**MiR-147a mediated by sodium new houttuyfonate could enhance radiosensitivity of non-small cell lung cancer cells via suppressing STAT3**

Kejun Dai, Ling Chen, Jun Liu, Yuqiong Ding, Cheng Gu, Xujing Lu

Radiotherapy Department, Changzhou Tumor Hospital Affiliated to Soochow University, China

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

**Abstract**

**Background.** Radioresistance is a huge obstacle in radiotherapy of non-small cell lung cancer (NSCLC) and how to raise radiosensitivity is an urgent issue.

**Objectives.** In this study, we investigated the role and molecular mechanism of sodium new houttuyfonate (SNH) in regulation of radiosensitivity of NSCLC cells.

**Material and methods.** The Cell Counting Kit-8 (CCK-8) was used to measure cell viabilities of NSCLC cell lines A549 and HCC827 after a treatment with SNH (0 mM, 0.1 mM and 0.3 mM). It examined cytotoxicity induced by X-ray (0 Gy, 1 Gy, 2 Gy, 4 Gy, 6 Gy, and 8 Gy) after SNH (0.3 mM) treatment, while flow cytometry was used for apoptosis detection use. Expression of miR-147a or signal transducer and activator of transcription (STAT3) in selected cell lines was assessed through real-time quantitative polymerase chain reaction (RT-qPCR). The CCK-8 was then applied to measure cytotoxicity in cells with miR-147a upregulation or STAT3 suppression under irradiation apoptosis changes were detected with flow cytometry. Thereafter, binding conditions between miR-147a and STAT3 were checked using luciferase reporter assays. Expressions of STAT3 in A549 transfected by siNC, siSTAT3, and by siSTAT3 and miR-147a mimics were checked using RT-qPCR and the phosphorylation of STAT3 was observed using enzyme-linked immunosorbent assay (ELISA).

**Results.** The SNH treatment significantly suppressed cell viabilities and increased apoptosis of lung cancer cells. Cytotoxicity and apoptosis in A549 cells were both enhanced after SNH treatment and raised as the dosages of X-ray grew. MiR-147a presented lower expression in lung cancer cells and was upregulated by SNH, which further contributed to higher cell apoptosis after irradiation. STAT3 directly bound miR-147a and was more expressed in NSCLC cells. Inhibited phosphorylation of STAT3 was observed using enzyme-linked immunosorbent assay (ELISA).

**Conclusions.** SNH-induced miR-147a increased radiosensitivity of A549 cells through inactivation of STAT3 pathway.

**Key words:** STAT3, miR-147a, sodium new houttuyfonate
Objectives

According to data regarding occurrence rate and death rate, lung cancer is the primary cause of cancer-related mortality, and the five-year survival rate is only 13%. In men, lung cancer is the most commonly diagnosed cancer and the main reason of cancer-related death; in women, lung cancer ranks the 4th most commonly diagnosed cancer and the 2nd leading cause of cancer death. Non-small cell lung cancer (NSCLC) is the most frequent type of lung cancer, known to have diverse pathological features. Despite a significant progress in early diagnosis and clinical treatment, NSCLC is often diagnosed at advanced stage and has a poor prognosis. At present, radiotherapy is a key strategy in treating lung cancer for local control and recurrence reduction. Through radiotherapy, survival rate and local cancer control have been improved, but resistance of cancer and radiotoxicity to normal tissues restrict its therapeutic effects. Hence, it is urgently necessary to find new ways to increase sensitivity of cancer cells to radiation. Since antiquity, natural products were regarded as traditional medicines. Houttuynia cordata Thunb is a medicinal plant having tumor suppression effect, which can induce apoptosis of colon cancer cells, leukemia and lung cancer, among others. Sodium new houttuynate (SNH) is a synthetic product of Houttuyninum, an active constituent of Houttuynia cordata Thunb, which can inhibit tumor growth. However, whether SNH can regulate radiosensitivity of lung cancer was unknown. Therefore, functions of SNH in regulating sensitivity of radiation in lung cancer were detected to provide a new therapeutic method.

MicroRNAs belong to small noncoding RNAs (21–25 nucleotides) which can accelerate mRNA degradation through binding target mRNA. The first miRNA, LIN-4, was discovered in Caenorhabditis elegans, and more than 2500 kinds of mature miRNAs were authenticated. MiRNAs expression changed after ionizing radiation in cells or tissues, suggesting that miRNAs might connect with radiation treatment. MiR-147a acted as a tumor suppressor in progression of lung cancer by suppressing its proliferation and metastasis. Its upregulation through irradiation resulted in increased cell apoptosis of thymus cells of mice and Chinese hamster lung fibroblasts V79 through binding PDPK1, which also obstructed PI3K/AKT signaling pathway. The PI3K/AKT signaling pathway has been reported to resist radiosensitization, implying that miR-147a might present the ability to promote radiosensitivity. Moreover, SNH was shown to increase RNA expression of miR-147a in lung cancer cells through repressing Linc00668 and to inhibit metastasis of NSCLC cells. Therefore, we have inferred that miR-147a might be a promising facilitator to enhance effects of SNH on promoting radiosensitivity of NSCLC.

Signal transducer and activator of transcription (STAT3) has been detected as an oncogene in tumors that could accelerate growth of cancers cells and block apoptosis. STAT3 was negatively regulated by miR-125a-5p and up-regulation of STAT3 elevated proliferation and invasion of NSCLC cells. Blocking STAT3 can impede progression of lung cancer by enhancing radiosensitivity in A549 cells.

Background

Using ENCORI database (starbase.sysu.edu.cn), assumptive binding sites between miR-147a and STAT3 were shown, indicating that miR-147a might regulate STAT3 in NSCLC cells. The aim of this study was to examine the role of SNH in the radiosensitivity of NSCLC cells and unveil the potential molecular mechanism related to miR-147a and STAT3.

Material and methods

Cell culture

A549, a human NSCLC cancer cell line, is a kind of epithelial cell line established through explant culture of lung cancer from a male. HCC827 is also a human NSCLC cell line and epithelial cell line obtained from lung adenocarcinoma tissue of a female. Human normal lung epithelial cell line BEAS-2B is an epithelial cell line isolated from normal human bronchial epithelium. These 3 cell lines were all purchased from American Type Culture Collection (ATCC; Manassas, USA). A549, HCC827 and BEAS-2B cells were all incubated using RPMI-1640 medium (Gibco, Waltham, USA) replenished with 10% fetal bovine serum (FBS), 100 U/mL of penicillin and 100 μg/mL of streptomycin. Cell incubation was processed in incubator with saturated humidity at 37°C and 5%CO2. After incubation, A549 cells were treated with SNH (0 mM, 0.1 mM and 0.3 mM) for 24 h and A549 cells were then irradiated with X-ray (0 Gy, 1 Gy, 2 Gy, 4 Gy, 6 Gy, and 8 Gy) for 30 min to prepare them for following experiments.

Transfection

To regulate the expression of miR-147a or STAT3, transfection was performed in A549 cell line. Briefly, A549 cells in log phase were selected for transfection. A549 cells were first incubated in a six-well plate at 37°C. Overexpressed miR-147a (named miR-147a mimics) and small interfering RNA of STAT3 named siSTAT3 were created by GenePharma (Shanghai, China) together with scrambled sequences, NC mimics and siNC. Later, transfection was conducted after cell confluence reached 85%.
The NC mimics, miR-147a mimics, siNC, and siSTAT3 were grouped and transfected into A549 cells using Lipofectamine 3000 Transfection Reagent (Invitrogen, Carlsbad, USA). Transfection efficiency was measured 48 h after transfection with real-time quantitative polymerase chain reaction (RT-qPCR).

**RT-qPCR**

The RT-qPCR was used to detect RNA expressions in selected cell lines. Strictly following manufacturer's instructions, total RNA was extracted using TRIzol reagent (Invitrogen). Thereafter, High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems™, Foster City, USA) was performed to produce cDNA through reverse transcription of total RNA. Next, PCR was carried out using iQ™ SYBR® Green (Bio-Rad, Hercules, USA) for amplification. Sequences of primers were listed: miR-147a, forward, 5'- CGCGGTGTGTTGGAATGTC-3' and reverse, 5'- AGTGCAGGGTCCGGATTATT-3'; STAT3, forward, 5'- AACTCTCAAGGACAGGGACT-3' and reverse, 5'- AGTAGTGAACCTGGACGCCGG-3'; GAPDH, forward, 5'- CAACACTCTCCACCTTTG-3' and reverse, 5'- CCACACCTGTGTGGTAGT-3'; and U6, forward, 5'- AGAGAAGATTAGCATGGCCCCTG-3' and reverse, 5'- ATCCAGTGCAGGGTCCGAGG-3'.

The first step was pre-denaturation at 95°C for 10 min, followed by 35 cycles with denaturation at 95°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 30 s. Relative expressions of miR-147a and STAT3 were calculated using 2^{-ΔΔCt} methods, with U6 and GAPDH as internal references. Results were collected from 3 independent experiments.

**Cell Counting Kit-8**

Cell Counting Kit-8 (CCK-8) assay was used to measure cell viability after SNH treatment and irradiation. A549, HCC827 and BEAS-2B cells were seeded into 96-well plate at a density of 1 x 10^4 cells per well. Detects of A549 cells were grouped into NC mimics, miR-147a mimics, siNC, siSTAT3, and siSTAT3 with miR-147a mimics. For cell proliferation measurement, different concentrations of SNH (0 mM, 0.1 mM and 0.3 mM) were used to treat cells and 10 μL of CCK-8 (Bio-Rad, Hercules, USA) was added and cultured for 1 h at 37°C. Model 680 Microplate Reader was used for measuring OD values of cells at 450 nm wavelength. Experiments were repeated 3 times for getting proper results.

**Flow cytometry**

Apoptosis rate was detected using flow cytometry. A549, HCC827 and BEAS-2B were first treated under different concentrations of SNH (0 mM, 0.1 mM and 0.3 mM). Later, A549 cells treated with 0 mM and 0.3 mM SNH were selected for apoptosis evaluation after exposure to X-ray (0 Gy, 1 Gy, 2 Gy, 4 Gy, 6 Gy, and 8 Gy) irradiation for 30 min. A549 cells transfected with NC mimics, miR-147a mimics, siNC, siSTAT3 without or with 0.3 mM SNH treatment, siNC+0.3 mM SNH, siSTAT3+0.3 mM SNH, and siSTAT3+miR-147a mimics+0.3 mM SNH were selected. Then, cells were rinsed and resuspended. Thereafter, Annexin V-FITC Apoptosis Detection Kit (Beyotime) was applied for staining. After cells were dyed with 5 μL (20 mg/mL) Annexin V and 10 μL (50 mg/mL) PI, Attune Flow Cytometer (Invitrogen) was processed for checking apoptosis rate. Experiment was run in triplicate.

**Dual luciferase report assay**

In order to confirm whether STAT3 and miR-147a have binding site, dual luciferase reporter assay was utilized. Putative binding sites between miR-147a and STAT3 were provided by Starbase (http://starbase.sysu.edu.cn). Then, psiCHECK™-2 vector (Promega, Madison, USA) was used for inserting wild-type and mutant-type of STAT3, called STAT3-wt and STAT3-mut, respectively. Later, NC mimics and miR-147a mimics, together with STAT3-wt and STAT3-mut were co-transfected into A549 cells using Lipofectamine 3000. Cells were gathered 24 h after transfection and luciferase activity was determined using Quantus™ Fluorometer (Promega, Madison, USA).

**ELISA**

Enzyme-linked immunosorbent assay (ELISA) was used to detect protein expressions of phosphorylated STAT3 and total STAT3. STAT3 (pY705) + total STAT3 ELISA kit (ab126459; Abcam, Cambridge, UK) was chosen for STAT3 phosphorylation detection. After phosphor-Stat3 (Tyr705) was coated on 96-well plate, protein collected from A549 cells were pipetted and then bound to the wells. After the wells were rinsed, Detection Antibody STAT3 (Tyr705)
was used to measure phosphorylated STAT3 while Detection Antibody STAT3 was to analyze total STAT3 protein. Thereafter, horseradish peroxidase (HRP)-conjugated anti-rabbit IgG was added and incubated at 37°C for 1 h, and then wells were washed. TMB One-Step Substrate Reagent was added at 37°C for 30 min. Stop Solution was added (Thermofisher, Waltham, USA) and OD values were checked at 450 nm wavelength using Model 680 Microplate Reader. The experiment was repeated 3 times.

**Statistical analysis**

Each experiment was run in triplicate and all data was displayed as mean ± standard deviation (SD). GraphPad Prism v. 7 (GraphPad Software, La Jolla, USA) was used to analyze data from experiments. Data comparisons in different groups were measured with Student’s t-test (for 2 groups) and one-way analysis of variance (ANOVA; for 3 groups and more). Post hoc analysis was performed adopting Bonferroni’s correction. The overall alpha level is 0.05. A value of p < 0.01 was considered statistically significant for three-group comparisons, while p < 0.05 was considered significant for comparisons between 2 groups.

### Results

**Sodium new houttuynfate upregulated radiosensitivity of NSCLC and miR-147a expression through promoting apoptosis**

After different concentrations of SNH (0.1 mM and 0.3 mM) were added, cell viability of A549 cell line was significantly decreased compared to the control group, which was not treated with SNH (SNH 0 mM compared to SNH 0.1 mM, p = 0.006; SNH 0 mM compared to SNH 0.3 mM, p = 0.002; Fig. 1A) and cellular viability was notably increased, with a growth of the SNH concentration (SNH 0.1 mM compared to SNH 0.3 mM, p = 0.008; Fig. 1A). On the contrary, increasing concentrations of SNH lead to higher apoptosis rates of A549 cell line (SNH 0 mM compared to SNH 0.1 mM, p = 0.007; SNH 0 mM compared to SNH 0.3 mM, p = 0.001; SNH 0.1 mM compared to SNH 0.3 mM, p = 0.009; Fig. 1B). Later, different doses of X-ray (0 Gy, 1 Gy, 2 Gy, 4 Gy, 6 Gy, and 8 Gy) were used to measure functions of SNH in NSCLC cells, indicating that growing doses of irradiation significantly inhibited viability of A549 cells treated with 0.3 mM X-ray.

**Fig. 1. Sodium new houttuynfate upregulated radiosensitivity of NSCLC through promoting apoptosis and enhanced miR-147a expression**

A. Cell viabilities of A549 cells with different concentrations of SNH (0 mM, 0.1 mM and 0.3 mM) were measured with CCK-8. ** presents the significant difference (p < 0.01) from the 0 mM SNH group, while ## signifies notable difference (p < 0.01) between 0.1 mM and 0.3 mM SNH treatment groups. B. Apoptosis rate of A549 cells was evaluated through flow cytometry. ** presents the significant difference (p < 0.01) from the 0 mM SNH group, while ## signifies notable difference (p < 0.01) between 0.1 mM and 0.3 mM SNH treatment groups. C. OD values of normal A549 cells and SNH-treated A549 cells irradiated with X-ray (0 Gy, 1 Gy, 2 Gy, 4 Gy, 6 Gy, and 8 Gy) were detected with CCK-8. ** presents the significant difference (p < 0.05) from the 0 mM SNH group. D. Apoptosis rates of A549 cells irradiated with X-ray (0 Gy, 1 Gy, 2 Gy, 4 Gy, 6 Gy, and 8 Gy) were checked using flow cytometry. ** presents the significant difference (p < 0.05) from the 0 mM SNH group. E. MiR-147a RNA expressions were measured with RT-qPCR in A549 with SNH treatment (0 mM, 0.1 mM and 0.3 mM). ** presents the significant difference (p < 0.01) from the 0 mM SNH group, while ## signifies notable difference (p < 0.01) between 0.1 mM and 0.3 mM SNH treatment groups. Each experiment was repeated 3 times.
SNH in comparison with A459 without SNH treatment. The comparisons between the NC and 0.3 mM groups are as follows: for 2 Gy $p = 0.032$; for 4 Gy $p = 0.048$; for 6 Gy $p = 0.027$; and for 8 Gy $p = 0.012$ (Fig. 1C). Moreover, the apoptosis rate of SNH-treated A549 was higher than normal A549 after irradiation. The comparisons between the NC and 0.3 mM groups are as follows: for 2 Gy $p = 0.032$; for 4 Gy $p = 0.048$; for 6 Gy $p = 0.027$; for 8 Gy $p = 0.012$ (Fig. 1C). Then, miR-147a expression was evaluated after different SNH treatments, revealing that miR-147a expression was significantly raised by SNH treatment in A549 cell line, and as the SNH concentration increased, miR-147a was upregulated more significantly (SNH 0 mM compared to SNH 0.1 mM, $p = 0.008 < 0.01$; SNH 0 mM compared to SNH 0.3 mM, $p = 0.004 < 0.01$; SNH 0.1 mM compared to SNH 0.3 mM, $p = 0.009 < 0.01$; Fig. 1E).

**Overexpressed miR-147a promoted apoptosis and enhanced radiosensitivity in NSCLC**

According to RT-qPCR results, miR-147a was dramatically decreased in lung cancer cells compared to BEAS-2B cell line (A459 compared to BEAS-2B, $p = 0.002$; HCC827 compared to BEAS-2B, $p = 0.009$; Fig. 2A) and A459 cell line had the lowest expression of miR-147a (A459 compared to HCC827, $p = 0.006$; Fig. 2A). To better understand the role of miR-147a in A549 cells, mimics of miR-147a were transfected into A549 cells, which upregulated expression of miR-147a compared with the control group ($p = 0.022$; Fig. 2B). Thereafter, A549 cells were exposed to irradiation and then analyzed for cell survival and apoptosis. Results showed that cell survival rates in miR-147a mimics group were remarkably lower than in NC mimics group (for 1 Gy $p = 0.023$; for 2 Gy $p = 0.029$; for 4 Gy $p = 0.021$; for 6 Gy $p = 0.017$; for 8 Gy $p = 0.039$; $p < 0.05$; Fig. 2C) while apoptosis was higher in miR-147a mimics group (for 1 Gy $p = 0.042$; for 2 Gy $p = 0.032$; for 4 Gy $p = 0.021$; for 6 Gy $p = 0.015$; for 8 Gy $p = 0.039$; $p < 0.05$; Fig. 2D).

**STAT3 directly targeted miR-147a and negatively regulated radiosensitivity of NSCLC**

The RNA expression levels of STAT3 were then detected in A549, HCC827 and BEAS-2B cell lines, indicating that STAT3 was highly expressed in lung cancer cell lines, especially in A549 cell line (A459 compared to BEAS-2B, $p = 0.002$; HCC827 compared to BEAS-2B, $p = 0.007$; A459 compared to HCC827, $p = 0.009$; Fig. 3A). Moreover, according to Starbase, putative binding sites between STAT3 and miR-147a were provided (Fig. 3B) and binding condition between miR-147a and STAT3 was verified using luciferase report assays, showing that the co-transfected cell group of miR-147a mimics and STAT3-wt had the lowest luciferase activity compared to other groups ($p = 0.008$; Fig. 3C). As STAT3 was high expressed in A549 cell line, suppression of STAT3 resulted in a significantly lower level...
MiR-147a enhanced SNH-induced radiosensitivity in NSCLC through suppressing STAT3

As miR-147a and STAT3 were measured individually, correlation between STAT3 and miR-147a was investigated further. After SNH treatment, A549 cells were transfected with siNC, siSTAT3 and siSTAT3 together with miR-147a mimics. The RT-qPCR results revealed that STAT3 expression was notably decreased in comparison with the control group and siSTAT3 group. ** statistically different (p < 0.05) in comparison with the control group and siSTAT3 group; G. The phosphorylated STAT3 conditions were examined using ELISA. ** statistically different (p < 0.05) in comparison with the control group and siSTAT3 group; H. After cells were treated with different irradiation (0 Gy, 1 Gy, 2 Gy, 4 Gy, 6 Gy, and 8 Gy), STAT3 phosphorylation was examined with ELISA. ** notably different (p < 0.01) from 0 Gy group. Each experiment was repeated 3 times.

of STAT3 (p = 0.029; Fig. 3D). Later, toxicity was detected under upregulated doses of X-ray, revealing that suppressed STAT3 significantly reduced OD values of A549 compared to control group (for 1 Gy p = 0.023; for 2 Gy p = 0.021; for 4 Gy p = 0.014; for 6 Gy p = 0.009; for 8 Gy p = 0.025; Fig. 3E). Meanwhile, apoptosis was significantly increased after STAT3 suppression (for 1 Gy p = 0.043; for 2 Gy p = 0.031; for 4 Gy p = 0.028; for 6 Gy p = 0.017; for 8 Gy p = 0.036; Fig. 3F). Then, protein concentrations of phosphorylated STAT3 and total STAT3 were detected, indicating that p-STAT3 was significantly decreased after knockdown of STAT3 (p = 0.009; Fig. 3G). Moreover, increasing doses of irradiation also resulted in decreases of p-STAT3 (for 1 Gy p = 0.009; for 2 Gy p = 0.006; for 4 Gy p = 0.004; for 6 Gy p = 0.003; for 8 Gy p = 0.002; Fig. 3H).
after STAT3 inhibition compared with that in the control si-NC group, and miR-147a upregulation intensified cell survival decrease under irradiation in different doses. For siSTAT3 compared to siNC, the results were as follows: for 1 Gy p = 0.008; for 2 Gy p = 0.006; for 4 Gy p = 0.003; for 6 Gy p = 0.009; for 8 Gy p = 0.003. For the combined group compared to the siSTAT3 group, the results were as follows: for 1 Gy p = 0.008; for 2 Gy p = 0.005; for 4 Gy p = 0.006; for 6 Gy p = 0.007; for 8 Gy p = 0.006 (Fig. 4B). Meanwhile, apoptosis rate of cells after SNH treatment was significantly lower after STAT3 downregulation (for 1 Gy p = 0.007; for 2 Gy p = 0.005; for 4 Gy p = 0.004; for 6 Gy p = 0.007; for 8 Gy p = 0.008). MiR-147a overexpression added to the drop in apoptosis rates in comparison with siSTAT3 group. For the combined group compared to siSTAT3 group, the results were as follows: for 0 Gy p = 0.005; for 1 Gy p = 0.007; for 2 Gy p = 0.004; for 4 Gy p = 0.008; for 6 Gy p = 0.007; for 8 Gy p = 0.009 (Fig. 4C). The changes in phosphorylation levels of STAT3 were examined using ELISA assay and results showed that p-STAT3 proteins were inhibited by STAT3 suppression and further reduced by miR-147a upregulation (p = 0.007 for the siSTAT3 group compared to siNC group; p = 0.005 for the combined group compared to siSTAT3 group; Fig. 4D).

Discussion

Houttuyninum can suppress tumor growth through downregulating HER2/neu phosphorylation, which indicates that Houttuyninum can provide therapeutic value in tumor treatment.24 Considering chemical instability of Houttuyninum, SNH was compounded for increasing stability, which inhibited metastasis in not only A549 and NCI-H1299 cell lines but also in lungs of mice.18 In this study, SNH significantly decreased cell viabilities in cancer cell lines but seldom downregulated the viability of normal BEAS-2B cell line, indicating that SNH could be a useful medicine for blocking cell progression of cancer cells. Moreover, SNH also increased apoptosis of cancer cell lines. Lower cell viability and higher apoptosis of A549 cells resulted from SNH treatment. Based on those results,
SNH was proven to be a useful medicine to suppress cell viability and promote apoptosis of A459 cells. As for miR-147a, it was reported that miR-147a could suppress cell proliferation and promote apoptosis in A549 cell line by negatively regulating pRB and HOXD-AS1.\textsuperscript{15} After irradiation, miR-147a was significantly upregulated in irradiation-induced thymus injury,\textsuperscript{25} implying that miR-147a is a potential regulator of radiotherapy. We have measured that SNH treatment in A549 cells significantly increased expression of miR-147a, which was remarkably low in cancer cells, especially in A549 cells. Furthermore, overexpression of miR-147a downregulated cell viability but upregulated apoptosis of A549 cells after irradiation. According to these results, SNH was shown to facilitate apoptosis of A549 cells and enhanced radiosensitivity of A549 cells by increasing miR-147a RNA level.

STAT3 was proven as the target gene of miR-147a through luciferase report assay after putative binding sites were provided by Starbase. STAT3 was discovered as a component of activated acute phase response factor complex of interleukin 6 (IL-6) over 20 years ago and its activation was to form dimer by combination of mutual SH2 domain-phosphotyrosine, which was determined as the key activation mechanism of STAT3 transcriptional function stimulation.\textsuperscript{26–28} It is constituted by 770 amino acids, containing 6 functional domains.\textsuperscript{29} STAT3 was proven to play an important role in occurrences of tumors, and it promoted cisplatin resistance after activation by periostrin in NSCLC.\textsuperscript{30,31} Activation of STAT3 accelerated proliferation and repressed apoptosis through accumulation of the large granular lymphocyte in leukemia.\textsuperscript{32} Suppression of STAT3 significantly declined the viability of MDA-MB-231 breast cancer cells.\textsuperscript{33} Moreover, STAT3 inhibited radiosensitivity in VEGFR2 low expressed Calu-1 cell line and in A549 cell line, which contains high level of VEGFR2.\textsuperscript{22} STAT3 has 1 phosphorylation site called Tyr705, which has been detected to activate STAT3 Tyr705, which has been detected to activate STAT3 of VEGFR2.

In our study, we have shown that miR-147a, increased by SNH, could enhance cell radiosensitivity by inactivating the STAT3 signaling pathway in A549 cells. Therefore, it is possible that miR-147a might also have the ability to promote radiosensitivity in animal models and at clinical stage.

Conclusions

In our study, we have shown that miR-147a, increased by SNH, could enhance cell radiosensitivity by inactivating the STAT3 signaling pathway in A549 cells. Therefore, it is possible that miR-147a might also have the ability to promote radiosensitivity in animal models and at clinical stage.

References

1. Risch A, Plass C. Lung cancer epigenetics and genetics. Int J Cancer. 2008;123(1):1–7. doi:10.1002/ijc.23605
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69–90. doi:10.3322/caac.20070
3. Berman AT, Rengan R. New approaches to radiotherapy as definitive treatment for inoperable lung cancer. Semin Thorac Cardiovasc Surg. 2008;20(3):188–197. doi:https://doi.org/10.1053/j.semctvs.2008.09.003
4. Xue G, Ren Z, Chen Y, et al. A feedback regulation between miR-145 and DNA methyltransferase 3b in prostate cancer cell and their responses to irradiation. Cancer Lett. 2015;361(1):121–127. doi:10.1016/j.cancerlet.2015.02.046
5. Boufridi A, Quinn RJ. Harnessing the properties of natural products. Annu Rev Pharmacol Toxicol. 2018;58:451–470. doi:10.1146/annurev-pharmaco-010116-150509
6. Tang YJ, Yang JS, Lin CF, et al. Houttuynia cordata Thumb extract induces apoptosis through mitochondrial-dependent pathway in HT-29 human colon adenocarcinoma cells. Oncol Rep. 2009;22(5):1051–1056. https://doi.org/10.3892/or_0000535
7. Banjerdpongchai R, Kongtawelert P. Ethanolic extract of fermented Thunb induces human leukemic HL-60 and Mo-M-4 cell apoptosis via oxidative stress and a mitochondrial pathway. Asian Pac J Cancer Prev. 2011;12(11):2871–2874.
8. Chen YF, Yang JS, Chang WS, Tsai SC, Peng SF, Zhou YR. Houttuynia cordata Thumb extract modulates G0/G1 arrest and Fas/CD95-mediated death receptor apoptotic cell death in human lung cancer A549 cells. J Biomed Sci. 2013;20:18. doi:10.1186/1423-0127-20-18
9. Wu KL, Tsai YM, Lien CT, Kuo PL, Hung AJ. The roles of microRNA in lung cancer. Int J Mol Sci. 2019;20(7):1611. doi:10.3390/ijms20071611
10. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell*. 1993;75(5):843–854. doi:10.1016/0092-8674(93)90529-y

11. Hashemi ZS, Khalihi S, Forouzandeh Moghadam M, Sadreddiny E. Lung cancer and miRNAs: A possible remedy for anti-metastatic, therapeutic and diagnostic applications. *Expert Rev Respir Med*. 2017;11(2):147–157. doi:10.1080/17476348.2017.1279403

12. Shi Y, Zhang X, Tang X, Wang P, Wang H, Wang Y. miR-21 is continually elevated long-term in the brain after exposure to ionizing radiation. *Radiat Res*. 2012;171(1):124–128. doi:10.1667/r2764.1

13. Girardi C, De Pittà C, Casara S, et al. Analysis of miRNA and mRNA expression profiles highlights alterations in ionizing radiation response of human lymphocytes under modeled microgravity. *PLoS One*. 2012;7(2):e31293. doi:10.1371/journal.pone.0031293

14. Lu Y, Luan XR. miR-147a suppresses the metastasis of non-small-cell lung cancer by targeting CCL5. *J Int Med Res*. 2019;48(4):300060 S186559371930309. doi:10.1177/0300060519883098

15. Wang Q, Jiang S, Song A, et al. HOXD-AS1 functions as an oncogenic ceRNA to promote NSCLC cell progression by sequestering miR-147a. *Onco Targets Ther*. 2017;10:4735–4763. doi:10.2147/OTT.S143787

16. Wang LJ, Li NN, Xu SJ, et al. A new and important relationship between miRNA-147a and PDPK1 in radiotherapy. *J Cell Biochem*. 2018;119(14):3519–3527. doi:10.1002/jcb.2652

17. Liao J, Jin H, Li S, et al. Apatinib potentiates irradiation effect via suppressing PI3K/AKT signaling pathway in hepatocellular carcinoma. *J Exp Clin Cancer Res*. 2019;38(1):454. doi:10.1186/s13046-019-1419-1

18. Jiang R, Hu C, Li Q, et al. Sodium new houttuyfonate suppresses Apatinib potentiates irradiation effect via suppressing PI3K/AKT signaling pathway in hepatocellular carcinoma.

19. Liu, S, Gao Y, Huang X, Wang X. GYY4137, a hydrogen sulfide (H₂S) donor, shows potent anti-hepatocellular carcinoma activity through blocking the STAT3 pathway. *Int J Oncol*. 2014;44(4):1259–1267. doi:10.3892/ijo.2014.2305

20. Islam M, Sharma S, Teknos TN, RhoC regulates stem cells in head and neck squamous cell carcinoma by overexpressing IL-6 and phosphorylation of STAT3. *PLoS One*. 2014;9(2):e88527. doi:10.1371/journal.pone.0088527

21. Zhong L, Sun S, Shi J, Cao F, Han X, Chen Z, MicroRNA-125a-5p plays a role as a tumor suppressor in lung cancer cells by directly targeting STAT3. *Tumour Biol*. 2017;39(6):1010428317697579. doi:10.1177/1010428317697579

22. Hu C, Zhuang W, Qiao Y, et al. Effects of combined inhibition of STAT3 and VEGFR2 pathways on the radiosensitivity of non-small-cell lung cancer cells. *Onco Targets Ther*. 2019;12:933–944. doi:10.2147/OTT.S186559

23. Sun X, Wang J, Huang M, et al. STAT3 promotes tumour progression in glioma by inducing FOXP1 transcription. *J Cell Mol Med*. 2018;22(1):5629–5638. doi:10.1111/jcmm.13833

24. Zhou NN, Tang J, Chen WD, et al. Houttuyninum, an active constituent of Chinese herbal medicine, inhibits phosphorylation of HER2/neu receptor tyrosine kinase and the tumor growth of HER2/neu-overexpressing cancer cells. *Life Sci*. 2012;90(19–20):770–775. doi:10.1016/j.lfs.2012.03.035

25. Chen C, Lu J, Hao L, Zheng Z, Zhang N, Wang Z. Discovery and characterization of miRNAs in mouse thymus responses to ionizing radiation by deep sequencing. *Int J Radiat Biol*. 2016;92(10):548–557. doi:10.1080/09553002.2016.1207821

26. Lütticken C, Wegenka UM, Yuan J, et al. Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130. *Science*. 1994;263(5143):89–92. doi:10.1126/science.7272872

27. Zhong Z, Wen Z, Darnell JE Jr, Stat3: A STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. *Science*. 1994;264(5155):95–98. doi:10.1126/science.8140422

28. Hillmer EJ, Zhang H, Li HS, Watowich SS. STAT3 signaling in immunity. *Cytokine Growth Factor Rev*. 2016;31:1–15. doi:10.1016/j.cytogfr.2016.05.001

29. Becker S, Groner B, Müller CW. Three-dimensional structure of the Stat3beta homodimer bound to DNA. *Nature*. 1998;394(6689):145–151. doi:10.1038/28101

30. Hu W, Jin P, Liu W. Peristin contributes to cisplatin resistance in human non-small cell lung cancer A549 cells via activation of Stat3 and Akt and upregulation of survivin. *Cell Physiol Biochem*. 2016;38(3):1199–1208. doi:10.1159/000443068

31. Yu H, Lee H, Herrmann A, Buettner R, Jove R. Revisiting STAT3 signaling in cancer: New and unexpected biological functions. *Nat Rev Cancer*. 2014;14(14):736–746. doi:10.1038/nrc3818

32. Epling-Burnette PK, Liu HH, Catlett-Falcone R, et al. Inhibition of STAT3 signaling leads to apoptosis of leukemic large granular lymphocytes and decreased Mcl-1 expression. *J Clin Invest*. 2001;107(3):351–362. doi:10.1172/JCI99490

33. Kim KW, Mutter RW, Cao C, et al. Inhibition of signal transducer and activator of transcription 3 activity results in down-regulation of survivin following irradiation. *Mol Cancer Ther*. 2006;5(11):2659–2665. doi:10.1158/1535-7163.MCT-06-0261

34. Sakaguchi M, Oka M, Iwasaki T, Fukami Y, Nishigori C. Role and regulation of STAT3 phosphorylation at Ser727 in melanocytes and melanoma cells. *J Invest Dermatol*. 2012;132(7):1877–1885. doi:10.1038/jid.2012.45

35. Lewis KM, Bharadwaj U, Eckols TK, et al. Small-molecule targeting of signal transducer and activator of transcription 3 to treat non-small cell lung cancer. *Lung Cancer*. 2015;90(2):182–190. doi:10.1016/j.lungcan.2015.09.014

36. Ding X, Cheng J, Pang Q, et al. BIBR1532, a selective telomerase inhibitor, enhances radiosensitivity of non-small cell lung cancer through increasing telomere dysfunction and ATM/CHK1 inhibition. *Int J Radiat Oncol Biol Phys*. 2019;105(4):861–874. doi:10.1016/j.ijrobp.2019.08.009

37. Park G, Son B, Kang J, et al. DR-induced miR-30a and miR-30b target CCL5 in non-small cell lung cancer. *Lung Cancer*. 2019;127:342–354. doi:10.1016/j.lungcan.2018.10.015