Journal of Cancer Research and Clinical Oncology (2019) 145:661–673
https://doi.org/10.1007/s00432-018-2823-1

ORIGINAL ARTICLE – CANCER RESEARCH

ZWINT is the next potential target for lung cancer therapy

Fang Peng[1] · Qiang Li[2] · Shao-Qing Niu[1] · Guo-Ping Shen[1] · Ying Luo[3] · Ming Chen[4] · Yong Bao[1]

Received: 8 July 2018 / Accepted: 12 December 2018 / Published online: 14 January 2019

Abstract

Purpose We aimed to analyze the expression of ZWINT, NUSAP1, DLGAP5, and PRC1 in tumor tissues and adjacent tissues with public data.

Methods The expression patterns of four genes were detected in cancer tissues and adjacent tissues by qRT-PCR. The overall survival analysis was used to explore these genes in lung adenocarcinoma and squamous cell carcinoma patients. Knockdown assays were used to select the most suitable gene among these four genes. Cell function assays with the knockdown gene were conducted in A549 and NCL H226 cells. The role of the knockdown gene in lung cancer was dissected in a mice tumor model. Transcriptome sequencing analyses with the knockdown gene were analyzed.

Results Overexpression of these genes was significantly detected in cancer tissues ($P < 0.01$). Overall survival revealed that high expression of these genes is closely related with poor prognosis of lung adenocarcinoma patients ($P < 0.05$). Knockdown of ZWINT reduced proliferation in NCI H226 and A549 cells ($P < 0.05$). Knockdown also inhibited cell migration, invasion, apoptosis, and colony formation ($P < 0.05$). ZWINT knockdown reduced tumor volume ($P < 0.05$). Transcriptome sequencing of ZWINT knockdown-treated A549 and NCI H226 cells indicated that 100 and 426 differentially expressed genes were obtained, respectively. Gene ontology analysis suggested that binding, biological regulation, and multicellular organismal processes were the most enriched. KEGG analysis revealed that TNF, P53, and PI3K signal networks would be the most potential ZWINT-related pathways and were identified by Western blot analysis.

Conclusions ZWINT may be a novel target for lung cancer therapy.

Keywords Lung cancer · ZWINT · Differentially expressed genes · Gene ontology · KEGG

Introduction

Lung cancer is a serious threat to human life. This disease has globally become the most common malignancy (McGuire 2016). About 1.6 million lung cancer patients are newly diagnosed worldwide each year. The mortality rate of lung cancer ranks first among malignant tumor-caused deaths (Sundar et al. 2014). The histological subtypes of

Fang Peng, Qiang Li, and Shao-Qing Niu contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00432-018-2823-1) contains supplementary material, which is available to authorized users.

✉ Ming Chen
chenming@zjcc.org.cn

✉ Yong Bao
baoyong@mail.sysu.edu.cn

[1] Department of Radiation Oncology, The First Affiliated Hospital of Sun Yat-sen University, 58 Zhongshan Road II, Guangzhou 510080, Guangdong, People's Republic of China

[2] Department of Organ Transplantation and General Surgery, Second Xiangya Hospital, Central South University, Changsha 410011, Hunan, People's Republic of China

[3] Department of Clinical Laboratory, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou 510080, Guangdong, People's Republic of China

[4] Department of Radiation Oncology, Zhejiang Key Laboratory of Radiation Oncology, Zhejiang Cancer Hospital, 1 East Banshan Road, Hangzhou 310022, Zhejiang, People's Republic of China

lung cancer are mainly divided into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), which accounts for about 85% and 15% of the lung cancer patients, respectively. The 5-year survival rate of early NSCLC patients is about 40% after surgery (Ramalingam et al. 2011). However, about 70% of patients have local or distant metastases at the time of diagnosis (Scagliotti et al. 2008). Meanwhile, SCLC is more sensitive to radiotherapy and chemotherapy compared to NSCLC. To note, there are still limits for clinical treatments of SCLC. For example, SCLC is prone to drug resistance (Zarogoulidis et al. 2013). Therefore, it is necessary to perform more in-depth studies regarding clinical treatments of lung cancer.

Although the detailed mechanisms of lung cancer are still not fully understood, there are several genes that could be potentially related with the occurrence and development of lung cancer. The Zw10 binding factor (Zwint White 10 interactor, Zwint) is a protein that regulates centromere division and is encoded by the Zwint gene. Zwint co-localizes with Zw10 on centromeres. Then, the complex attaches to the chromosome between the microtubules of the spindle and spindle. Zwint is an important regulatory protein for chromosome movement and mitotic checkpoints. Previous studies have suggested Zwint over expression in breast and ovarian cancers (Woo Seo et al. 2015; Xu et al. 2016). PRC1 (Protein Regulator of Cytokinesis 1) is highly expressed during the S and G2/M phases of mitosis and involved in cytokinesis. This is a microtubule binding and bundling protein and essential to maintaining the mitotic spindle midzone (Hu et al. 2012). Genome-wide expression profile analysis suggests that PRC1 was significantly up-regulated in various cancers, including cholangiocarcinoma, colon cancer, non-small cell lung cancer, pancreatic cancer, and breast cancer (Shimo et al. 2007). Moreover, PRC1 plays an important tumor-promoting role in the progression of many malignant tumors, such as cervical cancer, prostate cancer, hepatocellular carcinoma, and breast cancer (Kikuchi et al. 2003; Lin et al. 2002; Nakamura et al. 2004; Obama et al. 2005). DLGAP5 is a microtubule-associated protein that is required for spindle formation during the cell cycle (Engeland 2018). DLGAP5 plays an important role in the carcinogenesis of lung cancer cells (Shi et al. 2017). Overexpressed DLGAP5 may promote tumor radiotherapy resistance (Gomez et al. 2013). A recent study suggested that highly expressed DLGAP5 in NSCLC is associated with patient prognosis (Schneider et al. 2017). In addition, the nucleolar and spindle-associated protein 1 (NUSAP1) is a microtubule and DNA-binding protein, which is involved in spindle assembly during mitosis. This protein is also a key regulator to maintain normal cell cycle progression (Chou et al. 2011). Previous studies have shown that NUSAP1 is over-expressed in liver cancer (Satow et al. 2010), gastric cancer (Wu et al. 2017), breast cancer (Chen et al. 2015a), and prostate cancer (Gordon et al. 2017). Chen et al. suggested that NUSAP1 may be associated with lung cancer occurrence and development (Chen et al. 2015b). Although various genes are associated with lung cancer, the detailed mechanisms of these four genes mentioned above have not been systematically studied. In this study, we aimed to describe the molecular functions of four genes in the landscape of lung cancer.

Method and materials

Cells

MRC-5, H1975, NCI H226, and A549 cell lines were purchased from ATCC (Virginia, USA). All the cells were cultured in RPMI 1640 media with 10% (v/v) FBS (Invitrogen, Carlsbad, CA, USA). The cell lines were maintained in an incubator at 37 °C and 5% CO_2.

Quantitative real-time PCR (qRT-PCR)

Total RNA of MRC-5, H1975, NCI H226, and A549 cells were extracted with the MiniBEST Universal RNA Extraction Kit (Takara, Japan). The total RNA was immediately reverse transcribed to cDNA with the PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (Takara, Japan). The quality and quantity of total RNA were measured using a NanoDrop 2000 (Thermo Fisher, USA). Samples were reextracted once the OD_{260}/OD_{280} value was not distributed between 1.8 and 2.1. qRT-PCR was carried out in a 50 µl reaction system, which included 25 µl 2xSsoAdvanced™ universal SYBR® Green (BioRad, USA), 1 µl forward primer (10 µm), 1 µl reverse primer (10 µm), and 50 ng cDNA. Thermal cycle conditions were as follows: 98 °C for 5 min; 40 cycles of 95 °C for 10 s, 60 °C for 30 s, and 72 °C for 20 s; and 12 °C forever. The primers used in this study were: ZWINT (forward)—5′-AACTCCGGGAAGCCTTTGAG-3′ and ZWINT (reverse)—5′-TTCTGGACTGCTCTGCGTTT-3′; NUSAP1 (forward)—5′-CTTGGGTCTGAAGGGGTCAC-3′ and NUSAP1 (reverse)—5′-TGCAGACTTTCTGGCTGGAG-3′; DLGAP5 (forward)—5′-GGAATCTGGGTGGCAAGTCA-3′ and DLGAP5 (reverse)—5′-TAGGCGATTTCTCGCTGCAA-3′; PRC1 (forward)—5′-TGTCCAATGCTACGGCCAAT-3′ and PRC1 (reverse)—5′-TTGCTGCCAGAAGGAGGAAG-3′; and GAPDH (forward)—5′-AATGGGCAGCCGTTAGGAAA-3′ and GAPDH (reverse)—5′-GCGCCCAATACGACCAAATC-3′. The relative gene expression in different cell lines was calculated via the $2^{-\Delta\Delta C_T}$ method.

Knockdown treatments

Eight paired small hairpin RNAs against ZWINT, NUSAP1, DLGAP5, and PRC1 were designed and synthesized by Forevergen (Guangzhou, China). The following primers were used in this study: siZWINT-1 (forward)—5′-CCAGAGGAAACGGACACAA-3′ and siZWINT-1 (reverse)—5′-UUGUGUCCGUUUCCUCUGG-3′; siNUSAP1-1 (forward)—5′-CCAAGACUCCAGCCAGAAA-3′ and siNUSAP1-1 (reverse)—5′-UUUCUGGCUGGAGUCUUGG-3′; siDLGAP5-1 (forward)—5′-GCAGAGAGAGAAAGCUAAA-3′ and siDLGAP5-1 (reverse)—5′-UUUAGCUUUCUCUCUCUGC-3′; siPRC1-1 (forward)—5′-GCCAAGCAGGAAAGACAAC-3′ and siPRC1-1 (reverse)—5′-GUUGUCUUUCCUGCUUGGC-3′; siZWINT-2 (forward)—5′-GAGAAUCUUCCAGAUGAUAAA-3′ and siZWINT-2 (reverse)—5′-UAUCAUCUGGAAGAUUCUCUG-3′; siNUSAP1-2 (forward)—5′-CCUUGAAAGGCUACAUUA-3′ and siNUSAP1-2 (reverse)—5′-UUAAUGUAGCCUUUCAAGGCU-3′; siDLGAP5-2 (forward)—5′-UGUCUAUUUUCUUUCUGAGAC-3′ and siDLGAP5-2 (reverse)—5′-CUCAGAAAGAAAAUAGACAUA-3′; and siPRC1-2 (forward)—5′-AAUUAGCUCCCAUAUUUCCCG-3′ and siPRC1-2 (reverse): 5′-GGAAAUAUGGGAGCUAAUUGG-3′. LipofectamineTM RNAiMAX Regent (Thermo Fisher, USA) was used to perform cell transfections. The siRNA or scrambled siRNA concentration used in this study was 50 nM. Opti-MEM culture medium was used to culture cells. MEM medium containing 10% FBS was used to replace the previous cell growth medium after 4 h of transfection. Stable knockdown cells were screened in medium after 48 h. The supernatant was collected 48 h post-transfection. Subsequently, the treated supernatant was clarified and stored at − 80 °C. For cell proliferation assays, the MTT kit (Sigma-Aldrich, USA) was used to measure differentially treated cells. OD values were recorded across three time points (1, 2, and 3 days).

Migration and invasion

The InnoCyte™ Cell Migration Assay (Millipore, USA) was used to perform the cell migration assay. Scrambled siRNA-treated normal cells (siNC: NCI-H226 cells and A549 cells) and knockdown cells (NCI-H226 cells and A549 cells) were planted on the top chamber of each transwell (1×10^7 cells/ml). The pore size used for the transwell (BD Falcon, USA) was 8 μm. After culturing for 24 h, the cells that went through the bottom side membrane were fixed in methanol. Hematoxylin was used to stain cells, which were counted using a microscope. Regarding cell invasion, the QCM ECMatrix Cell Invasion Assay (Millipore, USA) was used to perform analysis according to the manufacturer's instructions. The cells were cultured at 37 °C and 5% CO_2 for 24 h. Subsequently, the migrated cells were fixed and incubated at 37 °C and 5% CO_2 for 1 h. Microscopy was used to analyze the number of migrated cells.

Cell apoptosis and colony formation

Annexin V-FITC Apoptosis Detection Kit (Sigma-Aldrich, USA) was used to analyze cell apoptosis, following the manufacturer's instructions. For the colony formation assays, 150, 350, and 650 cells were plated on each well of a 6-well plate. These cells were cultured in a medium and 10% FBS for 2 weeks. Then, 0.1% crystal violet (Sigma-Aldrich) was used to stain the colonies with PBS for 15 min. An inverted microscope was used to observe colony formation. Colony-forming efficiency was calculated as: (number of clones/cell inoculation number) × 100%.

In vivo study

The PsicoR vector was purchased from Forevergen. The A549 cell line was used to construct stable siZWINT knockdown cells. Ten 8-week-old SPF/VAF nude mice were obtained from the Guangdong Medical Laboratory Animal Center (China) ($n = 5$ each group). Additionally, 4.5×10^6 normal A549 cells and 4.5×10^6 ZWINT knockdown-treated A549 cells were injected into murine armpits in each group, respectively. Tumor growth was measured at six different time points (20, 25, 30, 35, 40, and 45 days).

Western blot

Total cellular protein in NCI H226 and A549 cells with or without knockdown treatment against ZWINT was isolated by 1% PMSF and RIPA lysis buffer. After boiling with SDS-PAGE sample buffer for 5 min, the samples were subjected to SDS-PAGE. Then, the proteins were transferred onto a polyvinylidene difluoride membrane (Millipore). After being blocked for 1 h at room temperature, the membrane was incubated overnight with a 1:1000 dilution of rabbit polyclonal anti-mouse TNF-α, P53, PI3K, or GAPDH antibodies (BOSTER, USA). Before using the ECL chemiluminescence detection kit (Beyotime Institute of Biotechnology, China), proteins were incubated with the corresponding secondary antibody (1:2000 dilution) for 1 h at room temperature (BOSTER). GeneGnome 5 (Synoptics Ltd., UK) was used to assess band intensities.

Sequencing

For the sequencing analysis, the Ribo-Zero Gold Kit (Epicentre, USA) was used to remove rRNA, starting with 8 μg of total RNA. RNase R was used to remove rRNAs at 37 °C for 1 h. The Illumina mRNA-Seq sample preparation kit

(Illumina, USA) was used to construct cDNA sequencing libraries. The 2 × 150 bp pair-end sequencing strategy was carried out on the Illumina Hiseq4000 platform. Differentially expressed genes were harvested according to a previous strategy (Lin et al. 2018). Gene ontology (GO) terms enriched of differentially expressed genes were analyzed using an online database (http://amp.pharm.mssm.edu/Enrichr/). Meanwhile, The KOBAS software (Xie et al. 2011) was used for the KEGG pathway enrichment analysis of differentially expressed genes. Three biological and technical replicates were performed for the analysis.

Bioinformatics

The expression patterns of ZWINT, NUSAP1, DLGAP5, and PRC1 in SCLC, squamous-cell carcinoma, lung adenocarcinoma, and their paired adjacent tissues were retrieved from the Oncomine web portal (http://www.oncomine.org). Moreover, overall survival and recurrence-free survival time of lung cancer patients were analyzed with Oncomine online analysis tools (TCGA, https://cancergenome.nih.gov/).

Statistics

The SPSS 18.0 software was used to perform the statistical analysis. The data were expressed as mean ± standard deviation and compared with the paired sample *t* test or independent sample *t* test. The Kaplan–Meier method was used for survival analysis. $P < 0.05$ was considered statistically significant.

Results

mRNA abundance analysis

To study ZWINT, NUSAP1, DLGAP5, and PRC1 expression in SCLC, lung adenocarcinoma, and their paired adjacent tissues, we collected expression data from the Oncomine web portal (http://www.oncomine.org). Of the non-paired study, the expression of these four genes was significantly higher in SCLC and lung adenocarcinoma tissues than that in non-paired adjacent tissues ($P < 0.001$) (Fig. 1a, b). Of the paired study, we observed similar results. The expression of ZWINT, NUSAP1, DLGAP5, and PRC1 in SCLC and lung adenocarcinoma was higher than those in paired adjacent tissues (Figure S1a and Figure S1b). However, it was notable that the expression of these genes in some patients was almost the same as in their paired adjacent tissues, which indicated the heterogeneity of gene expression. In summary, ZWINT, NUSAP1, DLGAP5, and PRC1 expression in SCLC and lung adenocarcinoma were higher than that in non-paired and paired adjacent tissues.

Overall survival analysis

We employed a Kaplan–Meier plotter database (http://kmplot.com/analysis/index.php?p=background) to analyze the overall survival (OS) of ZWINT, NUSAP1, DLGAP5, and PRC1 in lung adenocarcinoma and squamous cell carcinoma patients. For each gene, the top 50% represents high expression and the bottom 50% represents low expression. In the lung adenocarcinoma analysis, the results indicated that the OS of patients with highly expressed ZWINT [HR 1.35(1.07–1.71), $P < 0.05$], NUSAP1 [HR 1.65(1.3–2.09), $P < 0.001$], DLGAP5 [HR 1.63(1.29–2.07), $P < 0.001$], and PRC1 [HR 2.3(1.8–2.94), $P < 0.001$] were significantly worse than in patients with low expression of the four genes (Fig. 2a). In our squamous cell carcinoma analysis, the results suggested that the OS of patients highly expressing ZWINT [HR 0.99(0.78–1.25), $P = 0.91$], NUSAP1 [HR 1.01(0.8–1.28), $P = 0.91$], DLGAP5 [HR 1.11(0.88–1.41), $P = 0.37$], and PRC1 [HR 1.13(0.89–1.43), $P = 0.31$] were similar to the OS of patients with low expression of these genes (Fig. 2b). In summary, high expression of ZWINT, NUSAP1, DLGAP5, and PRC1 in patients was related to poor prognosis of lung adenocarcinoma. However, high expression of these genes was not associated with squamous cell carcinoma patient prognosis.

Expression of ZWINT, NUSAP1, DLGAP5, and PRC1 in cell lines

To study further the expression of these four genes in vitro, MRC-5, H1975, NCI H226, and A549 cells were selected for qRT-PCR analysis. Figure 3 shows that the expression of ZWINT in NCI H226 and A549 cells were significantly higher than that in the MRC-5 cell line ($P < 0.05$). NUSAP1 expression in the NCI H226 and A549 cell lines were also significantly higher than that in the MRC-5 cell line ($P < 0.001$). However, NUSAP1 expression in H1975 cells was significantly lower than that in MRC-5 cells ($P < 0.05$). Meanwhile, DLGAP5 expression in NCI H226 and A549 cells were significantly higher than that in MRC-5 cells ($P < 0.001$). There were no differences in DLGAP5 expression between MRC-5 and H1975 cells. In addition, PRC1 expression in the four cell lines was similar to that of NUSAP1: PRC1 expression patterns in NCI H226 and A549 cells were significantly higher than that in MRC-5 cells ($P < 0.001$). However, PRC1 expression in the H1975 cell line was significantly lower than that in the MRC-5 cell line ($P < 0.05$). The results revealed that the expression of ZWINT, NUSAP1, DLGAP5, and PRC1 in lung squamous cell and NSCLC cells were significantly higher than that in human embryonic lung fibroblasts. The expression levels of these four genes were similar or lower than that in human embryonic lung fibroblasts.

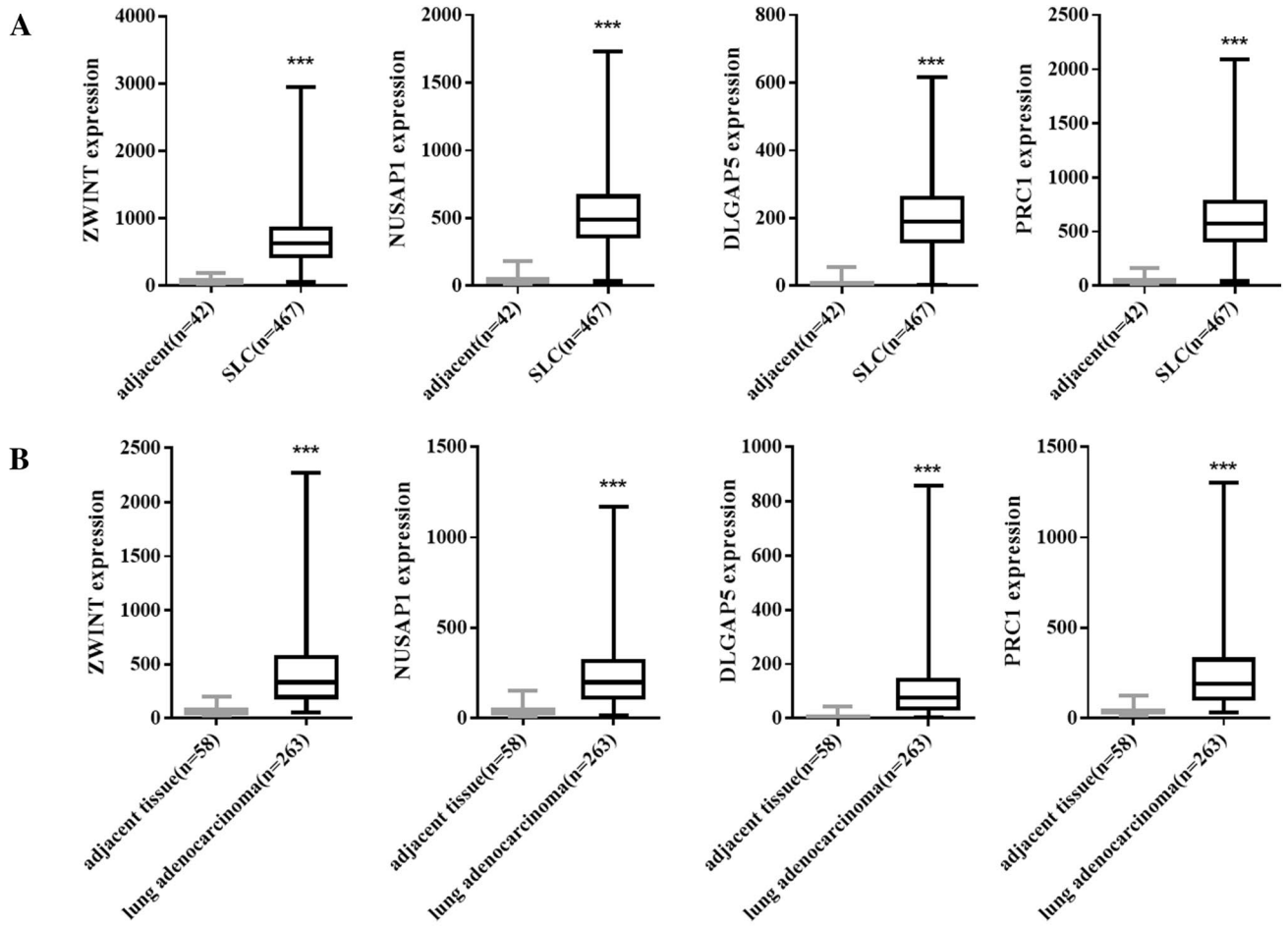


Fig. 1 mRNA abundance of ZWINT, NUSAP1, DLGAP5, and PRC1 in small cell lung cancer (SCLC), lung adenocarcinoma, and the non-paired adjacent normal tissues. **a** Gene expression in SCLC and the non-paired adjacent normal tissues. **b** Gene expression in lung adenocarcinoma and the non-paired adjacent normal tissues. ***$P < 0.001$

Knockdown and cell proliferation assays

We designed two siRNAs for each gene to knockdown the expression of ZWINT, NUSAP1, DLGAP5, and PRC1 in NCI H226 and A549 cells. q-PCR results suggested that siRNA-1 treatment of ZWINT, NUSAP1, and DLGAP5 could significantly decrease gene expression in both cell lines ($P < 0.001$) (Fig. 4a). However, siRNA-1 treatment of PRC1 in A549 cells increased expression compared with untreated cells ($P < 0.05$); yet, in NCI H226 cells gene expression was decreased ($P < 0.01$) (Fig. 4a). These results could be potentially related with siRNAs design and experimental errors. siRNA-2 treatment also decreased the expression of all four genes in both cell lines, though not so efficient as the former one. Thus, we choose the more efficient siRNA-1 to perform the following studies. We also studied NCI H226 and A549 cell proliferation in the context of gene knockdowns. Figure 4b shows that knockdown of ZWINT, NUSAP1, DLGAP5, and PRC1 all inhibited NCI H226 cell proliferation from day 2. Gene knockdown also inhibited A549 cell proliferation, albeit not as efficiently as observed in NCI H226 cells. ZWINT knockdown in NCI H226 and A549 cells significantly inhibited cell proliferation ($P < 0.05$), which suggested that ZWINT plays a key role in cancer cell growth.

Cell function analyses

To study the function of ZWINT, we performed migration and invasion assays, wherein ZWINT was silenced, in NCI H226 and A549 cells (Fig. 5a). Regarding cell migration, the number of ZWINT knockdown-treated NCI H226 and A549 cells was significantly lower than that in siNC ($P < 0.05$). Similar observations were seen for our cell invasion analysis, wherein the number of ZWINT knockdown-treated NCI H226 and A549 cells were significantly reduced ($P < 0.05$).

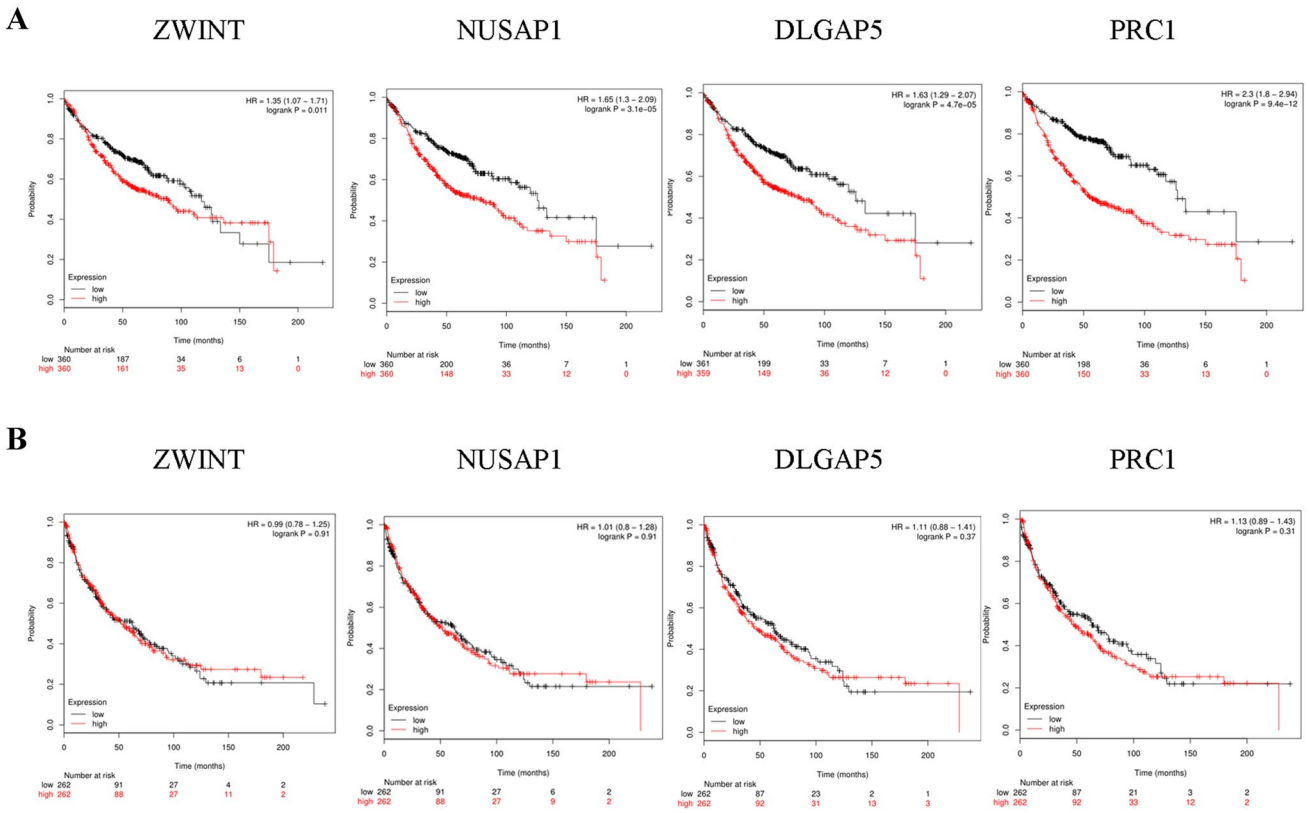


Fig. 2 Overall survival (OS) analysis of the prognosis of lung cancer patients with either high or low gene expression. **a** OS analysis of the impact of high gene expression on lung adenocarcinoma prognosis. **b** OS analysis of the impact of low gene expression on squamous cell carcinoma expression

Moreover, we performed cell apoptosis and colony formation analyses, with or without knockdown of ZWINT, in NCI H226 and A549 cells (Fig. 5b). These results indicated that knockdown of ZWINT promoted apoptosis. In addition, knockdown of ZWINT in NCI H226 cells significantly decreased colony formation ($P < 0.05$). Meanwhile, knockdown of ZWINT in A549 cells decreased, albeit not significantly, colony formation.

In vivo study

We studied ZWINT-knockdown A549 cells in a nude mice tumor model. The ZWINT-knockdown and normal A549 cells were implanted in mice, and the volume of tumors was recorded over time. Figure 6a shows tumor sizes in five experimental animals. Figure 5b shows the tumor volumes for five different time points. There were no significant differences in tumor volumes before day 20. However, the tumor volume was significantly smaller in ZWINT knockdown-treated A549 cells compared with normal cells (days 35, 40, and 45) ($P < 0.05$) (Fig. 6b). The results suggested that knockdown A549 cells could effectively restrain tumor growth.

Transcriptome sequencing and Western blot analysis

To study ZWINT-related molecular mechanisms, we performed transcriptome sequencing with ZWINT knockdown-treated NCI H226 and A549 cells. Fold change analysis and Student's *t* test were used to obtain differentially expressed genes (DEGs) (Fig. 7a). In this study, we identified 100 DEGs and 426 DEGs that were significantly up-regulated in knockdown NCI H226 and A549 cells, respectively. Meanwhile, 228 DEGs and 57 DEGs were significantly down-regulated in knockdown NCI H226 and A549 cells, respectively. GO enrichment analysis of DEGs in both cell lines was carried out using the KOBAS software (http://kobas.cbi.pku.edu.cn/home.do). Corrected *P* values ($< 10^{-4}$) indicated significant enrichment. Figure 7b shows biological processes, cellular components, and molecular functions. In A549 cells, there were 350 GO terms assigned under biological processes. Among these terms, negative regulation of cytokine production (GO: 0001818, *P* value: 0.000272076), negative regulation of lipoprotein lipase activity (GO: 0051005, *P* value: 0.00075424), and regulation of sequence-specific DNA-binding transcription factor activity (GO: 0051090,

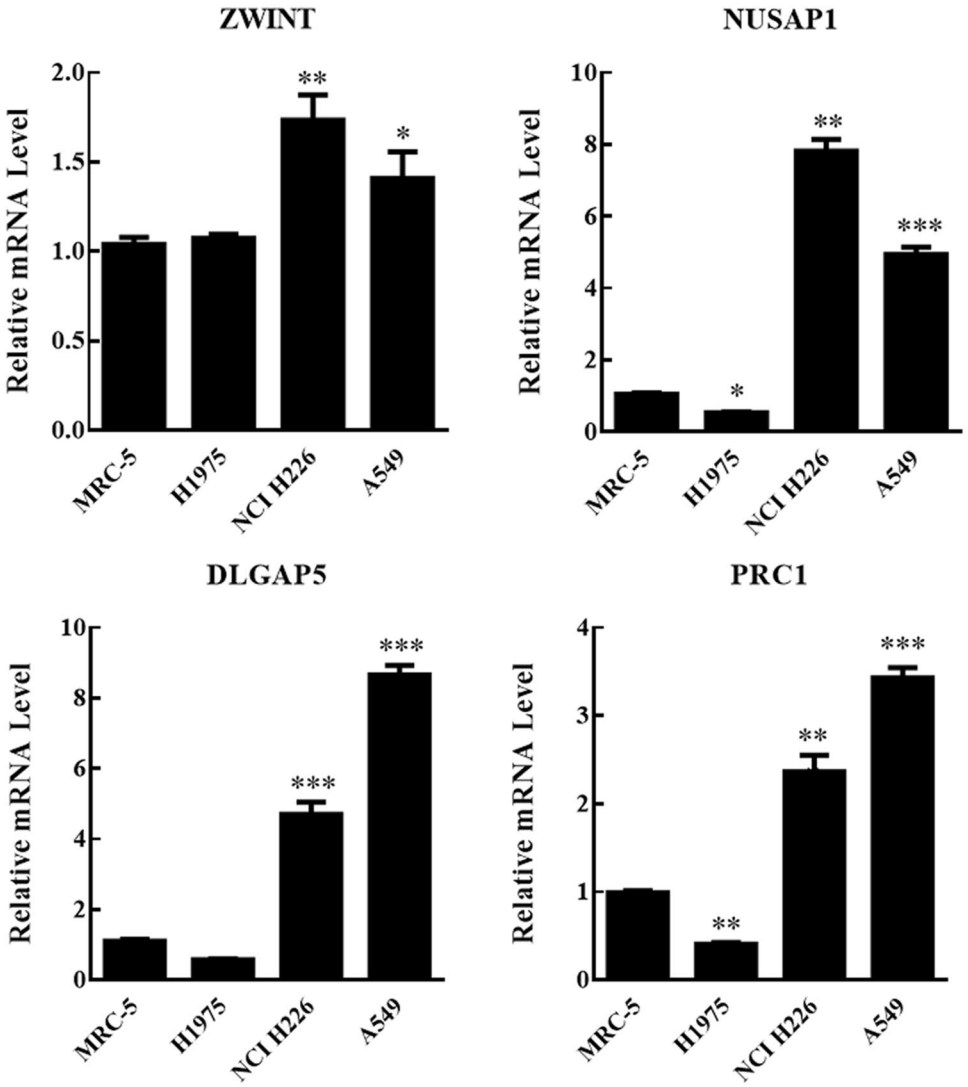
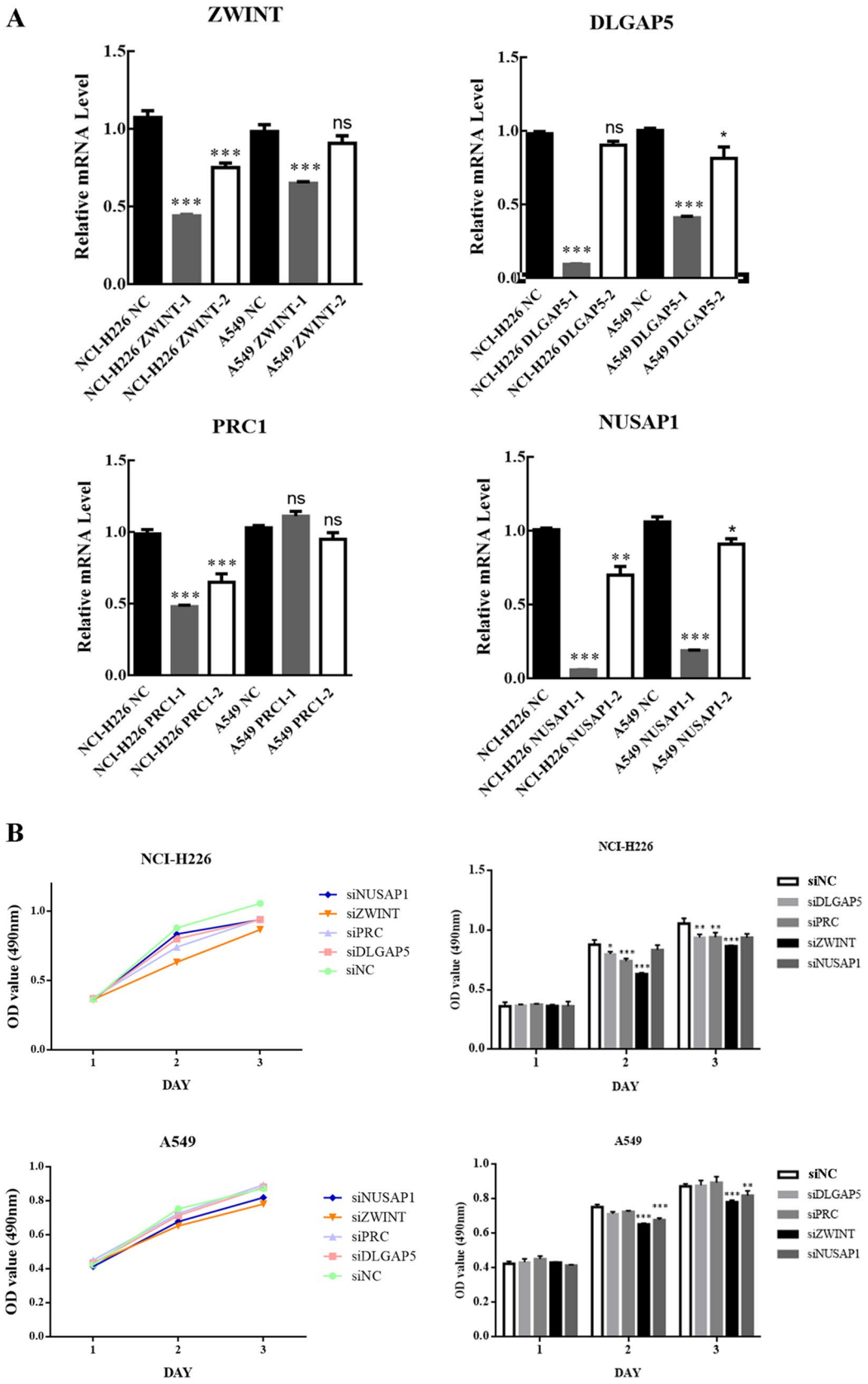


Fig. 3 Relative expression of ZWINT, NUSAP1, DLGAP5, and PRC1 in MRC-5, H1975, NCI H226, and A549 cell lines. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$

P value: 0.000825003) were the top three significantly enriched terms. From the cellular component perspective, blood microparticle (GO: 0072562, *P* value: 0.014252974) was the most significantly enriched term. From the molecular function perspective, histone serine kinase activity (GO: 0035174, *P* value: 0.001131193) was the most significantly enriched term. In NCI H226 cells, there were 3430 GO terms assigned under biological processes: single-organism process (GO: 0044699, *P* value: 2.79E−38), cellular process (GO: 0009987, *P* value: 3.11E−37), and single-organism cellular process (GO: 0044763, *P* value: 9.02E−34) were the top three significantly enriched terms. From the cellular component perspective, cell part (GO: 0044464, *P* value: 9.87E−37) was the most significantly enriched term. From the molecular function perspective, binding (GO: 0005488, *P* value: 1.46E−39) was the most significantly enriched term. For KEGG analysis of DEGs in A549 cells, 100 DEGs involved eight pathways. It is worth noting that the PPAR signaling, TNF signaling, and circadian entrainment pathways were significantly enriched (Fig. 7c). Meanwhile, in NCI H226 cells, PI3K-AKT signaling and cytokine–cytokine receptor interaction pathways were the most enriched signaling pathways. To confirm these pathways role, we performed Western blot analyses of TNF-α, P53, and PI3K in normal and ZWINT-knockdown NCI H226 and A549 cells (Fig. 7d). TNF-α and P53 protein expression in knockdown NCI H226 and A549 cells were significantly higher than in normal cells, which indicated that ZWINT could be closely related to TNF-α signaling and P53 signaling pathways. Although functional study of PI3K revealed that it may also be potentially associated with ZWINT, PI3K expression in knockdown NCI H226 and A549 cells was similar with that observed in normal cells. Therefore, we speculated that ZWINT could be indirectly related to the PI3K signaling pathway.


A
ZWINT
Relative mRNA Level
1.5
1.0
0.5
0.0
***
***
***
ns
NCI-H226 NC
NCI-H226 ZWINT-1
NCI-H226 ZWINT-2
A549 NC
A549 ZWINT-1
A549 ZWINT-2
DLGAP5
Relative mRNA Level
ns
*
***
***
NCI-H226 NC
NCI-H226 DLGAP5-1
NCI-H226 DLGAP5-2
A549 NC
A549 DLGAP5-1
A549 DLGAP5-2
PRC1
Relative mRNA Level
***
***
ns
ns
NCI-H226 NC
NCI-H226 PRC1-1
NCI-H226 PRC1-2
A549 NC
A549 PRC1-1
A549 PRC1-2
NUSAP1
Relative mRNA Level
***
**
***
*
NCI-H226 NC
NCI-H226 NUSAP1-1
NCI-H226 NUSAP1-2
A549 NC
A549 NUSAP1-1
A549 NUSAP1-2
B
NCI-H226
OD value (490nm)
DAY
siNUSAP1
siZWINT
siPRC
siDLGAP5
siNC
NCI-H226
OD value (490nm)
DAY
siNC
siDLGAP5
siPRC
siZWINT
siNUSAP1
A549
OD value (490nm)
DAY
siNUSAP1
siZWINT
siPRC
siDLGAP5
siNC
A549
OD value (490nm)
DAY
siNC
siDLGAP5
siPRC
siZWINT
siNUSAP1

◂**Fig. 4** q-PCR and cell proliferation analysis of ZWINT, NUSAP1, DLGAP5, and PRC1 siRNA treatment in NCI H226, and A549 cell lines. **a** q-PCR analysis of ZWINT, NUSAP1, DLGAP5, and PRC1 expression in normal and siRNA-treated NCI H226 and A549 cell lines. **b** Cell proliferation analysis of NCI H226 and A549 cells with or without ZWINT, NUSAP1, DLGAP5, and PRC1 siRNA treatment. $*P<0.05$; $**P<0.01$; $***P<0.001$

Discussion

Clinically, surgical treatment of lung cancer has advanced over the years. Innovative chemotherapy approaches continue to emerge, including synchrotherapy, immune therapy, and molecularly targeted therapy based on gene mutations (Dolly et al. 2017). However, the overall 5-year survival rate of NSCLC is still 10–15% only (Sharma et al. 2007; Siegel et al. 2012). Much research has primarily focused on the study of oncogenes (EGFR and K-RAS) and tumor suppressor genes (P53 and RB) in lung cancer (Bass et al. 2009; Ding et al. 2008). P53 mutations are prevalent in lung squamous cell carcinoma (about 80%). Meanwhile, K-RAS and EGFR mutations are more common in adenocarcinomas (Cooper et al. 2013; Hanibuchi et al. 2014). Along with the discovery of these key oncogenes, molecular-targeted therapy has made great progress in the treatment of

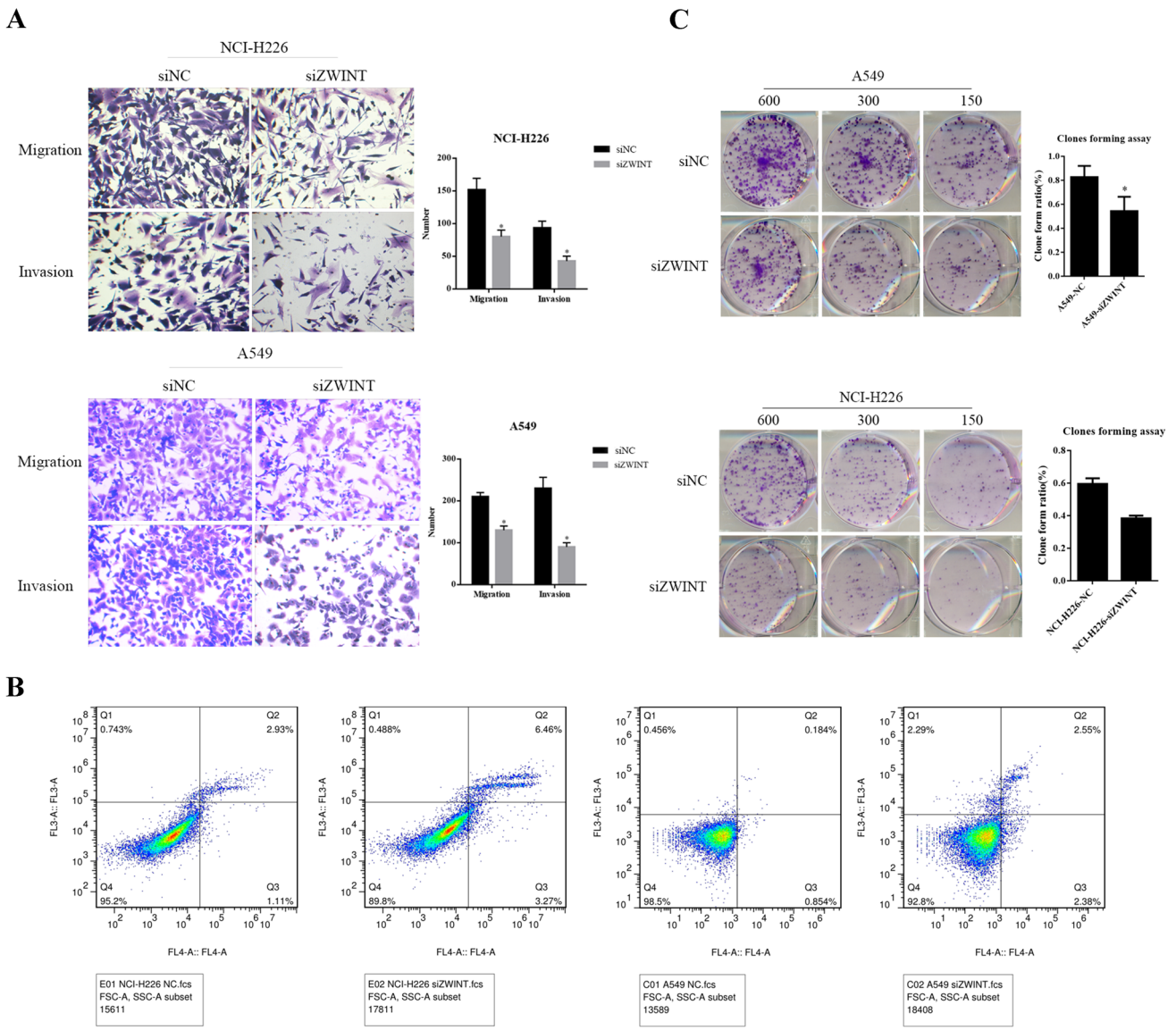


Fig. 5 Cell function changes in NCI H226 and A549 cells with or without ZWINT siRNA treatment. **a** Cell migration and invasion analyses of NCI H226 and A549 cells with or without ZWINT siRNA treatment. **b** Cell apoptosis analysis of NCI H226 and A549 cells with or without ZWINT siRNA treatment. **c** Cell colony formation study of NCI H226 and A549 cells with or without knockdown of ZWINT. $*P<0.05$

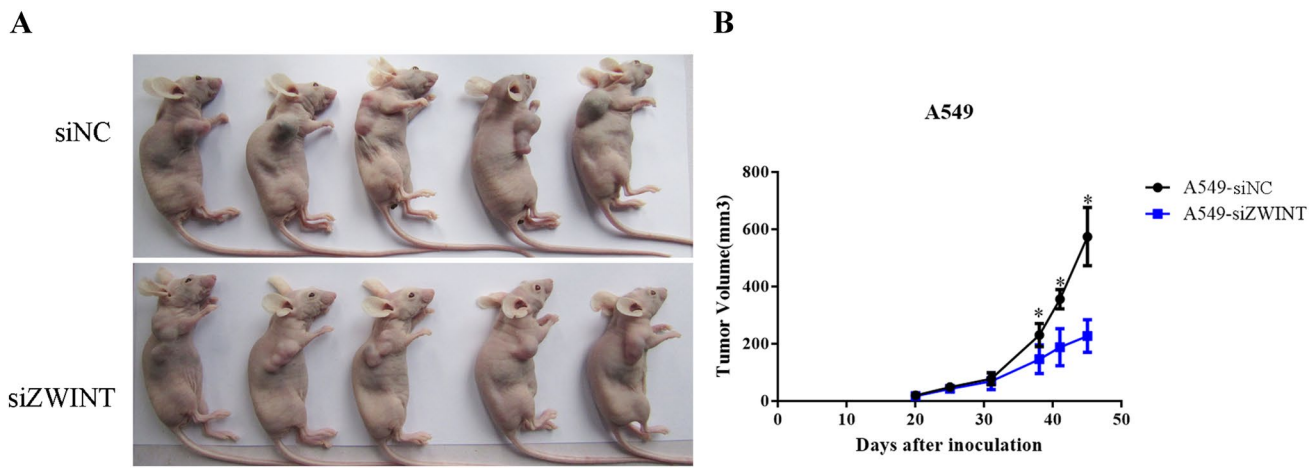


Fig. 6 In vivo mice models planted with normal A549 cells and ZWINT siRNA-treated A549 cells. **a** Images of tumor volume at 20, 25, 30, 35, 40, and 45 days after transplantation. **b** The tumor volumes in A549 cell, with or without ZWINT siRNA treatment, across five different time points. $*P < 0.05$

NSCLC, especially lung adenocarcinoma. EGFR mutations and EGFR inhibitors (EGFR-TKI) have enabled targeted drug treatment to step into a new phase (Pao et al. 2004). Gefitinib, erlotinib, and other EGFR-TKIs have achieved tremendous success in treating NSCLC patients (Kwak et al. 2010; Maemondo et al. 2010; Spigel et al. 2011). However, there are many patients without EGFR mutations that do not benefit from these targeted drugs. EGFR-TKI resistance is also a key limitation for clinical application (Hata et al. 2013; Remon et al. 2014). Moreover, the occurrence and development of lung cancer is a complex process, which could be caused by numerous oncogenes and tumor suppressor genes (de Bruin et al. 2014; Imielinski et al. 2012; Rooney et al. 2013). The current understanding of lung cancer-related genes and their molecular mechanisms is still limited. Therefore, it is of great importance to discover new lung cancer-related genes and novel therapeutic drugs.

In this work, we studied expression of ZWINT, NUSAP1, DLGAP5, and PRC1 in SLC, lung adenocarcinoma, and their paired adjacent tissues. These genes were highly expressed in cancer tissues compared with that in adjacent tissues. This result was consistent with the previous studies in other cancer types. Brendle et al. indicated that ZWINT is overexpressed in breast cancer patients with poor prognosis (Brendle et al. 2009). Meanwhile, high PRC1 expression has also been reported with poor prognosis in bladder, cervical, and prostate cancers (Espinosa et al. 2013; Kanehira et al. 2007; Luo et al. 2016). However, there were no significant differences in overall survival between high and low expression of these four genes in squamous cell carcinoma, which reveals the differences between different lung cancer subtypes. Moreover, we studied gene expression in several cell lines. ZWINT, NUSAP1, DLGAP5, and PRC1 were highly expressed in NCI H226 and A549 cells. They were minimally expressed in the H1975 cell line. The results seemed to be contradictory with clinical specimens. We speculated this was due to experimental and/or sampling errors. Our cell proliferation analysis revealed the impact of ZWINT knockdown (siRNA) treatment on lung cancer. Therefore, ZWINT was chosen for further study. Migration, invasion, apoptosis, and colony formation indicated that knockdown of ZWINT could effectively inhibit key cell behavior, cell apoptosis, and cell multiplication. ZWINT knockdown also retrained tumor volume in vivo. The ZWINT gene encodes a protein consisting of 278 amino acids. The ZWINT protein is a key regulatory protein in mitotic checkpoints and the cell cycle (Wang et al. 2004). Mitotic check points are critical for it provides a means to ensure the transition from one mitotic phase to the next (Bartkova et al. 2005; Gorgoulis et al. 2005). Meanwhile, a previous study reported ZWINT is associated with chromosome instability (CIN). CIN leads to abnormal chromosomal quantity and is considered as a marker of various malignant cancers (Kops et al. 2005). We performed RNA-seq to study the detailed molecular mechanisms related to ZWINT knockdown in NCI H226 and A549 cells. A total of 426 and 100 DEGs were identified in NCI H226 and A549 cells, respectively. GO enrichment analysis indicated that these DEGs represented diverse functions, revealing ZWINT as a multipotent molecular. In addition, KEGG analysis showed that the TNF signaling pathway is enriched in both cell lines wherein ZWINT was knocked down, as confirmed by Western blot analysis. Cell apoptosis is divided into two major pathways: extrinsic and intrinsic apoptotic signaling pathways (Huang et al. 2007). TNF and its associated apoptosis-inducing ligands (TRAILs) bind to specific death receptors located on the cell membrane. This complex recruits ligand molecules and transports death signals to the death-inducing

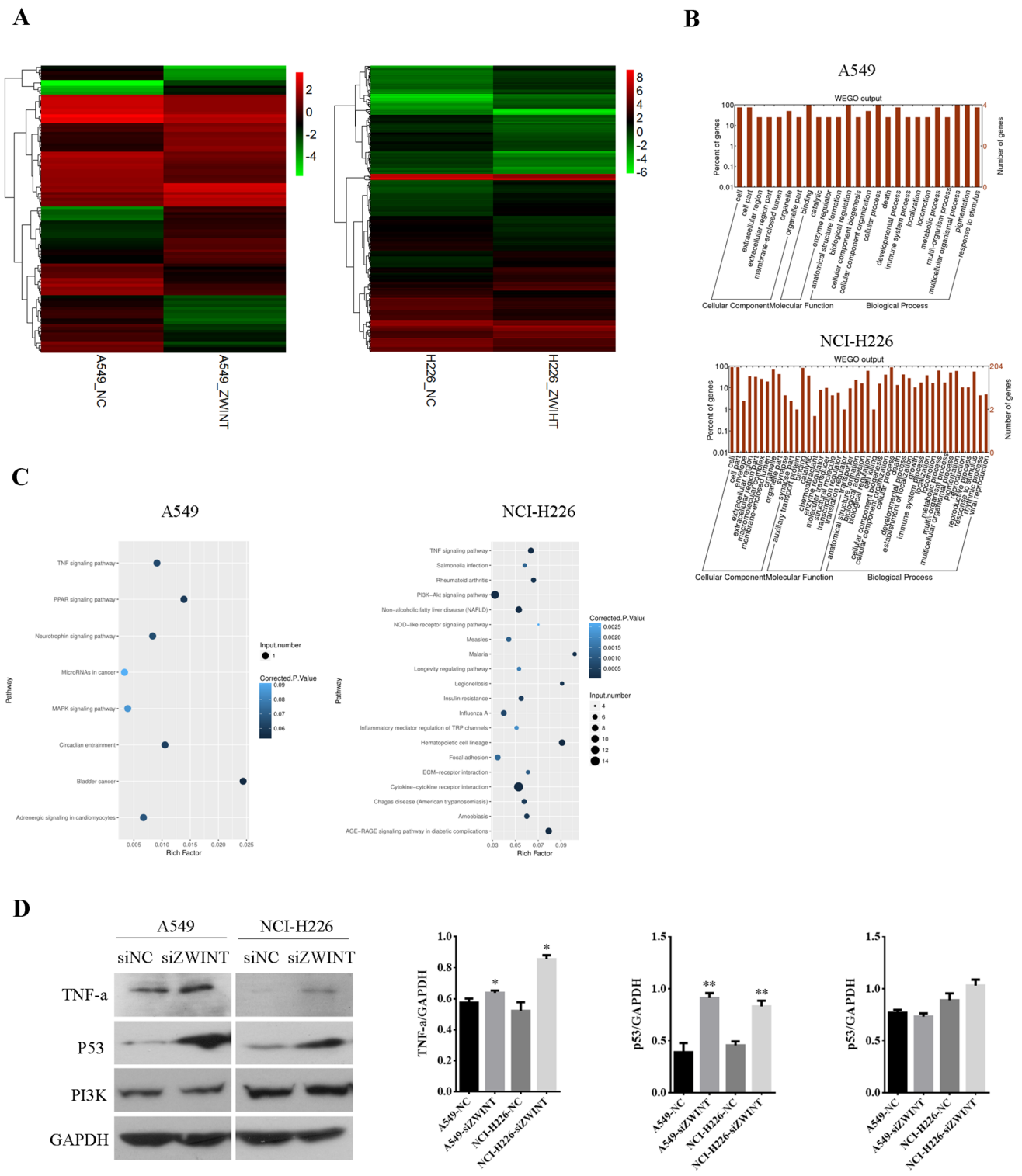


Fig. 7 Differentially expressed gene (DEG) analysis of NCI H226 and A549 cells with or without knockdown of ZWINT. **a** Heatmap analysis of NCI H226 and A549 cells with or without knockdown of ZWINT. **b** GO enrichment analysis of DEGs in NCI H226 and A549 cells with or without knockdown of ZWINT. **c** KEGG enrichment analysis of DEGs in NCI H226 and A549 cells with or without knockdown of ZWINT. **d** Western blot analysis of TNF-α, P53, and PI3K in NCI H226 and A549 cells with or without knockdown of ZWINT. $^{*}P < 0.05$; $^{**}P < 0.01$

signaling complex (DISC). The Fas-associated protein death domain (FADD) and TNF receptor-associated death domain (TRADD) recruit caspase-8 to activate non-inherent apoptotic pathways (Hassan et al. 2014). Therefore, we speculated that ZWINT could activate the TNF signaling pathway to inhibit cancer cell growth and development in lung cancer.

Conclusion

In this study, we have identified high expression of ZWINT, NUSAP1, DLGAP5, and PRC1 in different lung cancer tissues with poor overall survival. We also study the mRNA abundance of these four genes in four different cell lines with or without knockdown treatments. The results suggested ZWINT as a potential gene associated with lung cancer cell proliferation. Knockdown of ZWINT inhibited cell behavior and growth. Meanwhile, studying tumor volume in vivo revealed that ZWINT restrains tumor growth. Regarding our transcriptomic investigations, there were 426 and 100 DEGs in NCI H226 and A549 cells treated with ZWINT siRNA, respectively. Of these DEGs, 197 were up-regulated and 228 were down-regulated NCI H226 cells wherein ZWINT was knocked down, and 43 were up-regulated and 57 were down-regulated in ZWINT siRNA-treated A549 cells. In addition, GO and KEGG enrichment analyses of DEGs in both cell lines showed that cancer-related pathways were significantly enriched. TNF, P53, and PI3K signaling pathways were the most enriched cancer-related pathways, and were validated by Western blot analysis.

Author contributions FP, QL, and S-QN: bioinformatics analysis and writing of the manuscript. G-PS and YL: the discussion. MC and YB: discussion and comments on an earlier version of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported in part by the National Natural Science Foundation of China (no. 81602661), the Natural Science Foundation of Guangdong Province, China (no. 2016A030310164). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Compliance with ethical standards

Conflict of interest The author(s) declare that they have no competing interests.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards The Research Ethics Committee of Sun Yat-Sen University approved the collection of tissue samples for research.

Informed consent None.

References

Bartkova J et al (2005) DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. Nature 434:864–870. https://doi.org/10.1038/nature03482

Bass AJ et al (2009) SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. Nat Genet 41:1238–1242. https://doi.org/10.1038/ng.465

Brendle A et al (2009) Single nucleotide polymorphisms in chromosomal instability genes and risk and clinical outcome of breast cancer: a Swedish prospective case–control study. Eur J Cancer 45:435–442. https://doi.org/10.1016/j.ejca.2008.10.001

Chen L et al (2015a) High levels of nucleolar spindle-associated protein and reduced levels of BRCA1 expression predict poor prognosis in triple-negative breast cancer. PLoS One 10:e0140572. https://doi.org/10.1371/journal.pone.0140572

Chen L, Zhuo D, Chen J, Yuan H (2015b) Screening feature genes of lung carcinoma with DNA microarray analysis. Int J Clin Exp Med 8:12161–12171

Chou HY, Wang TH, Lee SC, Hsu PH, Tsai MD, Chang CL, Jeng YM (2011) Phosphorylation of NuSAP by Cdk1 regulates its interaction with microtubules in mitosis. Cell Cycle 10:4083–4089. https://doi.org/10.4161/cc.10.23.18200

Cooper WA, Lam DC, O'Toole SA, Minna JD (2013) Molecular biology of lung cancer. J Thorac Dis 5(Suppl 5):S479–S490 https://doi.org/10.3978/j.issn.2072-1439.2013.08.03

de Bruin EC et al (2014) Spatial and temporal diversity in genomic instability processes defines lung cancer evolution. Science 346:251–256. https://doi.org/10.1126/science.1253462

Ding L et al (2008) Somatic mutations affect key pathways in lung adenocarcinoma. Nature 455:1069–1075. https://doi.org/10.1038/nature07423

Dolly SO, Collins DC, Sundar R, Popat S, Yap TA (2017) Advances in the development of molecularly targeted agents in non-small-cell lung cancer. Drugs 77:813–827. https://doi.org/10.1007/s40265-017-0732-2

Engeland K (2018) Cell cycle arrest through indirect transcriptional repression by p53: I have a DREAM. Cell Death Differ 25:114–132. https://doi.org/10.1038/cdd.2017.172

Espinosa AM et al (2013) Mitosis is a source of potential markers for screening and survival and therapeutic targets in cervical cancer. PLoS One 8:e55975. https://doi.org/10.1371/journal.pone.0055975

Gomez CR et al (2013) Prognostic value of discs large homolog 7 transcript levels in prostate cancer. PLoS One 8:e82833. https://doi.org/10.1371/journal.pone.0082833

Gordon CA, Gong X, Ganesh D, Brooks JD (2017) NUSAP1 promotes invasion and metastasis of prostate cancer. Oncotarget 8:29935–29950. https://doi.org/10.18632/oncotarget.15604

Gorgoulis VG et al (2005) Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature 434:907–913. https://doi.org/10.1038/nature03485

Hanibuchi M, Kim SJ, Fidler IJ, Nishioka Y (2014) The molecular biology of lung cancer brain metastasis: an overview of current comprehensions and future perspectives. J Med Investig 61:241–253

Hassan M, Watari H, AbuAlmaaty A, Ohba Y, Sakuragi N (2014) Apoptosis and molecular targeting therapy in cancer. Biomed Res Int 2014:150845. https://doi.org/10.1155/2014/150845

Hata A et al (2013) Rebiopsy of non-small cell lung cancer patients with acquired resistance to epidermal growth factor receptor-tyrosine kinase inhibitor: comparison between T790M mutation-positive and mutation-negative populations. Cancer 119:4325–4332. https://doi.org/10.1002/cncr.28364

Hu CK, Ozlu N, Coughlin M, Steen JJ, Mitchison TJ (2012) Plk1 negatively regulates PRC1 to prevent premature midzone formation before cytokinesis. Mol Biol Cell 23:2702–2711. https://doi.org/10.1091/mbc.E12-01-0058

Huang X, Zhang X, Farahvash B, Olumi AF (2007) Novel targeted pro-apoptotic agents for the treatment of prostate cancer. J Urol 178:1846–1854. https://doi.org/10.1016/j.juro.2007.06.039

Imielinski M et al (2012) Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. Cell 150:1107–1120. https://doi.org/10.1016/j.cell.2012.08.029

Kanehira M et al (2007) Oncogenic role of MPHOSPH1, a cancer-testis antigen specific to human bladder cancer. Cancer Res 67:3276–3285. https://doi.org/10.1158/0008-5472.can-06-3748

Kikuchi T et al (2003) Expression profiles of non-small cell lung cancers on cDNA microarrays: identification of genes for prediction of lymph-node metastasis and sensitivity to anti-cancer drugs. Oncogene 22:2192–2205. https://doi.org/10.1038/sj.onc.1206288

Kops GJ, Weaver BA, Cleveland DW (2005) On the road to cancer: aneuploidy and the mitotic checkpoint. Nat Rev Cancer 5:773–785. https://doi.org/10.1038/nrc1714

Kwak EL et al (2010) Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 363:1693–1703. https://doi.org/10.1056/NEJMoa1006448

Lin YM, Furukawa Y, Tsunoda T, Yue CT, Yang KC, Nakamura Y (2002) Molecular diagnosis of colorectal tumors by expression profiles of 50 genes expressed differentially in adenomas and carcinomas. Oncogene 21:4120–4128. https://doi.org/10.1038/sj.onc.1205518

Lin KH et al (2018) RNA-seq transcriptome analysis of breast cancer cell lines under shikonin treatment. Sci Rep 8:2672. https://doi.org/10.1038/s41598-018-21065-x

Luo HW et al (2016) Protein regulator of cytokinesis 1 overexpression predicts biochemical recurrence in men with prostate cancer. Biomed Pharmacother 78:116–120. https://doi.org/10.1016/j.biopha.2016.01.004

Maemondo M et al (2010) Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR N. Engl J Med 362:2380–2388. https://doi.org/10.1056/NEJMoa0909530

McGuire S (2016) World Cancer Report 2014. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, 2015. Adv Nutr 7:418–419. https://doi.org/10.3945/an.116.012211

Nakamura T et al (2004) Genome-wide cDNA microarray analysis of gene expression profiles in pancreatic cancers using populations of tumor cells and normal ductal epithelial cells selected for purity by laser microdissection. Oncogene 23:2385–2400. https://doi.org/10.1038/sj.onc.1207392

Obama K, Ura K, Satoh S, Nakamura Y, Furukawa Y (2005) Up-regulation of PSF2, a member of the GINS multiprotein complex in intrahepatic cholangiocarcinoma. Oncol Rep 14:701–706

Pao W et al (2004) EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci USA 101:13306–13311. https://doi.org/10.1073/pnas.0405220101

Ramalingam SS, Owonikoko TK, Khuri FR (2011) Lung cancer: new biological insights and recent therapeutic advances. CA Cancer J Clin 61:91–112. https://doi.org/10.3322/caac.20102

Remon J, Moran T, Majem M, Reguart N, Dalmau E, Marquez-Medina D, Lianes P (2014) Acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in EGFR-mutant non-small cell lung cancer: a new era begins. Cancer Treat Rev 40:93–101. https://doi.org/10.1016/j.ctrv.2013.06.002

Rooney M, Devarakonda S, Govindan R (2013) Genomics of squamous cell lung cancer Oncologist 18:707–716. https://doi.org/10.1634/theoncologist.2013-0063

Satow R et al (2010) Combined functional genome survey of therapeutic targets for hepatocellular carcinoma. Clin Cancer Res 16:2518–2528. https://doi.org/10.1158/1078-0432.ccr-09-2214

Scagliotti GV et al (2008) Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol 26:3543–3551. https://doi.org/10.1200/jco.2007.15.0375

Schneider MA et al (2017) AURKA, DLGAP5, TPX2, KIF11 and CKAP5: Five specific mitosis-associated genes correlate with poor prognosis for non-small cell lung cancer patients. Int J Oncol 50:365–372. https://doi.org/10.3892/ijo.2017.3834

Sharma SV, Bell DW, Settleman J, Haber DA (2007) Epidermal growth factor receptor mutations in lung cancer. Nat Rev Cancer 7:169–181. https://doi.org/10.1038/nrc2088

Shi YX, Yin JY, Shen Y, Zhang W, Zhou HH, Liu ZQ (2017) Genome-scale analysis identifies NEK2, DLGAP5 and ECT2 as promising diagnostic and prognostic biomarkers in human lung cancer. Sci Rep 7:8072. https://doi.org/10.1038/s41598-017-08615-5

Shimo A, Nishidate T, Ohta T, Fukuda M, Nakamura Y, Katagiri T (2007) Elevated expression of protein regulator of cytokinesis 1, involved in the growth of breast cancer cells. Cancer Sci 98:174–181. https://doi.org/10.1111/j.1349-7006.2006.00381.x

Siegel R et al (2012) Cancer treatment and survivorship statistics, 2012. CA Cancer J Clin 62:220–241. https://doi.org/10.3322/caac.21149

Spigel DR et al (2011) Randomized, double-blind, placebo-controlled, phase II trial of sorafenib and erlotinib or erlotinib alone in previously treated advanced non-small-cell lung cancer. J Clin Oncol 29:2582–2589. https://doi.org/10.1200/jco.2010.30.7678

Sundar R, Soong R, Cho BC, Brahmer JR, Soo RA (2014) Immunotherapy in the treatment of non-small cell lung cancer. Lung Cancer 85:101–109. https://doi.org/10.1016/j.lungcan.2014.05.005

Wang H et al (2004) Human Zwint-1 specifies localization of Zeste White 10 to kinetochores and is essential for mitotic checkpoint signaling. J Biol Chem 279:54590–54598. https://doi.org/10.1074/jbc.M407588200

Woo Seo D, Yeop You S, Chung WJ, Cho DH, Kim JS, Su Oh J (2015) Zwint-1 is required for spindle assembly checkpoint function and kinetochore-microtubule attachment during oocyte meiosis. Sci Rep 5:15431. https://doi.org/10.1038/srep15431

Wu X et al (2017) Nucleolar and spindle associated protein 1 promotes the aggressiveness of astrocytoma by activating the Hedgehog signaling pathway. J Exp Clin Cancer Res 36:127. https://doi.org/10.1186/s13046-017-0597-y

Xie C et al (2011) KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases. Nucleic Acids Res 39:W316–W322. https://doi.org/10.1093/nar/gkr483

Xu Z, Zhou Y, Cao Y, Dinh TL, Wan J, Zhao M (2016) Identification of candidate biomarkers and analysis of prognostic values in ovarian cancer by integrated bioinformatics analysis. Med Oncol 33:130. https://doi.org/10.1007/s12032-016-0840-y

Zarogoulidis K et al (2013) Immunomodifiers in combination with conventional chemotherapy in small cell lung cancer: a phase II randomized study. Drug Des Dev Ther 7:611–617. https://doi.org/10.2147/dddt.s43184