The effect of Lfcin-B on non-small cell lung cancer H460 cells is mediated by inhibiting VEGF expression and inducing apoptosis

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Abstract Lfcin-B, an antimicrobial peptide found in various exocrine secretions of mammals, showed antitumor effects. However, the effect and relative mechanism of Lfcin-B on non-small cell lung cancer is unclear. In this study, assay of cell viability, quantitative real-time PCR, Western blot, annexin V/propidium iodide assay, flow cytometry and tumor-xenograft model were applied to elucidate the mechanism of Lfcin-B on non-small cell lung cancer NCI-H460 (H460) cells. Lfcin-B significantly suppressed the proliferation of H460 cells in vitro. Additionally, the transcription and translation of the VEGF gene in H460 cells were restrained after exposure to Lfcin-B. Moreover, the apoptosis of H460 cells was induced by Lfcin-B through stimulating caspase-3, caspase-9 and preventing survivin expression on both the transcription and translation level. Meanwhile, Lfcin-B increased the production of reactive oxygen species and suppressed the RNA of antioxidant enzymes (GPX1, GPX2, SOD3 and catalase) in H460 cells. Finally, Lfcin-B significantly prevented the tumor growth in the H460-bearing mice model. These results indicated that Lfcin-B could be a potential candidate for the treatment of lung cancer.

Keywords Lfcin-B · Non-small cell lung cancer · Apoptosis · VEGF · ROS

Introduction

Lung cancer is the leading cause of cancer deaths worldwide (Hecht 1999; Jemal et al. 2008). Epithelial lung cancer is primarily divided into two groups: non-small cell lung cancer (NSCLC), which is predominant and represents 85 % of all diagnosed lung cancers, and small cell lung cancer, which accounts for approximately 15 % of all lung cancers (Scrima et al. 2012; Kamangar et al. 2006). Traditional chemotherapy regimen was primarily used in the clinical treatment of NSCLC, but the toxicity limited its utilization (Gao et al. 2009). Hence, scientists are eager to find anticancer drugs that could prevent lung cancer.

Angiogenesis is the growth of new blood vessels from pre-existing blood supply (Emmett et al. 2011) and is essential for tumor growth and metastasis (Hanahan and Weinberg 2000). It has been reported that the inhibition of angiogenesis might be an effective therapeutic approach against cancer (Folkman 1971). Vascular endothelial growth factor (VEGF), a heparin-binding family of glycoproteins (including VEGF-A, VEGF-B, VEGF-C, and VEGF-D), is a key player in angiogenesis for promoting endothelial cell proliferation, migration, survival, vasodilatation, and vasculogenesis through recruiting bone marrow-derived haematopoietic progenitor cells (Ferrara and Henzel 1989). Moreover, VEGF, is believed to be one of the most specific and critical regulators of angiogenic signaling cascades (Ferrara 2002). Moreover, VEGF was associated with angiogenesis of NSCLC (Farhat et al. 2012; Pallis and Syrigos 2013). Hence, anti-lung cancer drugs, which could inhibit VEGF, attracted scientists’ attentions.

Antimicrobial peptides (AMPs), isolated from a wide range of organisms, including prokaryotes, insects, fish, amphibians and mammals (Wang et al. 2011), are active...
against a broad range of bacteria, fungi, viruses, and protozoa (Okumura 2011). AMPs, mostly cationic and amphipathic molecules (Tossi and Sandri 2002), can bind to target bacteria via electrostatic interactions to disrupt the membrane structure or inhibit fundamental metabolism to kill bacteria (Epand and Vogel 1999; Zasloff 2002; Brogden 2005). Moreover, recently the anticancer activity of AMPs has attracted wide attentions for selectively killing cancer cells, whose membrane proteins are negatively charged through glycosylation (Mader and Hoskin 2006; Kim and Varki 1997).

Lactoferrin (LF) is an antimicrobial protein found in various exocrine secretions of mammals leukocytes upon infections. Lactoferricin B (Lfcin-B), derived from the N-terminal region of LF (Wakabayashi et al. 1998). It has been reported that LF and Lfcin-B had wide antimicrobial activities against bacteria, fungi, and virus (Henry and Alexis 2009). Additionally, previous researchers have found that Lfcin-B inhibited the human leukemia cells growth (Yoo et al. 1997), and Lfcin-B-derived peptides exhibited an anti-gastric cancer activity and chondroprotective effects (Pan et al. 2013; Yan et al. 2013). However, the antitumor effect and clear mechanism of Lfcin-B on lung cancer cells were not yet been investigated.

In the present work we investigated the activity and the mechanism of Lfcin-B on human NSCLC NCI-H460 (H460) cells. The results obtained revealed Lfcin-B suppressed H460 cells’ proliferation via inducing apoptosis and preventing the VEGF gene in vitro. Lfcin-B induced the apoptosis in H460 cells was dependent on up-regulating of ROS generation, inhibiting survivin protein and activating of caspase-3 and caspase-9 on both transcription and translation level. These interferences then further inhibited tumor growth of H460 in nude mice. These results highlight the therapeutic potential of Lfcin-B in NSCLC.

Materials and methods

Regents

Purified Lfcin-B (FKCRRWQWRMKKLGAPSITCVARRAF), a cationic peptide corresponding to residues 17–41 near the N-terminus of LF, was synthesized by GL Biochem (Shanghai, China) via a stepwise solid phase methodology. The resulting peptide was purified by a Sephadex gel column and HPLC, and the homogeneity of the purified peptide was greater than 98 %. Synthetic AMP was reconstituted in phosphate-buffered saline (PBS, pH 7.4) for subsequent experiments. 3-(4,5-Dimethylthiazol-2-yl)-2,5-di-phenyl tetrazolium bromide (MTT), sodium pyruvate and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). BCA kit was purchased from Tiangen (Beijing, China). Annexin V-fluorescein (AV) and Propidium iodide (PI) were purchased from Mbchemic (Shanghai, China). The easy Plus Mini Kit,Script Select cDNA Synthesis Kit, SyberGreen qPCR primer and iCycleriQ multi-color real time PCR detection system were purchased from KeyGEN. SYBR® Premix Ex TaqTMIII was purchased from TaKaRa. The cell lysis buffer was purchased from Generay (Shanghai, China).

Cell culture

Human NSCLC cells NCI-H460 (H460) was obtained from ATCC (Manassas, Virginia, USA) and maintained in RPMI-1640 medium supplemented with 10 % fetal calf serum (Gibco, Vienna, NY, USA), 100 U/ml of penicillin and 100 U/ml of streptomycin. Cells were cultured at 37 °C in a humidified incubator containing 5 % CO₂.

Experimental animals

Nude mice were purchased from the Academy of Military Medical Science and housed in a rodent facility at 22 ± 1 °C with a 12 h light–dark cycle and provided with continuous standard rodent chow and water for acclimatization. All procedures involving animals and their care in this study were in strict accordance with protocols approved by the Ethics Committee of Jilin University.

Analysis of cell viability

To evaluate the effect of Lfcin-B on H460 cells, MTT assay was performed as previously described (Wang et al. 2013). Cells in the logarithmic phase of growth were collected and seeded on 96-well plates at a concentration of 5 × 10³ cells/well with RPMI-1640 medium that contained 10 % fetal calf serum. After 12 h, different concentrations (range from 0 to 100 µg/ml) of Lfcin-B were added to the cells and further incubated for 4, 6, 12 and 24 h. After incubation, the culture medium was removed, and the cells were washed with PBS. Then the cells were further incubated with an MTT solution (5 µg/ml) for 4 h. Next, the cells were lysed and the purple formazan crystals were solubilized for detection at 490 nm with an ELISA reader. The cell viability was calculated with the following formula: 

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\text{cell viability (\%)} = \frac{OD_A}{OD_B} \times 100 \%
\]

where ODₐ is the absorbance at 490 nm of the experimental group and ODₐ is that of the control group.

Quantitative real-time polymerase chain reaction

Total RNA was extracted from H460 cells treated with or without Lfcin-B (ranging from 0 to 80 µg/ml) for 24 h
using the RNeasy Mini Kit (Qiagen Sciences, Germantown, MD) according to the manufacturer’s instructions. First-strand cDNA was synthesized from total RNA using Super Script II first-strand synthesis system (Invitrogen). PCR primers for VEGF, survivin, caspase 3, caspase 9, GPX1, GPX2, peroxiredoxin (PRDX) 1, PRDX2, thioredoxin reductase (TXNRD) 1, SOD1, SOD2, SOD3, and catalase were customer-designed and obtained from Integrated DNA Technologies (Coralville, IA). The threshold cycle (Ct) value for each gene was normalized to the Ct value of glyceraldehyde 3 phosphate dehydrogenase (GAPDH) or β-actin. The relative mRNA expression was calculated using the comparative CT ($2^{-ΔΔCt}$) method.

The sequences of the primers are shown as follows: VEGF (forward 5′-CAATCCACATGGCGATGCATG-3′, reverse 5′-GTCTGTTTGGCGCCTTTC-3′); Survivin (forward 5′-AAATGCACTCCAGCCTCTGT-3′, reverse 5′-TGTCGAAGCTTTCAGGT-3′); PRDX1 (forward 5′-CTAGTTCGGACACAGGAAATGG-3′, reverse 5′-CAGGCTGTTGTTGCAAGGCATTC-3′); GPX2 (forward 5′-AACCAGTTCGGACACAGGAA-3′, reverse 5′-GGCAAAGACAGGATGCTCGTTCTG-3′); TXNRD1 (forward 5′-GTCATCTGGCATGGATTAACACAC-3′, reverse 5′-CCCTGAAGAGATACCTTCATCG-3′); PRDX2 (forward 5′-CACCCACCTGGCGTGGATCAATAC-3′, reverse 5′-AAAGAGACCCCTGTGTAAGGACTCC-3′); TXNRD1 (forward 5′-CTCTTGGGACCAGATGGGGTCTC-3′, reverse 5′-CACGGCTGTTGTTGCAAGGCATTC-3′); catalase (forward 5′-CCAGTCATGCTTCACTGTGTCTTC-3′, reverse 5′-TGCAAGTTCCATTACAA-3′); SOD2 (forward 5′-ATGGAGCTAGGACGACGAA-3′, reverse 5′-ATGGGGACAATACACAAGGCTGTAC-3′); SOD3 (forward 5′-CTGGCTCGGGGTTACTGCCAG-3′, reverse 5′-AAATGCACTCCAGCCTCTGT-3′);

The ROS production was assessed by flow cytometry using 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) as previously described (Piemme et al. 2013). Briefly, H460 cells were incubated in 6-well plates with different concentrations of Lfcin-B (ranging from 0 to 80 μg/ml) for 24 h. 30 min before the end of the experiment, the cell culture was treated with DCFH2-DA (50 μM) and keep in the dark. Cells were then collected, centrifuged (200 g; 4 °C; 5 min) and the pellet was washed with 1 ml of PBS and centrifuged. Then, the fluorescence intensity was measured at 240 nm for excitation and 530 nm for emission with a flow cytometer to elucidate the production of ROS.

Flow cytometric detection of apoptotic cells

Cell apoptosis assays (Paredes-Gamero et al. 2012) were conducted to evaluate the extent of apoptosis of Lfcin-B to H460 cells followed the AV-FITC/PI apoptosis detection kit (Mbchemic, Shanghai, China). Briefly, H460 cells were incubated in 6-well plates with different concentrations of Lfcin-B (ranging from 0 to 80 μg/ml) for 24 h. The cells were harvested and washed twice with PBS, then for the AV/PI assay were dealt with as the instructions of the manufacturer told. After that, cells were analyzed by a flow cytometer (BD Biosciences, San Jose, CA, USA). The fraction of the cell population in different quadrants was analyzed by using quadrant statistics, that is cells in the lower right (LR) quadrant represented early apoptotic cells, and cells in the upper right (UR) quadrant represented late apoptotic cells. Percent apoptosis (%)$=\frac{[(\text{number of apoptotic cells})-(\text{number of total cells observed})]}{\text{number of total cells observed}} \times 100\%.$

Reactive oxygen species (ROS) assay

The ROS production was assessed by flow cytometry using 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) as previously described (Piemme et al. 2013). Briefly, H460 cells were treated with Lfcin-B at different concentrations (0, 20, 40, 80 μg/ml) for 24 h. 30 min before the end of the experiment, the cell culture was treated with DCFH2-DA (50 μM) and keep in the dark. Cells were then collected, centrifuged (200 g; 4 °C; 5 min) and the pellet was washed with 1 ml of PBS and centrifuged. Then, the fluorescence intensity was measured at 240 nm for excitation and 530 nm for emission with a flow cytometer to elucidate the production of ROS.
Detection of active caspase-3 and active caspase-9 expression

Active caspase-3 and -9 were assessed using the active caspase-3 and -9 staining kits, respectively, (BioVision, Mountain View, CA, USA) and measured according to the manufacturer’s instructions (Cheng et al. 2012). H460 cells were incubated with different concentrations of Lfcin-B (ranging from 0 to 80 μg/ml) for 24 h. After incubation, cell pellets were washed twice with PBS and scrapped with ice-cold lysis buffer. 4 × 10⁶ cells were lysed in 100 ml lysis buffer and incubated with the colorimetric caspase substrate. The active caspase-3 and active caspase-9 expressions were measured accordingly: the fluorescence intensity was measured at 405 nm with a flow cytometer to detect the production of active caspase-3/9.

H460-xenograft mouse model and Lfcin-B administration in vivo

Human NSCLC H460 cells were collected in the logarithmic phase of growth, diluted with normal saline, and the cell suspension (0.1 ml, 5 × 10⁷ cells) was transplanted subcutaneously into the right axilla of each nude mouse. When the tumor grew to 100–300 mm³, mice were randomly divided into five groups: a model control group administered with normal saline, three Lfcin-treated groups administered at 25, 50 or 75 mg/kg body weight. Each group contained six mice. These solutions were dissolved in saline, filtered through a 0.22 μm Millipore filter and administered by intra-tumor injection (50 μl) three times a week. After three weeks of treatment, mice from all the groups were sacrificed by cervical dislocation 24 h after the final administration. The tumor weights of the mice from each group were measured. During the treatment, the tumor volume of each mouse was measured every 3 days.

The antitumor activity was expressed as inhibitory rate (%) and calculated as [(A - B)/A] × 100 %, where A and B were the average tumor weight of the model and Lfcin-B groups, respectively.

The tumor volume (TV) was measured and calculated using the following formula: TV = 1/2 × a × b², where a and b are the long and short diameters of the tumors in each mouse, respectively.

Statistical analysis

The data were first tested for normality (using the Anderson–Darling test) and for homogeneity of variance prior to any further statistical analysis. All experiments elaborated above were performed at least in three replicates. The values were presented as the mean ± SD. Statistical analysis was performed by one-way ANOVA, followed by Dunnett’s multiple comparison or a Student’s T test. P < 0.05 were considered to be statistically significant.

Results

Lfcin-B suppresses cell proliferation of H460 cells

To clarify the effects of Lfcin-B on lung cancer cells in vitro, H460 cells, a kind of human NSCLC cell lines, were treated with Lfcin-B at different concentrations of 20, 40, 60 and 80 μg/ml for 4, 6, 12 and 24 h. As the Fig. 1 presented, Lfcin-B treatment group inhibited cell viability in a dose-dependent and time-dependent manner. Values are presented as mean ± SD from three separate determinations with six replicates each.

Lfcin-B inhibits the VEGF and survivin gene expression in H460 cells

It has been reported that NSCLC was associated with angiogenesis, and VEGF, which produced by tumor cells under hypoxic conditions, is consider to be one of the most pivotal and specific regulators in angiogenic signaling cascades. Then, we detect the effect of Lfcin-B on VEGF in H460 cells. The quantitative real-time PCR (qRT-PCR) was performed to determine the RNA levels of VEGF when H460 cells after exposure to Lfcin-B for 24 h. As the Fig. 2a presented, the decrease in VEGF RNA levels was
occurred in a dose-dependent manner and showed significant differences \((P < 0.01)\) at the 40 \(\mu\)g/ml and 80 \(\mu\)M of Lfcin-B, when compared to the controls. Additionally, to explore whether Lfcin-B could affect the protein expression of VEGF, western blotting was performed. The effect of Lfcin-B on H460 cells on the protein levels of VEGF was consistent with its effect on RNA levels (Fig. 2c, d). These data indicated that Lfcin-B could inhibit the transcription and translation of VEGF gene.

Survivin was an inhibitory molecule in apoptosis and associated with VEGF (O’Connor et al. 2000). Hence, the effect of Lfcin-B on H460 cells in the expression of survivin was investigated. The results indicated that both the RNA level and the protein expression of survivin in H460 cells were down-regulated after Lfcin-B treatment (Fig. 2b–d).

Lfcin-B induces cell apoptosis of H460 cells

To test whether Lfcin-B induced cell death through apoptosis, the percentage of apoptotic cells was measured by the AV/PI assay. Positive staining with AV-FITC correlated with the loss of membrane polarity, and the complete loss of membrane integrity lead to apoptosis, and PI entered the cells after the loss of membrane integrity. Therefore, dual staining with AV and PI were adopted for discriminating between unaffected and apoptotic cells. The results indicated that Lfcin-B induced apoptotic effects on H460 cells (Fig. 3a). Specifically, the induced apoptotic cell accumulation reached to approximately 71.7 %, at the dose of 80 \(\mu\)g/ml (Fig. 3b). Therefore, induced apoptosis could be one mechanism for Lfcin-B in preventing the proliferation of H460 cells.
Lfcin-B stimulates the production of ROS in H460 cells

One mechanism of apoptosis induction is mediated with up-regulating the intracellular ROS production. As presented in Fig. 4a, b, the ROS levels were increased after the treatment of Lfcin-B. Specifically, the mean fluorescence intensity in the 80 µg/ml Lfcin-B group was 37.8, which was elevated 6.57-fold compared to that in the control group.

In order to gain more insights into the mechanisms by which Lfcin-B regulates ROS production in H460 cells, we analyzed the expression of mRNA of several important antioxidant enzymes by real-time PCR. As shown in Fig. 4c, the expression of GPX1, GPX2, SOD3 and catalase mRNA in H460 cells was down-regulated after treatment with 60 µg/ml Lfcin-B, while the expression of all other antioxidant enzymes examined was not significantly changed. The results mentioned above suggested that Lfcin-B could induce oxidative stress in H460 cells at least in part by down-regulating the expression of GPX1, GPX2, SOD3 and catalase.

Lfcin-B activates the caspase-3 and caspase-9 in H460 cells

Considering that caspase-3 and caspase-9 are activated during the early stage of apoptosis, Hence, they are treated as the markers of apoptotic cells. The results indicated that
both caspase-3 and caspase-9 were activated in the Lfcin-B-treated H460 cells (Fig. 5a–d). Moreover, the similar results were obtained in the real-time PCR assay, the RNA levels of caspase-3 and caspase-9 in the H460 cells were increased after exposure to Lfcin-B (Fig. 5e, f).

**Discussion**

Lung cancer was the most common malignant cancer with high fatality rate around the world, and most of the drugs being used in clinic had remarkable side effects. Hence, it is necessary to develop new drugs with high efficiency and low toxicity.

Recently, AMPs have attracted significant attentions for their novel mechanisms, including decreased likelihood of drug resistance and low intrinsic cytotoxicity (Chen et al. 2012; Schweizer 2009). As previously reported, cationic AMPs exert their cytolytic activity by folding into an amphipathic helix and inserting into the target membrane, then leading to the breakdown of membrane structure, leakage of cell contents and cell death (Zhou et al. 2011). Lfcin-B, an LF-derived AMP has been shown possessing broad-spectrum antimicrobial activity and antivirus effects (Hsiao et al. 2009). However, its anti-lung cancer activity had not been elucidated. In this present study, we investigated the anticancer activity and mechanisms of Lfcin-B on human NSCLC H460 cells.

In order to detect whether Lfcin-B could prevent the H460 growth in vivo, the H460-bearing nude mice were established. As the Fig. 6 shown, Lfcin-B significantly inhibited the H460 growth as the dose increased. Specifically, at the 75 mg/kg group, the tumor inhibitory rate was up to 67.4 %. These results suggested that Lfcin-B administration prevented the H460 tumor growth in mice.
As we known, angiogenesis is critical in the development of tumor growth and is regulated by various growth factors (Roskoski 2007) and VEGF is an important regulator in the angiogenic signaling cascades (Ferrara 2002). Researchers have reported that increased levels of VEGF correlated with angiogenesis of NSCLC (Farhat et al. 2012; Pallis and Syrigos 2013). Therefore, VEGF was an important therapeutic target for malignant lung cancer, especially in NSCLC. The results obtained illustrated that Lfcin-B remarkably inhibited the transcription and translation of the VEGF gene in H460 cells in a dose-dependent manner (Fig. 2a). Additionally, studies have reported that VEGF expression was correlated with apoptosis (Benjamin et al. 1999; Benjamin and Keshet 1997) and VEGF suppressed cell death through autocrine mechanism (Ferrer et al. 1999; Langer et al. 2000; Wu et al. 2003). It is reasonable to speculate that Lfcin-B inhibited the VEGF expression, which might lead to induce the apoptosis of H460 cells.

The Annexin V/PI double staining assay suggested that Lfcin-B induced the apoptosis in H460 cells (Fig. 3). There are various regulators contributed to apoptosis, among them, ROS and caspase cascade were usually investigated. ROS was known widely to be involved in cellular signaling, affecting most aspects of cellular function including gene expression, proliferation, differentiation, and migration. Furthermore, the ROS pathway was reported to be one of the three intrinsic mediated apoptosis pathways (Pieme et al. 2013). In this study, Lfcin-B could not only increased the ROS production in H460 cells, but also restrained the RNA level of several antioxidant enzymes, including GPX1, GPX2, SOD3 and catalase (Fig. 4). This result indicated that Lfcin-B-induced the production of ROS was involved in the apoptotic effects. Additionally, other apoptosis pathways were the caspase-dependent pathway and mitochondria-involved signaling. The caspases are a subfamily of cysteine proteases that play an active regulatory role in the apoptotic cascade. Based on their order of
activation, the caspases are classified into two families: the initiator and the effectors caspases (Sun et al. 2011; Yan et al. 2009). Among them, caspase-3 and caspase-9 have attracted abroad attentions for they were activated in the early stage of apoptosis and treated as the markers of apoptotic cells. Caspase-9 is the key initiator caspase for the intrinsic pathway to induce cell death. Upon cleavage and activation from its pro-form, caspase-9 cleaves and activates caspase-3 which is the most specific proteases involved in the apoptosis mechanism. In conclusion, the activation sequence of these components in mammalian cells appears to be as follows: procaspase-9, caspase-9, procaspase-3, caspase-3 and apoptosis (Delivoria-Papado- poulos and Mishra 2007). Our present study has found that both the RNA and expression of caspase-3 and caspase-9 increased significantly in the Lfcin-B-treated group which indicated that the Lfcin-B could induce the apoptosis H460 cells were partly via the activation of caspase-9 and caspase-3 (Fig. 5). From the results mentioned above, we may conclude that both the upregulated ROS production and the activated caspase-3/9 contributed to the Lfcin-B induced apoptosis.

Survivin has recently been described as a multifunctional regulator of cellular angiogenesis and apoptosis (O’Connor et al. 2000). Although rarely expressed in terminally differentiated adult tissues, up regulation of survivin is found in most cancers (Song et al. 2009). In our study, we found that the Lfcin-B could significantly decrease the transcription and the translation of VEGF and the survivin in H460 cells (Fig. 2b–d). These data were in consistent with the data shown in cell apoptosis assay. Lfcin-B exhibited a remarkable inhibitory effect on H460 cells in vitro, suppressed VEGF and survivin gene expression, and induced apoptosis and ROS production.

Fig. 6 Lfcin-B significantly suppresses the tumor growth in vivo. After the volume of the tumors had reached 100–300 mm³, nude mice were randomly subdivided into 4 groups: a model group, three Lfcin-B groups administered intratumor injection (25, 50, 75 mg/kg). Mice were administrated three times a week for three weeks. Then mice were sacrificed and the tumor tissues were removed. a The tumor volume curve in H460 bearing mice treated with or without Lfcin-B. The tumor volume in Lfcin-B administration group was smaller than that in the model group. b The tumor weights were decreased in a dose-dependent manner. c The tumor inhibitory rates were increased after Lfcin-B injection. Each group contained six mice (data were presented as mean ± SD, *P < 0.05, **P < 0.01, compared with control group)
Whether Lfcin-B had the inhibitory effect in vivo was further explored by establishing H460-xenograft tumor models in nude mice. Compared to the model control, Lfcin-B dose-dependently suppressed the proliferation of H460 cells in tumor-bearing mice with 67.4% inhibition at 75 mg/kg. Hence, Lfcin-B could inhibit the H460 cells proliferation not only in vitro but also in vivo.

In summary, our results indicated that at least two mechanisms contributed to the outstanding anti-lung cancer action of Lfcin-B. Lfcin-B inhibited the proliferation of human NSCLC cells in vitro and in vivo, through inducing apoptosis (including increased the intracellular ROS concentration, activated caspase-3 and caspase-9, inhibited the survivin expression) and preventing the expression of VEGF. These results further suggested that Lfcin-B might be a potential candidate for the treatment of lung cancer.

Conflict of interest The authors declare that no conflict of interest exists in the present study.

References


The effect of Lfcin-B on NSCLC H460 cells