *NUP155* expression was linked to poor OS and FPP in patients with NSCLC according to the KM survival analysis (*Figure 1F,1G*). These findings imply that *NUP155* could function as an NSCLC prognostic biomarker and that its elevated expression is associated with poor clinical outcomes.

NUP155 was upregulated in NSCLC cells

In NSCLC cell lines (A549, H157, H1299, and H1975), the expression of *NUP155* was assessed and compared with that

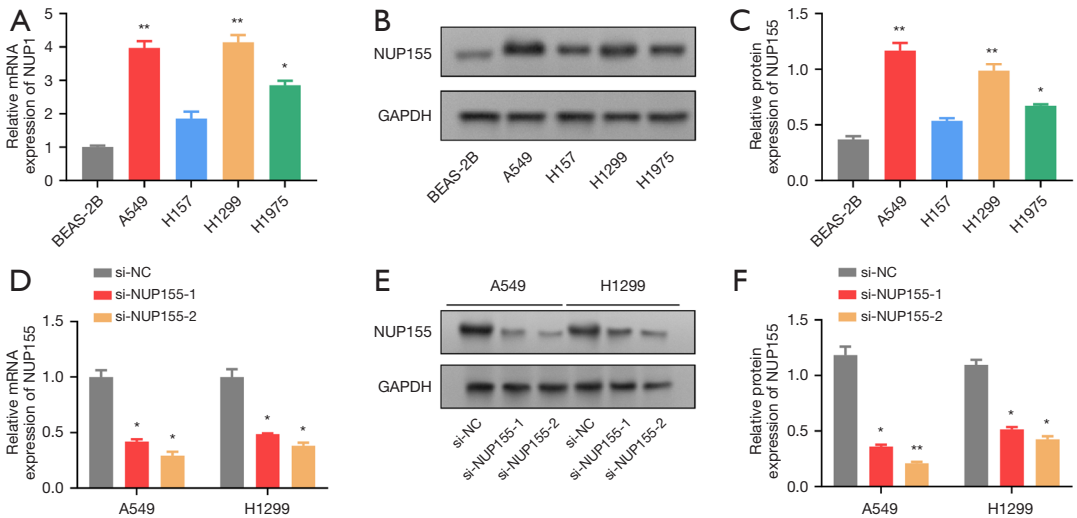

Figure 2 *NUP155* was upregulated in NSCLC cells. (A) qRT-PCR measurements of the relative mRNA expression of *NUP155* in NSCLC cell lines (A549, H157, H1299, and H1975) and normal lung cells (BEAS-2B). (B,C) WB of NUP155 protein levels in BEAS-2B and NSCLC cell lines. (D) Relative mRNA expression of *NUP155* in A549 and H1299 cells after transfection with control siRNA (si-NC) or two different *NUP155*-targeting siRNAs (si-*NUP155*-1 and si-*NUP155*-2). (E,F) WB of NUP155 protein knockdown efficiency in A549 and H1299 cells. *, P<0.05; **, P<0.01. qRT-PCR, quantitative real-time polymerase chain reaction; NSCLC, non-small cell lung cancer; WB, western blotting; si-NC, small interfering RNA negative control; siRNA, small interfering RNA.

of normal lung cells (BEAS-2B). *NUP155* messenger RNA (mRNA) and protein levels were revealed to be significantly elevated in A549, H1299, and H1975 cells, particularly in A549 and H1299, via qRT-PCR and Western blotting (*Figure 2A-2C*). Through the use of specific siRNAs (si-*NUP155*-1 and si-*NUP155*-2), *NUP155* expression was effectively knocked down in H1299 and A549 cells, as shown by the reduced amounts of mRNA and proteins (*Figure 2D-2F*). These results suggest that *NUP155* is overexpressed in NSCLC and could serve as a possible therapeutic target.

Knockdown of NUP155 inhibited the proliferation, migration, and invasion of NSCLC cells

The CCK8 assay demonstrated a significant reduction in NSCLC cell viability following *NUP155* knockdown, with si-*NUP155*-2 exhibiting a more pronounced inhibitory effect (*Figure 3A,3B*). Similarly, transwell assay indicated that *NUP155* knockdown notably decreased the invasion and migration abilities of NSCLC cells after 72 hours, with si-*NUP155*-2 showing a greater reduction (*Figure 3C-3F*). These findings suggest that *NUP155* knockdown can affect NSCLC cell phenotypes, indicating its potential as a therapeutic target for inhibiting tumor progression.

Knockdown of NUP155 promoted the apoptosis of NSCLC cells

Flow cytometry with annexin V and PI staining demonstrated that *NUP155* knockdown significantly increased the apoptotic rate of A549 and H1299 cells as compared with control siRNA (si-NC) (*Figure 4A-4C*). After treatment with si-*NUP155*-1 and si-*NUP155*-2, the percentage of dying cells in both cell lines was significantly higher, indicating effective induction of apoptosis. Analysis at the protein level further confirmed these findings, showing that after *NUP155* knockdown in A549 and H1299 cells, the expression of the proapoptotic proteins Bax and caspase-9 was elevated, whereas the expression of the anti-apoptotic protein Bcl-2 was decreased (*Figure 4D-4F*). These findings suggest that *NUP155* knockdown promotes apoptosis in NSCLC cells by regulating the expression of key apoptotic regulators, highlighting its potential as a target for NSCLC treatment intervention.

NUP155 activated the PTEN/AKT signaling pathway in NSCLC cells and influenced tumor migration and invasion

PTEN is a critical tumor suppressor that inhibits the PI3K/AKT signaling pathway by dephosphorylating PIP3 (20).

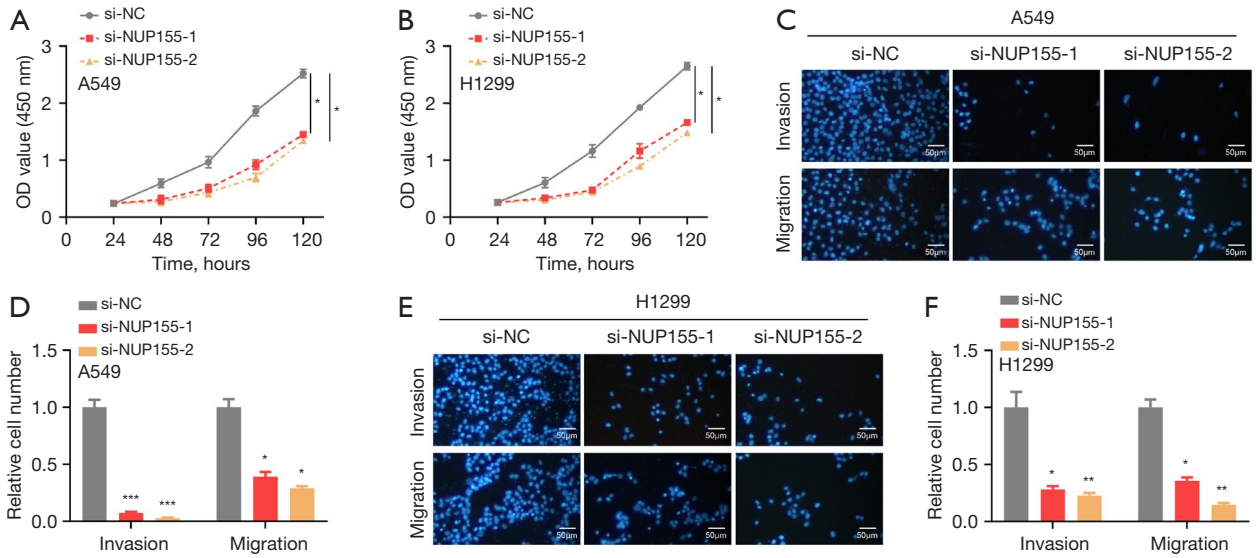

Figure 3 Knockdown of *NUP155* inhibited the proliferation, migration, and invasion of NSCLC cells. (A,B) CCK8 assay of NSCLC cells (A549 and H1299) at various time points (0, 24, 48, 72, 96, and 120 hours) following *NUP155* knockdown. (C-F) Transwell assay for detecting the changes in the invasion and migration ability of NSCLC cells (A549 and H1299) after knockdown of *NUP155*. Cells were stained using DAPI and imaged with a fluorescence microscope. Scale bar: 50 μm. *, P<0.05; **, P<0.01; ***, P<0.001. OD, optical density; NSCLC, non-small cell lung cancer; CCK8, Cell Counting Kit 8.

The phosphorylated form of AKT (p-AKT) serves as a marker that initiates this pathway (21). Western blotting revealed that the knockdown of NUP155 in NSCLC cells significantly upregulated PTEN protein levels while markedly downregulating p-AKT levels, with no significant change in total AKT levels (*Figure 5A-5C*). This suggests that *NUP155* may regulate the PTEN/AKT signaling pathway, influencing cancer cell behavior. To gain further insight into the function of PTEN in this context, we treated NSCLC cells with SF1670, a small-molecule inhibitor specifically targeting PTEN activity, for 24 hours. Transwell assays showed that the addition of SF1670 alleviated the reduced migration and invasion capabilities induced by *NUP155* knockdown (*Figure 5D-5G*). These results point to the involvement of *NUP155* in regulating the PTEN/AKT signaling pathway, with subsequent effects on NSCLC cell invasion and migration.

Discussion

Physical activity has well-documented comprehensive health benefits, including cancer prevention and management. Regular exercise has been shown to lower the risk of developing various cancers, such as breast, colorectal, and LCs (22). This protective effect is partly attributed to exercise's ability to enhance immune function, reduce systemic inflammation, and improve metabolic regulation (23). For example, exercise can lower levels of insulin and insulin-like growth factors, which are known to promote cancer cell proliferation (24). Additionally, physical activity can modulate hormone levels, decrease obesity, and improve cardiovascular health, all of which contribute to a reduced cancer risk (25). In the context of LC, physical activity has been associated with improved treatment outcomes and quality of life, suggesting a potential role in modulating disease progression (26,27). Our study adds to this body of evidence by exploring the relationship between physical activity and the expression of exercise-related genes, specifically *NUP155*, which may further clarify the underlying molecular processes underlying the benefits of exercise in cancer prevention and management.

NUP155, a nucleoporin gene, plays a crucial role in regulating the NPC and cellular transport mechanisms (28). Our study identified *NUP155* as a key exercise-related gene, exhibiting significant downregulation in post-exercise samples and upregulation in NSCLC samples, which was associated with poorer prognosis and tumor progression through enhanced proliferation, invasion, and migration in NSCLC cells. The results are consistent with findings from Holzer *et al.*, who demonstrated that *NUP155* regulates the

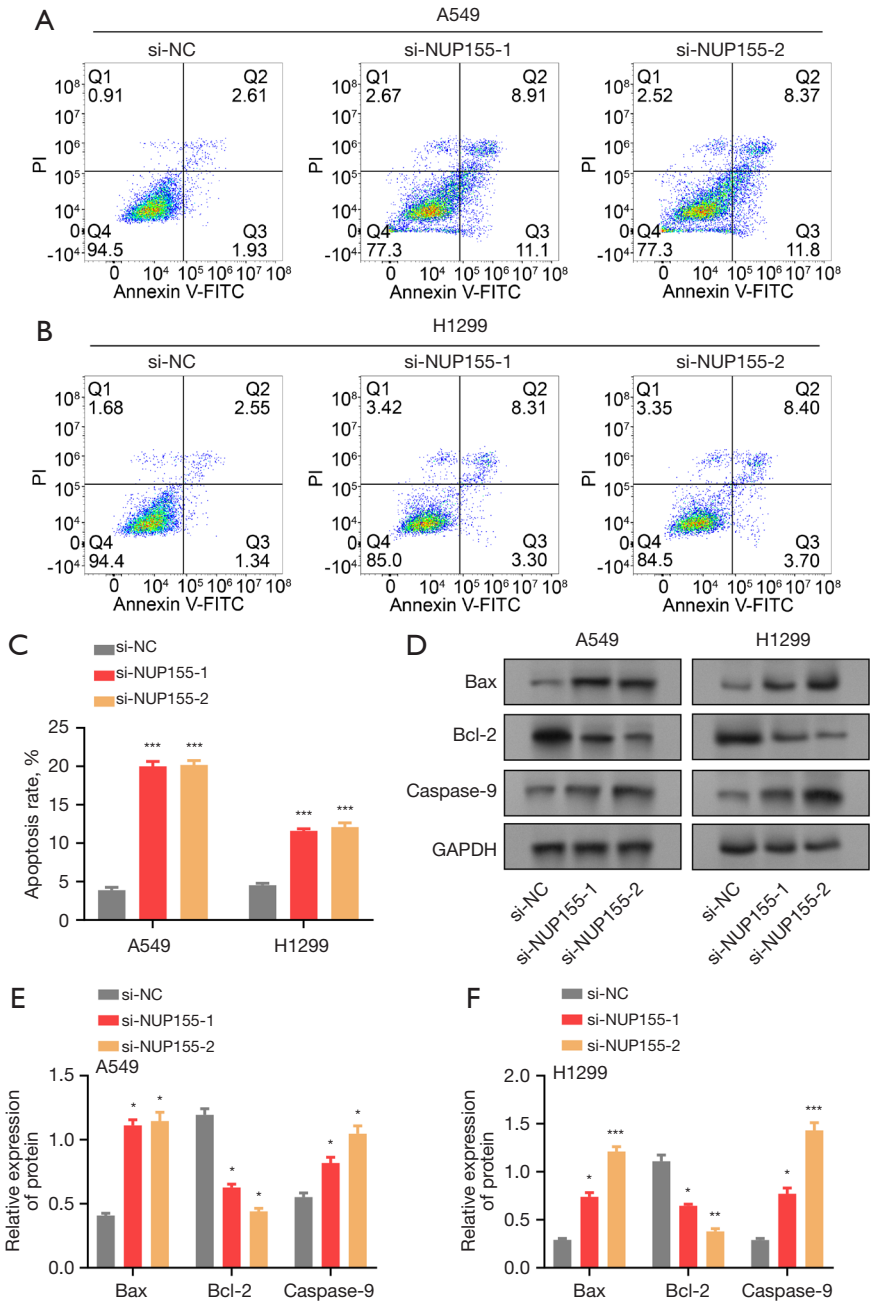

Figure 4 Knockdown of *NUP155* promoted apoptosis of NSCLC cells. (A-C) Following *NUP155* knockdown, apoptosis in NSCLC cells (A549 and H1299) was observed via flow cytometry. Quadrants represent viable cells (Q4), late apoptotic cells (Q2), early apoptotic cells (Q3), and necrotic cells (Q1). (D-F) WB was used to detect the protein expression of apoptotic proteins in NSCLC cells (A549 and H1299) after the knockdown of *NUP155*. *, P<0.05; **, P<0.01; ***, P<0.001. NSCLC, non-small cell lung cancer; WB, western blotting.

mRNA translation of *p21*, influencing cancer progression via translational control mechanisms involving *FTSJ1* and *p53* status (29). Additionally, Chi *et al.* highlighted the function of *NUP155* in cell proliferation and survival through RNA export and interaction with other nucleoporins, which is linked to a poor prognosis and an advanced stage of the illness (30). Our study further showed that knockdown of *NUP155* significantly increased apoptosis in NSCLC cells,

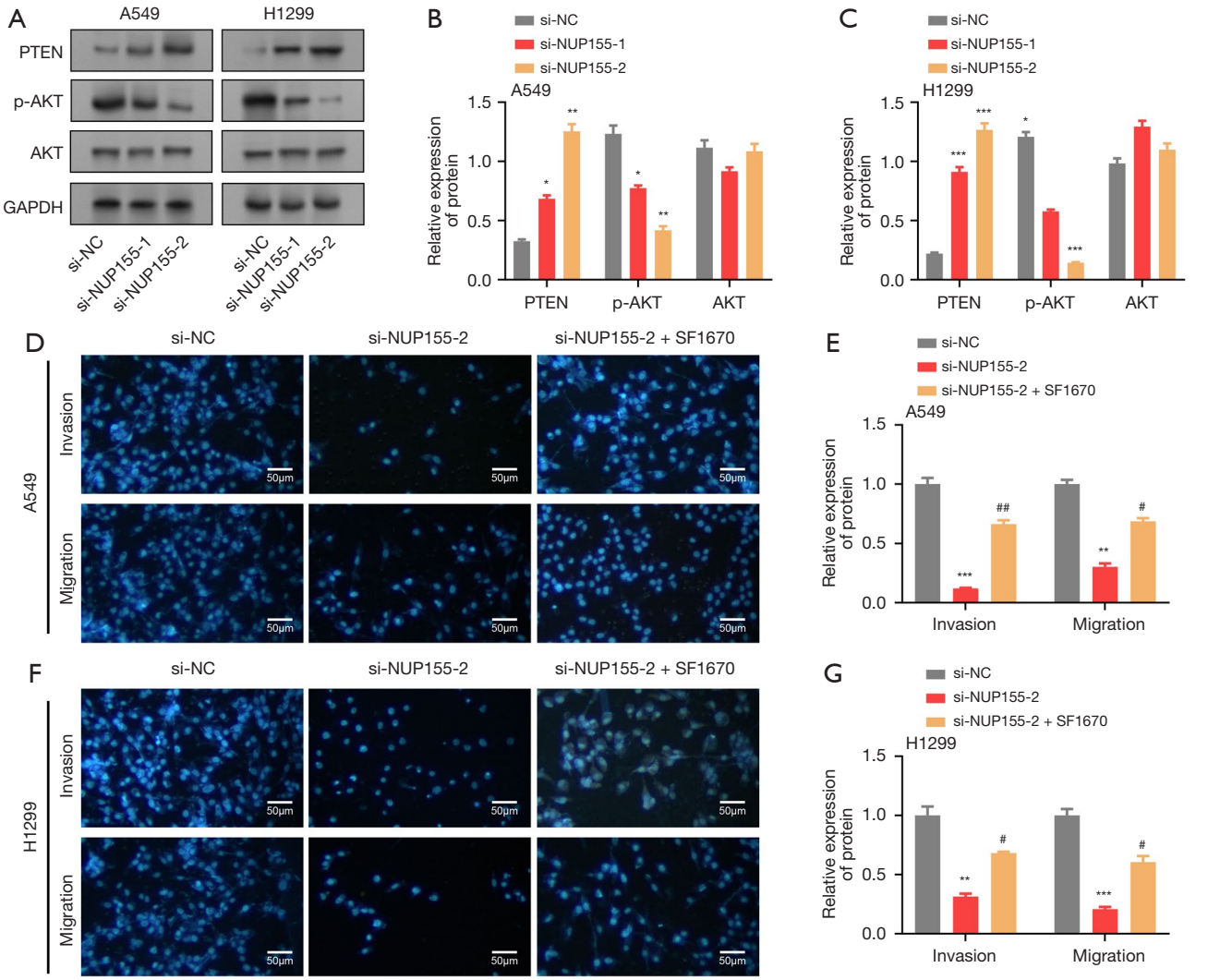

Figure 5 *NUP155* activated the PTEN/AKT signaling pathway in NSCLC cells and influenced tumor migration and invasion. (A-C) WB detection of PTEN, p-AKT, and AKT protein levels in NSCLC cells after knockdown of *NUP155*. *, P<0.05; **, P<0.01; ***, P<0.001. (D-G) Transwell detection of the invasion and migration ability of NSCLC cells after knockdown of *NUP155* and addition of PTEN inhibitor SF1670. Cells were stained using DAPI and imaged with a fluorescence microscope. Scale bar: 50 μm. **, P<0.01; ***, P<0.001 *vs.* si-NC. $^{\#}$, P<0.05; $^{\#\#}$, P<0.01 *vs.* si-*NUP155*-2. WB, western blotting; NSCLC, non-small cell lung cancer; si-NC, small interfering RNA negative control.

as seen by the downregulation of the antiapoptotic protein Bcl-2 and the overexpression of the proapoptotic proteins Bax and caspase-9. Apoptosis, which is essential for tissue homeostasis, involves key regulators such as Bax and Bcl-2, which govern mitochondrial membrane permeability and caspase activation (31). In NSCLC, apoptosis is the key to effective treatment. Zuo *et al.* found that IFN-α and celecoxib synergistically enhanced TRAIL-induced apoptosis, and Zu *et al.* found that miR-146a functions to regulate apoptosis and cell proliferation (32,33). Similarly, Zuo *et al.* reported that bamboo saponins IV and V induced apoptosis through a p53-mediated pathway (34). Our results indicate that *NUP155* figures prominently in NSCLC progression and apoptosis, highlighting its potential use as a target for therapy in the context of physical activity and cancer management.

The PTEN signaling pathway is crucial in regulating cell growth, survival, and proliferation, and serves as a

suppressor of tumors by inhibiting the PI3K/AKT signaling pathway (35,36). In cancers, including NSCLC, mutation or loss of PTEN function leads to uncontrolled cell growth and tumor development (37,38). PTEN inhibits AKT activation by dephosphorylating PIP3 to PIP2, thereby preventing downstream signaling that promotes cell survival and proliferation (39). Consequently, PTEN dysfunction is often associated with poor prognosis and resistance to therapies. For example, Zou *et al.* found that *KBTBD7* promotes the ubiquitin-dependent degradation of PTEN, resulting in the engagement of the EGFR/PI3K/AKT pathway and promotion of NSCLC cell proliferation and invasion (40). In a similar vein, Zhu *et al.* demonstrated that *USP52* stabilizes *PTEN*, downregulates *cyclin D1*, and inhibits the AKT/mTOR pathway, thereby suppressing cancer cell proliferation (41). In the study by Zhu *et al.*, miR-205 was significantly upregulated in LC tissues and decreased *PTEN* expression and activated the PI3K/AKT pathway, thus promoting cell proliferation, migration, and invasion (42). In our study, we found that knocking down *NUP155* in NSCLC cells significantly reduced p-AKT levels, elevated PTEN protein levels, and left AKT levels unchanged. Transwell assays showed that the PTEN inhibitor SF1670 mitigated the reduction in cell migration and invasion caused by *NUP155* knockdown, indicating that *NUP155* may modulate tumor migration and invasion via the PTEN/AKT signaling pathway. These findings highlight the importance of targeting the PTEN/AKT signaling pathway as a form of cancer therapy, particularly in terms of restoring normal cell function and inhibiting tumor progression.

NUP155 is upregulated in NSCLC and is associated with enhanced tumor proliferation, migration, and invasion. The downregulation of *NUP155* after exercise may have significant implications for the progression of NSCLC. Firstly, the downregulation of *NUP155* can inhibit the proliferation, migration, and invasion of NSCLC cells and promote apoptosis. This finding suggests that exercise might slow down the growth and spread of tumor cells by reducing the expression levels of *NUP155*, thereby exerting an inhibitory effect on the progression of NSCLC. Secondly, *NUP155* influences cell invasion and migration through the PTEN/AKT signaling pathway. The downregulation of *NUP155* after exercise may affect this pathway, thereby inhibiting the invasive and migratory capabilities of tumor cells, which is crucial for controlling local tumor spread and distant metastasis. Lastly, as a potential therapeutic target, the downregulation of *NUP155* after exercise provides possibilities for developing new treatment strategies. Exercise, as a non-pharmacological treatment method, may enhance the effectiveness of traditional therapeutic approaches, such as chemotherapy and radiation therapy, by downregulating *NUP155*. It may even be combined with immunotherapy to improve treatment outcomes. In summary, the downregulation of *NUP155* after exercise may affect the progression of NSCLC through multiple mechanisms, including inhibiting tumor cell proliferation and invasion, and serving as a potential therapeutic target. These findings provide new perspectives and potential treatment strategies for the treatment of NSCLC.

In our study, we utilized bioinformatics analysis to assess the expression levels of *NUP155* both before and after exercise, and we also compared these levels between NSCLC samples and normal tissue samples. When analyzing samples before and after exercise, we used NK cell data, however, this has certain limitations. Due to different cellular mechanisms, complexity of tumor microenvironment, and lack of direct cellular response data, the response of NK cells to motility does not fully represent the direct impact on NSCLC. The interaction between NK cells and NSCLC cells is complex, and changes in NK cell function may not be a direct indicator of NSCLC cells' response to movement. Our *in vitro* experiments focused on the functional role of *NUP155* in NSCLC, rather than the direct post-exercise effects, due to current experimental constraints. To overcome these limitations, future research will include direct measurement of post-exercise *NUP155* levels in NSCLC cell lines to elucidate the immediate impact of exercise on *NUP155* expression. Additionally, we will explore the effects of exercise on *NUP155* expression in preclinical models, such as xenografts, to bridge the gap between *in vitro* and *in vivo* studies and gain a more holistic view of exercise's influence on *NUP155* expression and function in a physiological setting.

Conclusions

Our study highlights the crucial role of the exercise-related gene *NUP155* in promoting NSCLC cell proliferation, migration, invasion, and apoptosis regulation. Elevated *NUP155* expression is linked to comparatively poor clinical results, suggesting its potential as a prognostic biomarker. Notably, *NUP155* appears to activate the PTEN/AKT signaling pathway, influencing tumor cell behavior. Knockdown of *NUP155* leads to increased PTEN expression and decreased p-AKT levels, resulting in

reduced cell migration and invasion. The use of the PTEN inhibitor SF1670 further confirmed the involvement of this pathway. These findings suggest that targeting *NUP155* and modulating the PTEN/AKT pathway could be a promising therapeutic strategy for NSCLC, paving the way for future research on the combination of molecular-targeted therapies and physical activity for improving patient outcomes. However, we still have an incomplete understanding as to how exercise specifically regulates *NUP155* expression and lack clinical data to support its practical applications; thus warranting further investigation.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1619/rc

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

References

1. Muhas C, Kumar P, Raja D. Etiological factors for the development of lung cancer in non-smokers: an overview. Int J Pharm Pharm Sci 2019;11:10-6.
2. Martinez VD, Sage AP, Marshall EA, et al. Oncogenetics of Lung Cancer induced by environmental carcinogens. In: Erkekoglu P, editor. Oncogenes and Carcinogenesis. IntechOpen; 2019. doi: 10.5772/intechopen.81064.
3. Yuan M, Huang LL, Chen JH, et al. The emerging treatment landscape of targeted therapy in non-small-cell lung cancer. Signal Transduct Target Ther 2019;4:61.
4. Wang YL, Zhang XF, Wang XP, et al. Combined Mindfulness-Based Stress Reduction and Exercise Intervention for Improving Psychological Well-Being in Patients With Non-Small Cell Lung Cancer. Clin Psychol Psychother 2024;31:e3023.
5. Yanagita Y, Arizono S, Tawara Y, et al. Physical activity in patients with non-small cell lung cancer after lung resection. Clin Biomech (Bristol) 2024;115:106249.
6. Filgueira TO, Castoldi A, Santos LER, et al. The Relevance of a Physical Active Lifestyle and Physical Fitness on Immune Defense: Mitigating Disease Burden, With Focus on COVID-19 Consequences. Front Immunol 2021;12:587146.
7. Schumacher O, Luo H, Taaffe DR, et al. Effects of Exercise During Radiation Therapy on Physical Function and Treatment-Related Side Effects in Men With Prostate Cancer: A Systematic Review and Meta-Analysis. Int J Radiat Oncol Biol Phys 2021;111:716-31.
8. He A, Pu Y, Jia C, et al. The Influence of Exercise on Cancer Risk, the Tumor Microenvironment and the Treatment of Cancer. Sports Med 2024;54:1371-97.
9. Sharif S, Harman N, Hydock D, et al. Exercise intervention may play a potential therapeutic role in patients with glioblastoma multiforme. World Acad Sci J 2024. doi: 10.3892/wasj.2024.256.
10. Akbarzadeh M, Mihanfar A, Akbarzadeh S, et al. Crosstalk between miRNA and PI3K/AKT/mTOR signaling pathway in cancer. Life Sci 2021;285:119984.
11. Coyne AN, Rothstein JD. Nuclear pore complexes - a doorway to neural injury in neurodegeneration. Nat Rev Neurol 2022;18:348-62.
12. Cristi AC, Rapuri S, Coyne AN. Nuclear pore

complex and nucleocytoplasmic transport disruption in neurodegeneration. FEBS Lett 2023;597:2546-66.
13. Forest F, Laville D, Da Cruz V, et al. WHO grading system for invasive pulmonary lung adenocarcinoma reveals distinct molecular signature: An analysis from the cancer genome atlas database. Exp Mol Pathol 2022;125:104756.
14. Wang ZQ, Wu ZX, Wang ZP, et al. Pan-cancer analysis of NUP155 and validation of its role in breast cancer cell proliferation, migration, and apoptosis. BMC Cancer 2024;24:353.
15. Zhu C, Du Y, Huai Q, et al. The Identification of Gamma-Glutamyl Hydrolase in Uterine Corpus Endometrial Carcinoma: a Predictive Model and Machine Learning. Reprod Sci 2024;31:532-49.
16. Savci-Heijink CD, Halfwerk H, Koster J, et al. A novel gene expression signature for bone metastasis in breast carcinomas. Breast Cancer Res Treat 2016;156:249-59.
17. Jurdana M. Physical activity and cancer risk. Actual knowledge and possible biological mechanisms. Radiol Oncol 2021;55:7-17.
18. Tang Z, Zhao P, Zhang W, et al. SALL4 activates PI3K/AKT signaling pathway through targeting PTEN, thus facilitating migration, invasion and proliferation of hepatocellular carcinoma cells. Aging (Albany NY) 2022;14:10081-92.
19. Caballero-Solares A, Hall JR, Xue X, et al. Reverse Transcription-Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR) for Gene Expression Analyses. Methods Mol Biol 2022;2508:319-40.
20. Braglia L, Zavatti M, Vinceti M, et al. Deregulated PTEN/PI3K/AKT/mTOR signaling in prostate cancer: Still a potential druggable target? Biochim Biophys Acta Mol Cell Res 2020;1867:118731.
21. Ortega MA, Fraile-Martínez O, Asúnsolo Á, et al. Signal Transduction Pathways in Breast Cancer: The Important Role of PI3K/Akt/mTOR. J Oncol 2020;2020:9258396.
22. Thomas R, Kenfield SA, Yanagisawa Y, et al. Why exercise has a crucial role in cancer prevention, risk reduction and improved outcomes. Br Med Bull 2021;139:100-19.
23. Von Ah Morano AE, Dorneles GP, Peres A, et al. The role of glucose homeostasis on immune function in response to exercise: The impact of low or higher energetic conditions. J Cell Physiol 2020;235:3169-88.
24. Kang DW, Bressel E, Kim DY. Effects of aquatic exercise on insulin-like growth factor-1, brain-derived neurotrophic factor, vascular endothelial growth factor, and cognitive function in elderly women. Exp Gerontol 2020;132:110842.
25. Rubinstein MM, Brown KA, Iyengar NM. Targeting obesity-related dysfunction in hormonally driven cancers. Br J Cancer 2021;125:495-509.
26. Li Q, Guo C, Cao B, et al. Safety and efficacy evaluation of personalized exercise prescription during chemotherapy for lung cancer patients. Thorac Cancer 2024;15:906-18.
27. Song W, Zou M, Zheng W, et al. Associations of different combinations of moderate-vigorous physical activity and muscle-strengthening activity with mortality among US lung cancer survivors. BMC Pulm Med 2024;24:326.
28. Storm E. Characterizing the Cellular Dynamics of NUP155 and a Clinically Identified Variant. University of South Dakota; 2023.
29. Holzer K, Ori A, Cooke A, et al. Nucleoporin Nup155 is part of the p53 network in liver cancer. Nat Commun 2019;10:2147.
30. Chi G, Pei JH, Li XQ. RAE1 is a prognostic biomarker and is correlated with clinicopathological characteristics of patients with hepatocellular carcinoma. BMC Bioinformatics 2022;23:252.
31. Naumova N, Šachl R. Regulation of Cell Death by Mitochondrial Transport Systems of Calcium and Bcl-2 Proteins. Membranes (Basel) 2020;10:299.
32. Zuo C, Qiu X, Liu N, et al. Interferon-α and cyclooxygenase-2 inhibitor cooperatively mediates TRAIL-induced apoptosis in hepatocellular carcinoma. Exp Cell Res 2015;333:316-26.
33. Zu Y, Yang Y, Zhu J, et al. MiR-146a suppresses hepatocellular carcinoma by downregulating TRAF6. Am J Cancer Res 2016;6:2502-13.
34. Zuo T, Zhang Z, Jiang P, et al. Characterization of chikusetsusaponin IV and V induced apoptosis in HepG2 cancer cells. Mol Biol Rep 2022;49:4247-55.
35. Glaviano A, Foo ASC, Lam HY, et al. PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. Mol Cancer 2023;22:138.
36. Chawra HS, Agarwal M, Mishra A, et al. MicroRNA-21's role in PTEN suppression and PI3K/AKT activation: Implications for cancer biology. Pathol Res Pract 2024;254:155091.
37. Xia Q, Ali S, Liu L, et al. Role of Ubiquitination in PTEN Cellular Homeostasis and Its Implications in GB Drug Resistance. Front Oncol 2020;10:1569.
38. Tran TTT, Hung JJ. PTEN decreases NR2F1 expression to inhibit ciliogenesis during EGFR(L858R)-induced lung cancer progression. Cell Death Dis 2024;15:225.
39. González-García A, Garrido A, Carrera AC. Targeting

PTEN Regulation by Post Translational Modifications. Cancers (Basel) 2022;14:5613.
40. Zou Z, Zhang B, Li Z, et al. KBTBD7 promotes non-small cell lung carcinoma progression by enhancing ubiquitin-dependent degradation of PTEN. Cancer Med 2022;11:4544-54.
41. Zhu M, Zhang H, Lu F, et al. USP52 inhibits cell proliferation by stabilizing PTEN protein in non-small cell lung cancer. Biosci Rep 2021;41:BSR20210486.
42. Zhu H, Xu Y, Li M, et al. Inhibition Sequence of miR-205 Hinders the Cell Proliferation and Migration of Lung Cancer Cells by Regulating PETN-Mediated PI3K/AKT Signal Pathway. Mol Biotechnol 2021;63:587-94.

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