Original Article

C-Phycocyanin Suppresses Cell Proliferation and Promotes Apoptosis by Regulating the AMPK Pathway in NCL-H292 Non-Small Cell Lung Cancer Cells

(AMPK / C-phycocyanin / mTOR / NSCLC / NBR2)

H. CHAOWEN, H. DONGXUAN, H. DONGSHENG, P. JIANFENG, Y. FAN, C. YAHUI, L. XIAOHUA

Department of Pulmonary and Critical Care Medicine, Shenzhen Longhua District Central Hospital, Shenzhen, China

Abstract. Non-small cell lung cancer (NSCLC) results in high mortality and has gained increasing attention. C-Phycocyanin (C-PC) has been identified as a potential therapeutic inhibitor for NSCLC, but its underlying mechanism remains obscure. The gene expression of the long noncoding RNA neighbour of BRCAI RNA 2 (NBR2) in NSCLC cells was evaluated by quantitative reverse transcription-PCR. The cell capacity for proliferation and migration was examined by EdU and wound-healing assays. Furthermore, the viability and apoptosis of cells was measured with CCK-8 and annexin V/PI, respectively. Next, the protein level of activation of adenosine monophosphate-activated protein kinase and the rapamycin kinase (mTOR) signalling pathway-associated molecules was evaluated by western blotting. H292 cells were pre-treated with C-PC or transfected with plasmids encoding NBR2 or the shNBR2 plasmid, to over-express or knock down NBR2 expression, respectively. NBR2 expression was robustly down-regulated in NSCLC cell lines compared with a normal cell line (BEAS-2B). NBR2 over-expression inhibited migration and promoted apoptosis of H292 cells. Treatment of H292 cells with C-PC enhanced NBR2 levels in a dose- and time-dependent manner. Downregulation of NBR2 in H292 cells inhibited the activity of C-PC on cell proliferation, viability and clone

Folia Biologica (Praha) 68, 16-24 (2022)

formation. Further mechanistic investigation showed that the down-regulation of NBR2 abolished the modulatory effects of C-PC on the AMPK/mTOR signalling pathway. In conclusion, C-PC inhibits H292 cell growth by enhancing the NBR2/AMPK signalling pathway.

Introduction

Lung cancer (LC) remains the most common cancer in the world, affecting up to 1.4 million persons every year (Brozos-Vazquez et al., 2020). Non-small cell lung cancer (NSCLC) accounts for about 80 % of LC (Liu et al., 2019). Although chemotherapy, surgery, radiotherapy and molecular targeted drugs, such as gefitinib and erlotinib, have inhibitory effects on the development of NSCLC, the overall 5-year survival rate of advanced patients with NSCLC is still only 20 % (Miller et al., 2019; Zhang et al., 2020a), and traditional treatments have severe side effects and increase treatment costs (Schutt et al., 2010). Thus, it is urgent to develop new drugs for treating patients with NSCLC and to identify the exact molecular mechanisms involved in cancer progression.

Long noncoding RNAs (LncRNAs) are more than 200 nucleotides in length and have been attributed with diverse molecular functions, ranging from negative regulation of miRNAs by serving as miRNA 'sponges' to marking mRNAs for degradation and transcriptional regulation of genes (Ulitsky and Bartel, 2013). Previous studies have revealed that lncRNAs can act as tumour suppressors or oncogenes in malignant tumours, demonstrating that abnormal expression of lncRNA is associated with tumour development in various cancers (Pandya et al., 2020; Zhang et al., 2020b). Schmidt et al. (2014) indicated that MALAT-1 lncRNA influences expression of prognostic markers in NSCLC, which could improve the risk prediction in resectable patients with NSCLC. In addition, another lncRNA molecule (ABHD11-AS1) has been observed to promote NSCLC proliferation and the Warburg effect (Xue et al., 2020). Furthermore,

Received October 31, 2021. Accepted December 25, 2021.

Corresponding author: He Chaowen, Department of Pulmonary and Critical Care Medicine, Shenzhen Longhua District Central Hospital, No. 187 Guanlan Avenue, Shenzhen 518110, China. Phone and Fax: (+86) 0755-280 173 52; e-mail: hechaowen114@sina.com

Abbreviations: AMPK – adenosine monophosphate-activated protein kinase, C-PC – C-phycocyanin, lncRNA – long noncoding RNA, mTOR – mechanistic target of repamycin kinase, NBR2 – neighbour of BRCAI RNA 2, NSCLC – non-small cell lung cancer, qRT-PCR – quantitative reverse transcription-PCR.

IncRNA00673 facilitates cell proliferation, migration, invasion, and epithelial-mesenchymal transition (EMT) of NSCLC through sponging miR-150-5p (Lu et al., 2017). However, the function of lncRNA neighbour of BRCAI RNA 2 (NBR2) in the progression of NSCLC remains unclear.

The sequence locus of NBR2 is adjacent to tumour suppressor gene BRCA1 (Xu et al., 1997). NBR2 has been reported to be decreased in tissue specimens from NSCLC patients, and these NSCLC patients with low expression of NBR2 show a poor prognosis. NBR2 over-expression suppresses the viability and migration of NSCLC cells (Gao et al., 2019). However, the functional target of NBR2 and the underlying mechanisms in the regulation of NSCLC remain obscure. Furthermore, NBR2 may also interact with intracellular energy sensor adenosine monophosphate-activated protein kinase (AMPK), which modulates the activation of AMPK. Depletion of NBR2 suppressed deprivation-induced activation of the AMPK signalling pathway, including anabolic processes and catabolism of adenosine triphosphate (ATP) production (Liu et al., 2016). This suggests that NBR2 can enhance AMPK expression, which may be involved in the development of NSCLC.

C-Phycocyanin (phycocyanin, C-PC) is a natural antineoplastic marine protein derived from *Spirulina platensis* with multiple biological functions (Mitra et al., 2015), including anti-inflammatory (Mitra et al., 2015), antioxidant (Sonani et al., 2017), immunomodulatory (Cian et al., 2012), and antibacterial activities (Basha et al., 2008; Abd El-Baky and El-Baroty, 2012; Jiang et al., 2018). Furthermore, many tumours, including NSCLC, can be treated with C-PC. The anticancer activities of C-PC are well defined (Wang et al., 2007; Hao et al., 2019), but the exact function and molecular mechanism that C-PC plays in NSCLC remain poorly understood.

To investigate the molecular mechanism of C-PC activity in NSCLC, we performed an *in vitro* assay in which H292 cells were treated with C-PC. This study demonstrated that C-PC inhibited tumour proliferation and migration by promoting NBR2 expression, which activated AMPK and suppressed the mechanistic target of repamycin kinase (mTOR) signalling pathway that ultimately triggered cancer cell death.

Material and Methods

Materials, cell lines and transfection

Phosphate-buffered solution (PBS) was used to prepare phycocyanin treatments (C-PC, MedChemExpress, Monmouth Junction, NJ, USA). Human NSCLC H1299 (ATCC[®] CRL-5803), H292 (ATCC[®] CRL-1848), PC-9 (ATCC[®] HTB-177), and Beas-2B (ATCC[®] CRL-9609) cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, USA). Cell lines were cultured in DMEM media including 10 % foetal bovine serum (FBS) (Gibco, Australia) and a 37 °C humidified incubator with 5 % CO_2 . Cells were passaged every two days.

For cell transfection experiments, vectors including empty control (pcDNA3.1, pc-NC) and lncRNA NBR2 plasmid (pc-NBR2) were transfected into H292 cells using Lipofectamine[™] 2000 (Thermo Fisher Scientific, MA, USA) according to the manufacturer's protocol. Briefly, cells were seeded in 6-well plates and transfected at 90% confluence. Initially, 500 µl of Opti-MEM medium was added to two 1.5 ml centrifuge tubes each. Then, Lipofectamine[™] 2000 and NBR2 expression plasmids were individually added to the two tubes and mixed with OPTI-MEM medium. After incubation for 5 min at room temperature, the reagents were mixed and incubated for an additional 20 min. The transfection mixture was added to wells containing 1 ml OPTI-MEM medium and gently shaken. After 7 h incubation at 37 °C, the OPTI-MEM medium was replaced by normal cell culture medium and the plasmid-transfected cells were maintained in culture for the duration of the experiments (Deng et al., 2018).

Cell counting kit-8 assay

A total of 5000 H292 cells per well were seeded in 96-well plates (Corning Inc., Corning, NY, USA) and were then subjected to different treatments for the indicated times. The OD value of 450 nm was detected after incubation with 10 μ M of the cell counting kit-8 (CCK-8) (Beyotime, Shanghai, China) solution for another 2 h.

Colony formation assay

A total of 200 cells were seeded into each well of a 6-well plate. After the cells were incubated for 14 days with the different treatments for the indicated times, a 0.05 % (w/v) crystal violet solution was added to each well of the 6-well plates and then incubated for an additional 1 hour at room temperature. The number of colonies of each well could be observed by light-field microscopy. Three replicates were performed for each experiment.

EdU assay

Each well of any 6-well plate was seeded with 1×10^5 H292 cells. The cells were then subjected to the indicated treatments, and then co-cultured with 10 μ M EdU for 2 h. The cells were then fixed in 4 % paraformaldehyde. Finally, these cells were stained with Alexa Fluor 488 probes and DAPI (nuclear stain; Sigma, USA). The number of EdU+ cells in five random fields was calculated with an IX71 microscope and DP80 camera (200×, Olympus GmbH, Hamburg, Germany).

Quantitative RT-PCR (qRT-PCR)

According to the manufacturer's protocols, TRIzol reagent (Invitrogen, Life Technologies, Carlsbad, CA, USA) was used to separate total RNA from each group of cells. Briefly, 1 ml of TRIzol was added to each centrifuge tube containing 1×10^7 cells. After 5 min of in-

cubation, 0.3 ml of chloroform was added to each tube. All tubes were shaken vigorously by hand for 15 s and centrifuged for 15 min at $12,000 \times g$ at 4 °C. The upper aqueous phase was transferred to a clean 1.5 ml centrifuge tube and mixed with 0.5 ml isopropyl alcohol to precipitate RNA. After centrifugation for 10 min at $12,000 \times g$ at 4 °C, the RNA pellet was washed with 1 ml 75% ethanol. Then, the RNA pellet was dissolved with 30 µl RNase-free water for further experiments. The cDNA was obtained by reverse transcription using a reverse transcription kit (TaKaRa, Tokyo, Japan) from 500 ng total RNA (Rio et al., 2010; Yang et al., 2022). The qRT-PCR assay was performed in a Bio-Rad CFX96 system using SYBR Master Mix (TaKaRa, Tokyo, Japan). The gene expression ratio of NBR2 to GAPDH was calculated as relative fold changes against control values using the $2^{-\Delta\Delta Ct}$ method (Rao et al., 2013). The specific primers used in this study were as follows: NBR2 (Forward: 5'-GGAGGTCTCCAGTTTCGGTA-3'; reverse: 5'-TTGATGTGTGCTTCCTGGG-3'). GAPDH (forward: 5'-CCATGGGGAAGGTGAAGGTC-3'; reverse: 5'-GAAGGGGTCATTGATGGCAAC-3').

Western blotting

According to manufacturer's protocols, RIPA lysis buffer (Beyotime, Shanghai, China) was used to separate total proteins from each group of cells. Cell lysates were centrifuged at $12,000 \times g$ for 10 min at 4 °C and the supernatant was transferred to new 1.5 ml centrifuge tubes. The BCA Protein Assay Kit (Beyotime, Shanghai, China) was used to assay the protein concentration. After that, these protein samples were electrophoresed by 12 % sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotted on polyvinylidene fluoride membranes. The membranes were immersed in TBST (TBS + 0.5 % Tween 20) containing 5 % non-fat dry milk (Fdbio, Shanghai, China) for 1 h, and then primary antibodies were added to bind to the proteins under study. These primary antibodies were anti-phospho-acetyl-CoA carboxylase (Ser79) antibody (CST, #3661S, 1:1000, USA), acetyl-CoA carboxylase antibody (CST, 3662S, 1:1000, USA), phospho-AMPKa (Thr172) (40H9) rabbit mAb (CST, 2535S, 1:1000, USA), and AMPKa (D63G4) Rabbit mAb (CST, 5832S, 1:1000, USA). Following incubation, the membranes were washed five times for 5 min in TBST and incubated for 1 h in secondary antibody (1: 1000 dilution in TBST containing 5 % bovine serum albumin). Following five washes of 5 min each in TBST, the membranes were visualized with chemiluminescence solution (FDbio-Dura ECL kit, Hanzhou, China) at room temperature using the ChemiDoc/XPS+ gel imaging system (Bio-Rad Laboratories, Hercules, CA).

Annexin V-FITC/PI analysis

To compare the effects on cells under different indicated treatments, cells were harvested and stained with the Annexin V-FITC-PI test kit (Solarbio, Beijing, China). A FACS flow cytometer (CytoflexLX, Beckman, USA) was used to analyse the results. Three replicates were established for each experiment (Yang et al., 2021).

Wound-healing assay

For each type of experimental conditions, cells were grown in 6-well culture plates. Once the cells of each well reached a confluence of 90 %, a 200 μ l pipette tip was used to produce a scratch on the cell layer. The cells were washed with PBS and cultured in low-serum DMEM medium. A DM IRB inverted microscope (Leica Microsystems, Wetzlar, Germany) was used to observe the wounds and photographed at multiple time points. ImageJ software (US National Institutes of Health, Bethesda, MD, USA) was used to analyse the wound areas.

Statistical analysis

All experimental data were analysed by the SPSS software (version 20.0, IBM, USA). The mean \pm standard deviation (mean \pm SD) was used to present all data. To compare two groups of experimental data, the Student's *t*-test method was used. Comparing multiple groups of experimental data, one-way analysis of variance was used. P values less than 0.05 were considered statistically significant.

Results

NBR2 was down-regulated in cancer cell lines, and its over-expression inhibited migration and promoted apoptosis of H292 cells

The gene expression of *NBR2* was down-regulated in H1299, H292, H1650 and PC-9 cells, and especially in H292 cells, compared to BEAS-2B cells (P < 0.01, Fig. 1A). Therefore, H292 cells were chosen for further experiments.

To investigate the role of NBR2 in H292 cells, *NBR2* was over-expressed by plasmid transfection. *NBR2* gene levels were up-regulated more than 15-fold in the pc-NBR2 group compared to the negative control group (P < 0.05, Fig. 1B), which revealed that effective transfection had been achieved. Functionally, NBR2 over-expression inhibited migration of H292 cells *in vitro* (Fig. 1C and D). Furthermore, NBR2 over-expression reduced the cell viability (Fig. 1E) and promoted H292 apoptosis (4.52 ± 0.92 % in the pc-NC group vs 22.31 ± 2.13 % in the pc-NBR2 group, P < 0.01) (Fig. 1F and G). These results suggested that NBR2 over-expression could inhibit migration and induce apoptosis in H292 cells.

NBR2 was up-regulated by C-PC in a time- and concentration-dependent manner in H292 cells

Studies demonstrating AMPK activation by C-PC (Ren et al., 2018) and NBR2 (Yang et al., 2020) have raised interest in the potential relationship between C-PC and NBR2 in H292 cells. The results of RT-qPCR revealed that *NBR2* gene levels were significantly ele-



Fig. 1. Over-expression of NBR2 inhibited cell migration and induced apoptosis of H292 cells. (A) qRT-PCR detection of NBR2 down-regulation in NSCLC lines (H1299, H292, H1650 and PC-9) compared to BEAS-2B cell lines. (B) NBR2 was up-regulated after treatment with pc-NBR2 (pcDNA-NBR2) compared to the empty vector pc-NC. (C, D) Woundhealing analysis was used to evaluate H292 cell migration. (E) CCK-8 assay showing the cell viability after transfection with the empty vector or pc-NBR2. (F, G) Flow cytometry analysis of apoptotic cells. Data are shown as mean \pm SD, *P < 0.05; **P < 0.01. Three replicates were performed for each experiment.

vated by C-PC at 10 μ M after 12 h. The *NBR2* gene levels were further up-regulated over a 48-h period (Fig. 2A). After transfection of shNBR2 into H292 cells, the

NBR2 gene levels decreased either in the presence or absence of C-PC (P < 0.01, Fig. 2B). Thus, C-PC influenced H292 cells.



Fig. 2. C-PC treatment increases the gene expression of *NBR2*. (A) RT-qPCR showing the changes in gene expression of *NBR2* in H292 cells treated with 0, 5, 10 μ M C-PC for 12, 24 and 48 h using two primer sets. (B) RT-qPCR histogram showing the efficiency of *NBR2* shRNA-mediated knock-down in H292 cells. Data are shown as mean \pm SD, *P < 0.05; **P < 0.01. Three replicates were performed for each experiment.

Knock-down of NBR2 attenuated the inhibitory effects of C-PC in H292 cells

To further examine whether the inhibitory effects of C-PC were dependent on NBR2, EdU staining, colony formation and wound-healing assays were carried out. The results of EdU assay demonstrated that C-PC could suppress proliferation of H292 cells (Fig. 3A and B, P < 0.05). However, NBR2 depletion could reverse the inhibitory effect of C-PC (Fig. 3A and B, P < 0.01). Furthermore, shNBR2 could reverse the C-PC inhibitory effect on colony formation in H292 cells (Fig. 3C and D, P < 0.05). We also found that shNBR2 attenuated the effect of C-PC on the inhibition of H292 cell migration (Fig. 3E and F, P < 0.01).

NBR2 knock-down influenced C-PC activity on AMPK/mTOR signalling

Finally, the expression of p-AMPK, p-ACC, p-S6K, and p-S6 was examined in H292 cells treated with C-PC. C-PC not only clearly declined phosphorylation of p-AMPK and p-ACC (Fig. 4A and C), but also reduced the protein levels of total S6K. In contrast, the C-PC treatment up-regulated phosphorylation of p-S6K (Fig. 4B and C). The depletion of NBR2 abolished the C-PC down-regulated p-AMPK and p-ACC, but the expression of p-S6K and p-S6 were not affected by the exposure to C-PC (Fig. 4C). These findings indicated that *NBR2* was a bridging gene between C-PC and the AMPK/mTOR signalling pathway.

Discussion

Lung cancer accounts for almost one-third of all cancer-related deaths. NSCLC is the leading subtype of lung cancer and accounts for around 80 % of all cancers (Nussinov et al., 2021). Distant metastasis and rapid proliferation are the main causes of mortality in patients with NSCLC (Hamouri et al., 2022). Despite the successful application of radiation and chemotherapy for the treatment of NSCLC since the 1990s, accumulating evidence has shown that NSCLC cells could acquire resistance to therapy agents and the additional dose results in unaffordable toxicity (Huang et al., 2017). It is necessary to identify new agents to inhibit the proliferation and metastasis abilities of NSCLC cells and to reveal the molecular mechanisms. C-PC is a natural substance having anti-cancer activity, which is obtained from aquatic species Spirulina platensis (Hao et al., 2019; Ji et al., 2020; Kaur et al., 2020). The role and mechanism of NBR2 in the C-PC inhibition of cancer cells has not been fully elucidated. In this study, we found that C-PC could inhibit proliferation and migration of H292 cells. Next, NBR2 was found to be highly up-regulated in NSCLC cells treated with C-PC based on qRT-PCR. These results indicated that NBR2 was associated with the inhibitory effects of C-PC on the proliferative and invasive ability of NSCLC cells. Furthermore, C-PC could not further activate AMPK and inhibited mTORassociated pathway molecules in H292 cells with NBR2 knock-down. In brief, C-PC exerted significant antitumour effects on NSCLC cells, and NBR2 was an important factor in the C-PC-mediated suppression of NSCLC cell migration and proliferation.

Some studies have found that NBR2 suppresses migration, invasion and EMT of colorectal cancer cells (Dimitrov et al., 2001; Bai et al., 2020). Our study showed that NBR2 was down-regulated in NSCLCs *in vitro*, which is consistent with previous reports that low expression of NBR2 correlated with poor clinical outcomes in NSCLC patients (Gao et al., 2019). Furthermore, we found that NBR2 over-expression inhibited NSCLC cell malignancy by reducing NSCLC cell migration and promoting apoptosis. Therefore, NBR2 has an inhibitory effect on the progression of NSCLC, which suggests that increasing NBR2 transcripts may represent a therapeutic target and interventional strategy in tumour cells.



Fig. 3. C-PC mediates the inhibitory effects on the growth of H292 cells via NBR2. (**A**, **B**) EdU assay showing that the proliferation of H292 cells was enhanced following treatment with 0 or 10 μ M C-PC for 24 h. (**C**, **D**) The clone formation observed after H292 shNBR2-transfected cells were treated with 0 or 10 μ M C-PC for 24 h. (**E**, **F**) Wound-healing assay demonstrated migration of H292 cells following transfection with shNBR2 followed by 0 or 10 μ M C-PC for 24 h. (**E**, **F**) Wound-healing assay demonstrated migration of H292 cells following transfection with shNBR2 followed by 0 or 10 μ M C-PC for 24 h. (**E**, **F**) Wound-healing assay demonstrated migration of H292 cells following transfection with shNBR2 followed by 0 or 10 μ M C-PC for 24 h. Three replicates were performed for each experiment. Data are shown as the mean ± SD. *P < 0.05, **P < 0.01.



Fig. 4. Knock-down of *NBR2* in H292 cells compromised the activity of C-PC in activation of the AMPK signalling pathway. (**A**, **B**) Western blotting revealed that the protein phosphorylation of AMPK, ACC, S6K, and S6 was enhanced after H292 cells were transfected with the shNBR2 plasmid followed by 0 or 10 μ M C-PC for 24 h. (**C**) Quantitative analysis of the level of protein phosphorylation of AMPK, ACC, S6K, and S6 following transfection of H292 cells with shNBR2 was followed by exposure to 0 or 10 μ M C-PC for 24 h. Data are shown as the mean \pm SD. Three replicates were performed for each experiment.

C-PC has been definitively demonstrated to exert multiple anti-cancer functions in lung (Bingula et al., 2016), breast (Baudelet et al., 2013), liver (Basha et al., 2008), and cervical cancer (Yuan and Gurunathan, 2017; Hao et al., 2019) and in melanoma. Some studies have found that C-PC down-regulates EMT in cervical cancer cells (Ji et al., 2020). As a key sensor of cellular energy status, the AMPK pathway plays an important role in regulating tumour cell growth and proliferation (Inoki et al., 2012; Li et al., 2021). AMPK can be activated by C-PC in HepG2 liver cancer cells (Ren et al., 2018). Recently, AMPK activity has been shown to be regulated by interacting directly with NBR2 (Liu et al., 2016). Our study demonstrated that C-PC is also an important negative factor in NSCLC cells. Furthermore, NBR2 expression could be significantly enhanced by C-PC in NSCLC cells, while NBR2 depletion reversed the inhibitory effect of C-PC on NSCLC cells. These studies established that C-PC could have an important influence on the progression of NSCLC by increasing NBR2 expression and triggering activation of AMPK signalling. In NSCLC, abnormal activation of the mTOR signalling pathway has been closely related to the resistance to apoptosis of tumour cells and to promote tumour metastasis (Deng et al., 2018; Wang et al., 2018). Recent studies have shown that mTOR inhibition promotes transmission of DNA damage signals to the apoptotic machinery and enhances antitumour effects in NSCLC (Piao et al., 2016). In this study, C-PC inhibited downstream mTOR signalling in NSCLC cells, a mechanism that can increase the loss of DNA damage recognition and enhance the resistance of tumour cells.

Conclusion

In this study, we provide evidence that C-PC inhibits migration and promotes apoptosis of NSCLC cells. These effects are exerted by up-regulating NBR2 expression and regulating activity of the AMPK/mTOR signalling pathway. Thus, C-PC may represent a potential anti-cancer agent for the treatment of NSCLC.

Conflict of interest

The authors have no conflict of interest to declare.

Author contributions

(I) study concepts and design: Chaowen He; (II) data acquisition: Chaowen He, Dongxuan Huang, Dongsheng Huang, Jianfeng Peng; (III) data analysis: Chaowen He, Fan Yang; (IV) manuscript preparation and editing: all authors.

References

- Abd El-Baky, H. H., El-Baroty, G. S. (2012) Characterization and bioactivity of phycocyanin isolated from Spirulina maxima grown under salt stress. *Food Funct.* **3**, 381-388.
- Bai, J., Xu, J., Zhao, J., Zhang, R. (2020) LncRNA NBR2 suppresses migration and invasion of colorectal cancer cells by downregulating miRNA-21. *Hum. Cell* 33, 98-103.
- Basha, O. M., Hafez, R. A., El-Ayouty, Y. M., Mahrous, K. F., Bareedy, M. H., Salama, A. M. (2008) C-Phycocyanin inhibits cell proliferation and may induce apoptosis in human HepG2 cells. *Egypt. J. Immunol.* 15, 161-167.
- Baudelet, P. H., Gagez, A. L., Berard, J. B., Juin, C., Bridiau, N., Kaas, R., Thiery, V., Cadoret, J. P., Picot, L. (2013) Antiproliferative activity of Cyanophora paradoxa pigments in melanoma, breast and lung cancer cells. *Mar. Drugs* 11, 4390-4406.
- Bingula, R., Dupuis, C., Pichon, C., Berthon, J. Y., Filaire, M., Pigeon, L., Filaire, E. (2016) Study of the effects of betaine and/or C-phycocyanin on the growth of lung cancer A549 cells in vitro and in vivo. J. Oncol. 2016, 8162952.
- Brozos-Vazquez, E. M., Diaz-Pena, R., Garcia-Gonzalez, J., Leon-Mateos, L., Mondelo-Macia, P., Pena-Chilet, M., Lopez-Lopez, R. (2020) Immunotherapy in nonsmall-cell lung cancer: current status and future prospects for liquid biopsy. *Cancer Immunol. Immunother.* **70**, 1177-1188.
- Cian, R. E., Lopez-Posadas, R., Drago, S. R., de Medina, F. S., Martinez-Augustin, O. (2012) Immunomodulatory properties of the protein fraction from Phorphyra columbina. J. Agric. Food Chem. 60, 8146-8154.
- Deng, H., Gao, X., Peng, H., Wang, J., Hou, X., Xu, H., Yang, F. (2018) Effect of liposome-mediated HSP27 transfection on collagen synthesis in alveolar type II epithelial cells. *Mol. Med. Rep.* **17**, 7319-7324.
- Dimitrov, S. D., Matouskova, E., Forejt, J. (2001) Expression of BRCA1, NBR1 and NBR2 genes in human breast cancer cells. *Folia Biol. (Praha)* **47**, 120-127.

- Gao, Y. P., Li, Y., Li, H. J., Zhao, B. (2019) LncRNA NBR2 inhibits EMT progression by regulating Notch1 pathway in NSCLC. Eur. Rev. Med. Pharmacol. Sci. 23, 7950-7958.
- Hamouri, S., Alrabadi, N., Syaj, S., Abushukair, H., Ababneh, O., Al-Kraimeen, L., Al-Sous, M., Hecker, E. (2022) Atrial resection for T4 non-small cell lung cancer with left atrium involvement: a systematic review and meta-analysis of survival. *Surg. Today*, online ahead of print, doi: 10.1007/ s00595-021-02446-8.
- Hao, S., Li, S., Wang, J., Zhao, L., Yan, Y., Wu, T., Zhang, J., Wang, C. (2019) C-Phycocyanin suppresses the in vitro proliferation and migration of non-small-cell lung cancer cells through reduction of RIPK1/NF-κB activity. *Mar. Drugs* 17, 362.
- Huang, C. Y., Ju, D. T., Chang, C. F., Muralidhar Reddy, P., Velmurugan, B. K. (2017) A review on the effects of current chemotherapy drugs and natural agents in treating non-small cell lung cancer. *Biomedicine (Taipei)* 7, 23.
- Inoki, K., Kim, J., Guan, K. L. (2012) AMPK and mTOR in cellular energy homeostasis and drug targets. *Annu. Rev. Pharmacol. Toxicol.* 52, 381-400.
- Ji, H., Liu, G., Han, J., Zhu, F., Dong, X., Li, B. (2020) Cphycocyanin inhibits epithelial-to-mesenchymal transition in Caski cells. *Cancer Cell Int.* 20, 292.
- Jiang, L., Wang, Y., Liu, G., Liu, H., Zhu, F., Ji, H., Li, B. (2018) C-Phycocyanin exerts anti-cancer effects via the MAPK signaling pathway in MDA-MB-231 cells. *Cancer Cell Int.* 18, 12.
- Kaur, P., Dhandayuthapani, S., Venkatesan, T., Gantor, M., Rathinavelu, A. (2020) Molecular mechanism of C-phycocyanin induced apoptosis in LNCaP cells. *Bioorg. Med. Chem.* 28, 115272.
- Li, H. B., Chen, J. K., Su, Z. X., Jin, Q. L., Deng, L. W., Huang, G., Shen, J. N. (2021) Cordycepin augments the chemosensitivity of osteosarcoma to cisplatin by activating AMPK and suppressing the AKT signaling pathway. *Cancer Cell Int.* 21, 706.
- Liu, X., Xiao, Z. D., Han, L., Zhang, J., Lee, S. W., Wang, W., Lee, H., Zhuang, L., Chen, J., Lin, H. K., Wang, J., Liang, H., Gan, B. (2016) LncRNA NBR2 engages a metabolic checkpoint by regulating AMPK under energy stress. *Nat. Cell Biol.* 18, 431-442.
- Liu, Z., Cao, H., Shi, Y., Yang, R. (2019) KIAA1211 plays an oncogenic role in human non-small cell lung cancer. J. Cancer 10, 6747-6753.
- Lu, W., Zhang, H., Niu, Y., Wu, Y., Sun, W., Li, H., Kong, J., Ding, K., Shen, H. M., Wu, H., Xia, D., Wu, Y. (2017) Long non-coding RNA linc00673 regulated non-small cell lung cancer proliferation, migration, invasion and epithelial mesenchymal transition by sponging miR-150-5p. *Mol. Cancer* 16, 118.
- Miller, K. D., Nogueira, L., Mariotto, A. B., Rowland, J. H., Yabroff, K. R., Alfano, C. M., Jemal, A., Kramer, J. L., Siegel, R. L. (2019) Cancer treatment and survivorship statistics, 2019. *CA Cancer J. Clin.* **69**, 363-385.
- Mitra, S., Siddiqui, W. A., Khandelwal, S. (2015) C-Phycocyanin protects against acute tributyltin chloride neurotoxicity by modulating glial cell activity along with its anti-oxidant and anti-inflammatory property: a comparative efficacy

evaluation with N-acetyl cysteine in adult rat brain. *Chem. Biol. Interact.* **238**, 138-150.

- Nussinov, R., Tsai, C. J., Jang, H. (2021) Anticancer drug resistance: an update and perspective. *Drug Resist. Updat.* 59, 100796.
- Pandya, G., Kirtonia, A., Sethi, G., Pandey, A. K., Garg, M. (2020) The implication of long non-coding RNAs in the diagnosis, pathogenesis and drug resistance of pancreatic ductal adenocarcinoma and their possible therapeutic potential. *Biochim. Biophys. Acta Rev. Cancer* **1874**, 188423.
- Piao, J., Chen, L., Quan, T., Li, L., Quan, C., Piao, Y., Jin, T., Lin, Z. (2016) Superior efficacy of co-treatment with the dual PI3K/mTOR inhibitor BEZ235 and histone deacetylase inhibitor Trichostatin A against NSCLC. *Oncotarget* 7, 60169-60180.
- Rao, X., Huang, X., Zhou, Z., Lin, X. (2013) An improvement of the 2^{(-delta} delta CT) method for quantitative real-time polymerase chain reaction data analysis. *Biostat. Bioinforma. Biomath.* 3, 71-85.
- Ren, Z., Xie, Z., Cao, D., Gong, M., Yang, L., Zhou, Z., Ou, Y. (2018) C-Phycocyanin inhibits hepatic gluconeogenesis and increases glycogen synthesis via activating Akt and AMPK in insulin resistance hepatocytes. *Food Funct.* 9, 2829-2839.
- Rio, D. C., Ares, M. Jr., Hannon, G. J., Nilsen, T. W. (2010) Purification of RNA using TRIzol (TRI reagent). *Cold Spring Harb. Protoc.* 2010, pdb.prot5439.
- Schmidt, L. H., Görlich, D., Spieker, T., Rohde, C., Schuler, M., Mohr, M., Humberg, J., Sauer, T., Thoenissen, N. H., Huge, A., Voss, R., Marra, A., Faldum, A., Müller-Tidow, C., Berdel, W. E., Wiewrodt, R. (2014) Prognostic impact of Bcl-2 depends on tumor histology and expression of MALAT-1 lncRNA in non-small-cell lung cancer. J. Thorac. Oncol. 9, 1294-1304.
- Schutt, M., Kern, W., Zimmermann, A., Busch, P., Kerner, W., Voll, A., Wagner, C., Kann, P. H., Dapp, A., Holl, R. W.; DPV initiative; German BMBF Competence Network Diabetes mellitus (2010) Association of antidiabetic therapies to glycemic control and to body weight in type 2 diabetes: a German multicenter analysis on 9294 patients. *Exp. Clin. Endocrinol. Diabetes* **118**, 490-495.
- Sonani, R. R., Patel, S., Bhastana, B., Jakharia, K., Chaubey, M. G., Singh, N. K., Madamwar, D. (2017) Purification and antioxidant activity of phycocyanin from Synechococcus sp. R42DM isolated from industrially polluted site. *Bioresour. Technol.* 245, 325-331.

- Ulitsky, I., Bartel, D. P. (2013) lincRNAs: genomics, evolution, and mechanisms. *Cell* **154**, 26-46.
- Wang, H., Liu, Y., Gao, X., Carter, C. L., Liu, Z. R. (2007) The recombinant beta subunit of C-phycocyanin inhibits cell proliferation and induces apoptosis. *Cancer Lett.* 247, 150-158.
- Wang, P., Liu, X. M., Ding, L., Zhang, X. J., Ma, Z. L. (2018) mTOR signaling-related microRNAs and cancer involvement. J. Cancer 9, 667-673.
- Xu, C. F., Brown, M. A., Nicolai, H., Chambers, J. A., Griffiths, B. L., Solomon, E. (1997) Isolation and characterisation of the NBR2 gene which lies head to head with the human BRCA1 gene. *Hum. Mol. Genet.* 6, 1057-1062.
- Xue, L., Li, J., Lin, Y., Liu, D., Yang, Q., Jian, J., Peng, J. (2020) m⁶ A transferase METTL3-induced lncRNA AB-HD11-AS1 promotes the Warburg effect of non-small-cell lung cancer. J. Cell. Physiol. 236, 2649-2658.
- Yang, W., Zheng, Z., Yi, P., Wang, S., Zhang, N., Ming, J., Tan, J., Guo, H. (2020) LncRNA NBR2 inhibits the malignancy of thyroid cancer, associated with enhancing the AMPK signaling. *Front. Oncol.* **10**, 956.
- Yang, Y., Kuang, L., Li, L., Wu, Y., Zhong, B., Huang, X. (2021) Distinct mitochondria-mediated T-cell apoptosis responses in children and adults with coronavirus disease 2019. J. Infect. Dis. 224, 1333-1344.
- Yang, Y., Wu, Y., Meng, X., Wang, Z., Younis, M., Liu, Y., Wang, P., Huang, X. (2022) SARS-CoV-2 membrane protein causes the mitochondrial apoptosis and pulmonary edema via targeting BOK. *Cell Death Diff.* **29**, 1395-1408.
- Yuan, Y. G., Gurunathan, S. (2017) Combination of graphene oxide-silver nanoparticle nanocomposites and cisplatin enhances apoptosis and autophagy in human cervical cancer cells. *Int. J. Nanomedicine* **12**, 6537-6558.
- Zhang, L., Wang, Y., Xia, S., Yang, L., Wu, D., Zhou, Y., Lu, J. (2020a) Long noncoding RNA PANDAR inhibits the development of lung cancer by regulating autophagy and apoptosis pathways. J. Cancer 11, 4783-4790.
- Zhang, X. Z., Liu, H., Chen, S. R. (2020b) Mechanisms of long non-coding RNAs in cancers and their dynamic regulations. *Cancers* (*Basel*) 12, 1245.