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Jinfukang regulates integrin/Src pathway and anoikis mediating circulating lung cancer cells migration

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ABSTRACT

Ethnopharmacological relevance: Metastasis is the main cause of death in lung cancer patients. Circulating tumor cells (CTCs) may be an important target of metastasis intervention. Previous studies have shown that Jinfukang could prevent the recurrence and metastasis of lung cancer, and we have established a circulating lung tumor cell line CTC-TJH-01. However, whether Jinfukang inhibition of lung cancer metastasis is related to CTCs is still unknown.

Aim of the study: To further explore the mechanism of Jinfukang in anti-metastasis of lung cancer from the perspective of intervention of CTCs.

Materials and methods: CTC-TJH-01 and H1975 cells were treated with Jinfukang. Cell viability was detected by CCK8, and the cell apoptosis was detected by flow cytometry. Transwell was used to detected cell migration and invasion. Cell anoikis was detected by anoikis detection kit. Protein expression was analysis by Western blot.

Results: Jinfukang could inhibit the proliferation, migration and invasion of CTC-TJH-01 and H1975 cells. Besides, Jinfukang could also induce anoikis in CTC-TJH-01 and H1975 cells. Analysis of the mRNA expression profile showed ECM-receptor interaction and focal adhesion were regulated by Jinfukang. Moreover, it was also find that Jinfukang significantly inhibited integrin/Src pathway in CTC-TJH-01 and H1975 cells. When suppress the expression of integrin with ATN-161, it could promote Jinfukang to inhibit migration and induce anoikis in CTC-TJH-01 and H1975 cells.

Conclusions: Our results indicate that the migration and invasion of CTCs are inhibited by Jinfukang, and the mechanism may involve the suppression of integrin/Src axis to induce anoikis. These data suggest that Jinfukang exerts anti-metastatic effects in lung cancer may through anoikis.

1. Introduction

Lung cancer is the most lethal malignant tumour (Siegel et al., 2019). The 5-year survival rate of lung cancer patients is still less than 20% in both developed and developing countries (Allemani et al., 2018). The main reason is tumour metastasis, which is the leading cause of death in patients with lung cancer (Miller et al., 2019). Therefore, suppression of cancer metastasis is thus an urgent therapeutic need. However, most existing drugs for lung cancer treatment developed on pre-clinical models which based on primary cancer cell lines (Reinhold et al., 2015). Lacking of the specific pre-clinical models for metastasis study is still the main obstacle in the field. New ideas are needed to develop

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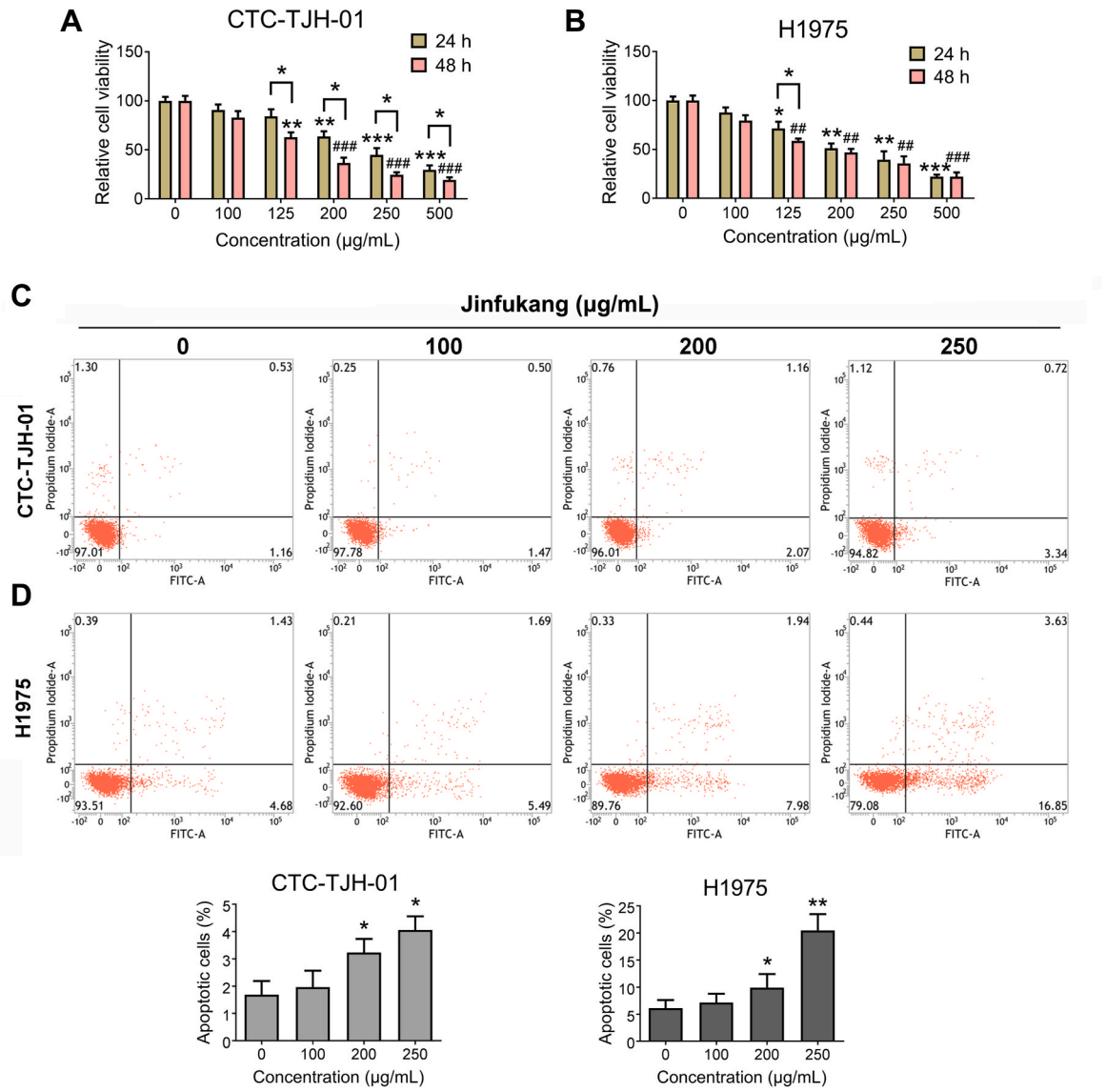

Fig. 1. Effects of Jinfukang on the proliferation and apoptosis of lung cancer cells. (A and B) CTC-TJH-01 and H1975 cells were incubated with Jinfukang (0, 100, 125, 200, 250, 500 μg/mL) for 24 h or 48 h. The CCK-8 assay was performed to determine the cytotoxic effect of Jinfukang. (C and D) CTC-TJH-01 and H1975 cells were treated with Jinfukang (0, 100, 200, and 250 μg/mL) for 24 h. Flow cytometry was performed to determine apoptosis. Each bar represents the mean ± SD of three separate experiments. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

effective anti-tumor drugs (Anderson et al., 2019).

Circulating tumour cells (CTCs) shed from the primary tumour into the vascular system since the beginning of the malignant process, and thousands of CTCs may be sown in distant organs (Gkountela et al., 2019; Massague and Obenauf, 2016). CTCs must overcome many obstacles before colonizing distant organs, such as surviving in peripheral blood, evading immune defence, infiltrating distant tissue and surviving in a dormant state (Joosse et al., 2015). Hence, there are two strategies for anti-metastasis treatment, one is to prevent CTCs dissemination, and the other is to suppress already existing metastases and induce dormancy (Bi et al., 2020; Weber, 2013). Many studies have demonstrated that CTCs levels are correlated with distant metastasis and poor prognosis in lung cancer (Alama et al., 2014; Zhou et al., 2017). Therefore, CTCs may be an important target in the design and development of anti-metastatic drugs.

Traditional Chinese medicine has great advantages in the treatment of cancer (Hnit et al., 2020). Jinfukang, Traditional Chinese medicine prescription, is specifically administered in the treatment of lung cancer, and has been proven to prevent metastasis and prolong the survival of lung cancer patients (Liu et al., 2001). Multiple lines of evidence indicate that Jinfukang can induce cell cycle arrest, apoptosis, and epigenetic regulation in lung cancer (Kou et al., 2017; Lu et al., 2018). However, the role and mechanism of Jinfukang against lung cancer metastasis is still elusive.

Although the clinical efficacy of Jinfukang in the treatment of lung cancer metastasis has been confirmed, the mechanism of its anti-metastatic effect remains unclear. We speculate that the mechanism may be related to CTCs anoikis. In this study, we examined the role of Jinfukang on CTCs metastasis and studied the underlying molecular mechanisms.

2. Materials and Methods

2.1. Chemicals and reagents

Jinfukang (No. 20180501) was provided by China Resources Sanjiu Medical & Pharmaceutical CO, LTD as described before (Que et al., 2020). ATN-161 was purchased from Selleck (Shanghai, China). Cell

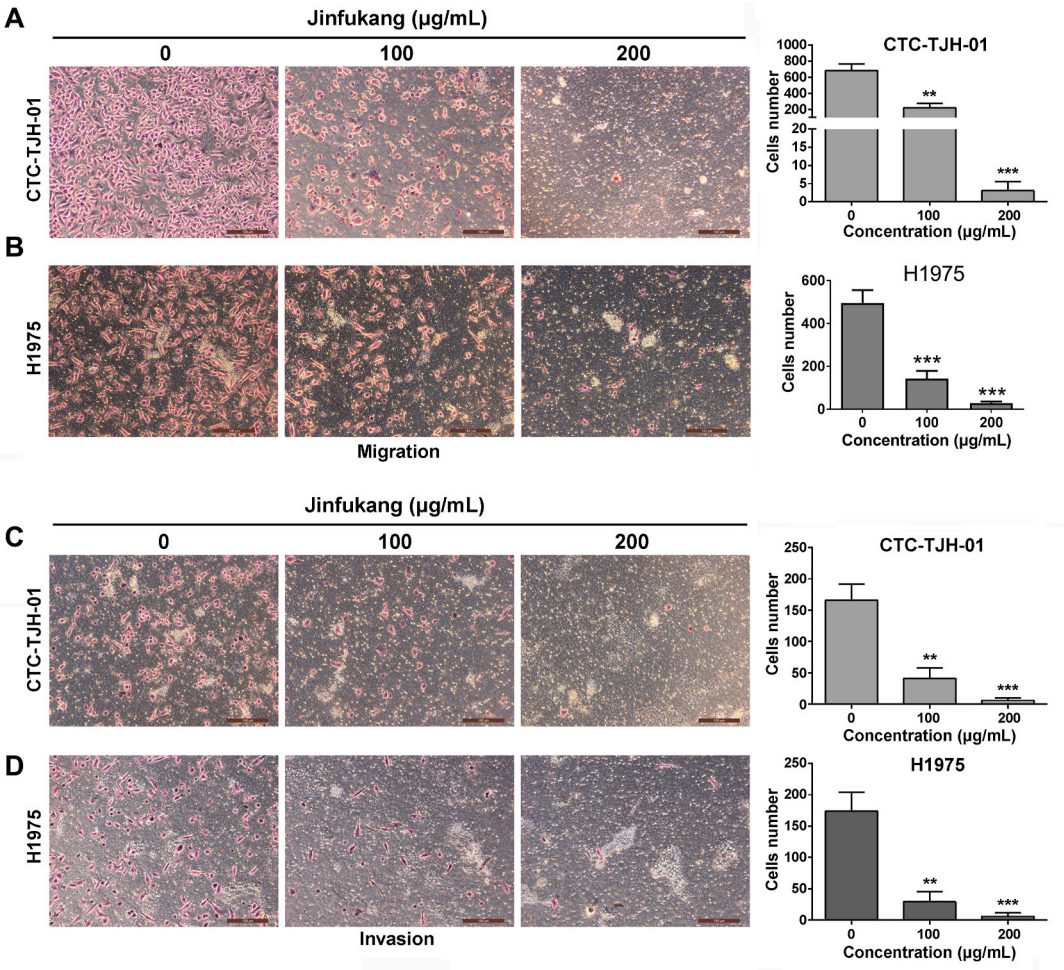

Fig. 2. Jinfukang reduced cell migration and invasion of lung cancer cells. The migration (A and B) or invasion (C and D) capacity of CTC-TJH-01 and H1975 cells was measured using transwell assays or matrigel invasion chambers after Jinfukang (0, 100, 200 μg/mL) treatment for 16 h. Each bar represents the mean ± SD of three separate experiments. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

counting kit-8 (CCK-8) reagent was purchased from Dojindo (Dojindo, Shanghai, China). Annexin V-FITC/PI apoptosis detection kit was obtained from BD Pharmingen (BD Biosciences, CA, USA). Anoikis detection kit was purchased from Biovision (Mountain View, CA, USA). Antibodies specific for TrkB, goat anti-mouse IgG-HRP, and donkey anti-rabbit IgG-HRP were purchased from Cell Signaling Technology (Danvers, MA, USA); Antibodies specific for MMP-2, MMP-9, and GAPDH were purchased from Proteintech (Wuhan, China); Antibodies specific for Integrin β1, Src, and p-Src were purchased from Affinity Biosciences; Antibodies specific for MEK, p-MEK, ERK1/2, p-ERK1/2, and FN1 were purchased from Abcam (Cambridge, MA, UK).

2.2. Cell culture

Human circulating lung cancer cell line CTC-TJH-01 was established by our laboratory (Wang et al., 2016). H1975 cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cells were maintained in F12K (Gibco) or RPMI-1640 (Corning Cellgro) supplemented with 10% foetal bovine serum (Biological Industries) and penicillin-streptomycin at 37 °C in a humidified atmosphere with 5% CO_2.

2.3. Cell viability assay

Cell viability assay were measured as previously described (Que et al., 2019b).

2.4. Apoptosis analysis

The annexin V-FITC/PI apoptosis assay were measured as previously described (Que et al., 2019b).

2.5. Migration and invasion assays

In vitro migration and invasion assays were performed as described previously (Liang et al., 2018). Briefly, the lower transwell chamber was filled with 750 μL serum containing 20% FBS medium, and 5×10^4 cells in 500 μL serum-free medium were added to the upper transwell chamber. After 30 min, Jinfukang was added to the upper chamber. The cells were allowed to migrate for 16 h at 37 °C. The non-migrated cells were removed with a cotton swab, and the migrated cells were fixed with methanol and stained with Giemsa. The transwell was photographed under a Leica DMI3000B microscope (Leica Microsystems, Wetzlar, Germany). Five random fields were counted at 100 × magnification. The cell invasion assay was performed using transwell inserts coated with matrigel.

2.6. Western blot analysis

Western blotting was conducted as described previously (Que et al., 2019b). In brief, cell lysates were centrifuged at 12,000 rpm for 30 min at 4 °C. Protein concentrations of cell lysates were determined using the BCA assay, and 40 μg of proteins was loaded onto 7.5%–15% SDS

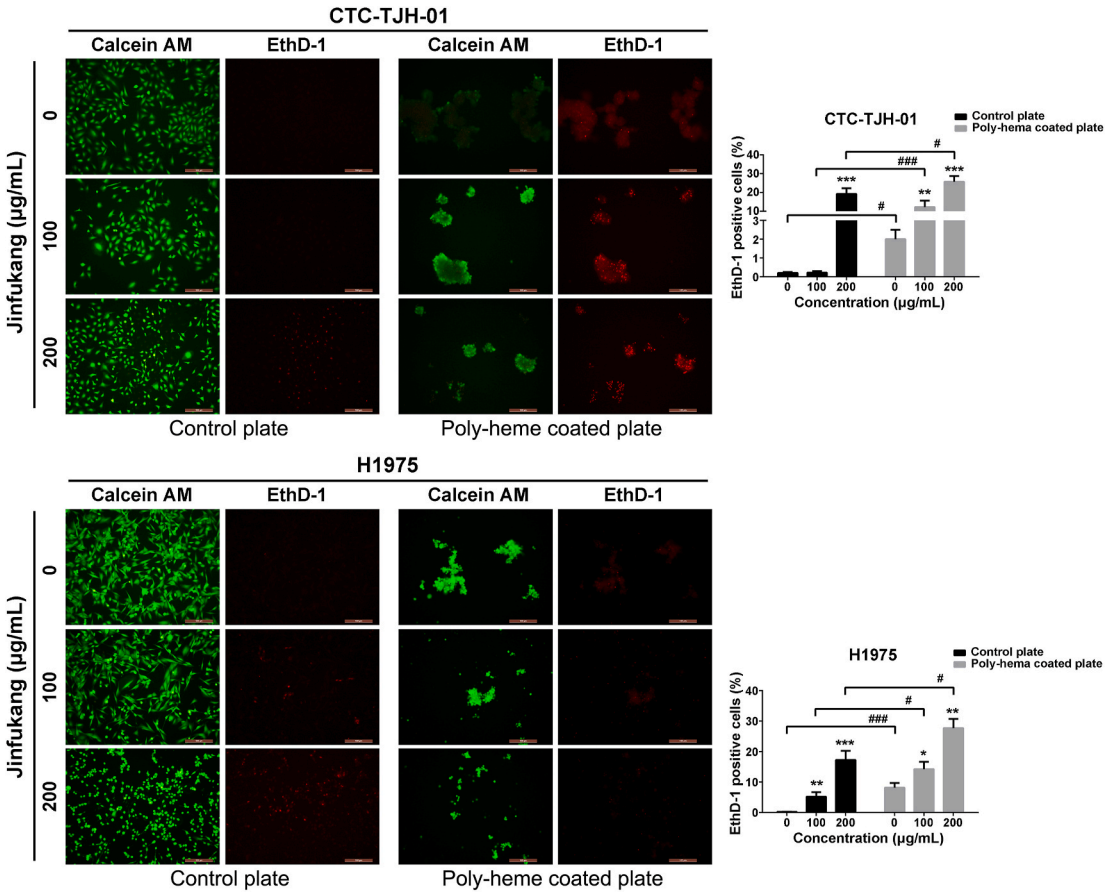

Fig. 3. Jinfukang induced anoikis in lung cancer cells. An anoikis assay was used to measure the anoikis levels in CTC-TJH-01 and H1975 cells after incubation with Jinfukang (0, 100, 200 μg/mL) for 24 h. Each bar represents the mean ± SD of three separate experiments. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

polyacrylamide gels and electrophoresed. The gels were then transferred to polyvinylidene difluoride membrane. The blotted membranes were blocked in blocking buffer (5% non-fat dry milk/1% Tween 20 in PBS) for 2 h at room temperature and then incubated overnight with appropriate primary antibodies in blocking buffer at 4 °C. The blot was then washed three times with TBST buffer, and incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. The immunoreactive bands were visualized using an ECL kit (Bio-Rad). The density of the immunoreactive bands was analyzed using Image J software (Bio-rad, CA, USA).

2.7. Anoikis assays

Anoikis assays were performed as described previously (Lu, S.M. et al., 2016). Cells were cultured in plates coated with or without poly-HEMA, and then treated with Jinfukang for 24 h. Calcein AM/ethidium homodimer-1 (EthD-1) solution (500X, 1 μL) was added to each well of a 24-well anchorage resistant or control plate. The plates were then incubated for 30–60 min at 37 °C. The green calcein AM fluorescence (Ex, 485 nm; Em, 515 nm) and red EthD-1 fluorescence (Ex, 520 nm; Em, 590 nm) were detected by inverted fluorescence microscope. Green calcein AM fluorescence stains live cells, red EthD-1 fluorescence stains dead cells.

2.8. Statistical analysis

All experiments were assayed in triplicate (n = 3). Data are expressed as means ± SEM. All statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Prism Software Inc, CA, USA). Differences in measured variables between experimental and control groups were assessed by the Student's *t*-test. The analysis of multiple groups was performed by ANOVA. $P < 0.05$ was defined as statistically significant.

3. Results

3.1. Jinfukang significantly inhibited the proliferation of CTC-TJH-01 and H1975 cells

To investigate the role of Jinfukang in the metastasis of lung cancer cells, we first examined the effects of Jinfukang on the proliferation of CTC-TJH-01 and H1975 cells. Jinfukang was found to significantly inhibit the proliferation of CTC-TJH-01 and H1975 cells (Fig. 1A and B). Additionally, CTC-TJH-01 and H1975 cells treated with Jinfukang for 24 h were examined for apoptosis by flow cytometry to determine the follow-up experimental intervention dose of Jinfukang. The results suggested that high-concentration of Jinfukang could induce apoptosis in CTC-TJH-01 and H1975 cells, and that H1975 cells were more sensitive to Jinfukang (Fig. 1C and D). Therefore, in the next experiment, we chose a lower dose of Jinfukang.

3.2. Jinfukang inhibited the migration and invasion of CTC-TJH-01 and H1975 cells

Next, we evaluated the effect of Jinfukang on lung cancer metastasis. As shown in Fig. 2A and B, Jinfukang significantly suppressed the migration of CTC-TJH-01 and H1975 cells. In addition, the same results were obtained in the invasion experiments (Fig. 2C and D). These results indicate that Jinfukang can inhibit the metastasis of lung cancer cells.

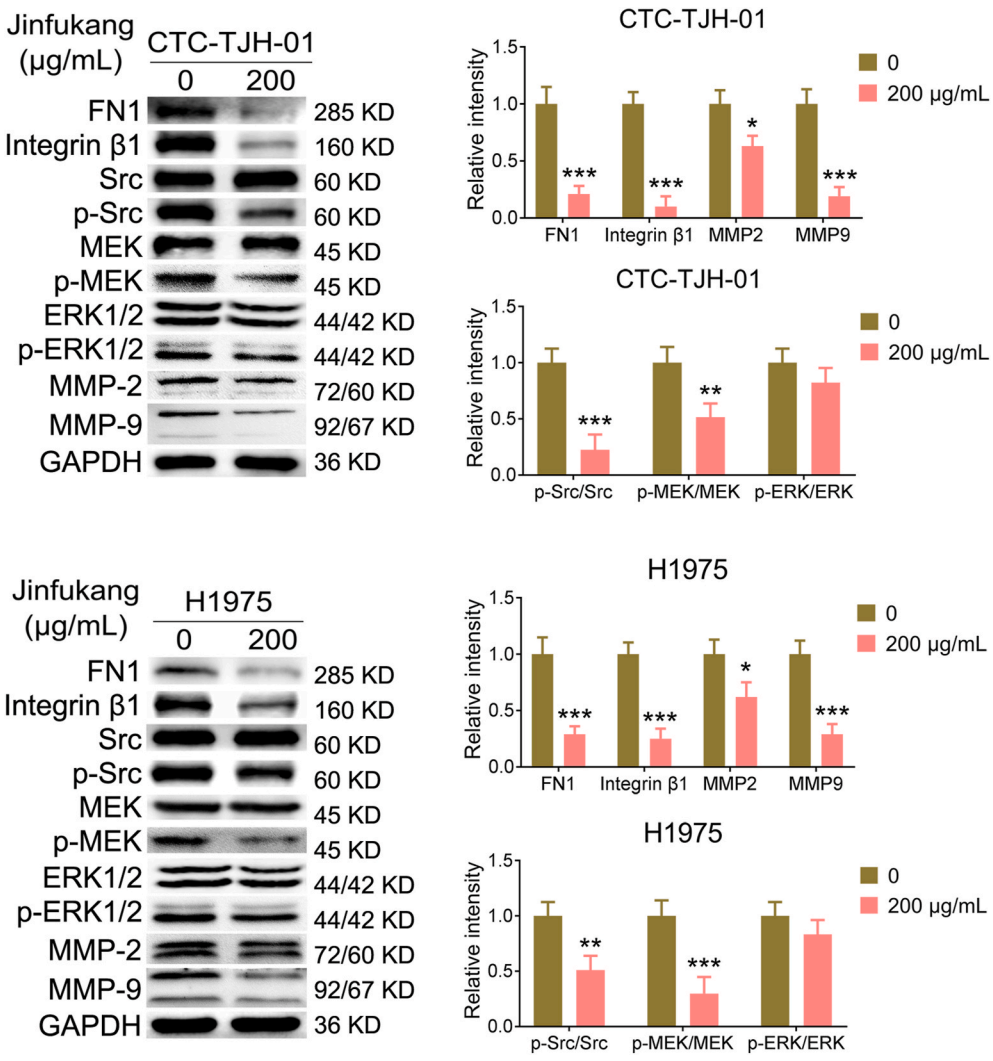

Fig. 4. Jinfukang reduced integrin expression and inhibited Src cascade in lung cancer cells. CTC-TJH-01 and H1975 cells were incubated with Jinfukang (0, 200 μg/mL) for 24 h. A Western blot assay was used to measure the protein expression levels of FN1, Integrin β1, Src, p-Src, MEK, p-MEK, ERK1/2, p-ERK1/2, MMP-2, and MMP-9 in CTC-TJH-01 and H1975 cells. GAPDH was used as an internal standard. Each bar represents the mean ± SD of three separate experiments. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

3.3. Jinfukang induced anoikis in CTC-TJH-01 and H1975 cells

The above results show that Jinfukang could significantly inhibit the invasion and migration of CTC-TJH-01 and H1975 cells without effect the apoptosis. Therefore, anoikis was analyzed using the anoikis assay. As shown in Fig. 3, Jinfukang significantly increased anoikis in CTC-TJH-01 and H1975 cells *in vitro*. These results indicate that Jinfukang may inhibit the invasion and migration of CTC-TJH-01 and H1975 cells by inducing anoikis.

3.4. Jinfukang inhibited the integrin/Src signaling cascade in CTC-TJH-01 and H1975 cells

In order to reveal the mechanism of Jinfukang inhibition of lung cancer cell metastasis, we analyzed the results of previous RNA sequencing and found that Jinfukang mainly affects the ECM receptor interaction signaling pathway of lung cancer cells (Figs. S1A, S1B, S1C). Therefore, we analyzed the integrin/Src signal pathway, which related to ECM receptor interaction. As shown in Fig. 4, Jinfukang significantly reduced the expression of FN1, integrin β1 and p-Src in CTC-TJH-01 and H1975 cells. In addition, the cell survival and metastasis-associated protein p-MEK, p-ERK1/2, MMP2 and MMP9 were also downregulated in CTC-TJH-01 and H1975 cells following exposure to Jinfukang (Fig. 4). These findings indicate that the integrin/Src pathway may be the mechanism of Jinfukang in inhibiting the metastasis of lung cancer cells.

3.5. Jinfukang inhibited the migration via integrin/Src axis to induced anoikis in CTC-TJH-01 and H1975 cells

In order to further clarify the role of integrin/Src signaling pathway in the inhibition of lung cancer cell metastasis by Jinfukang. We used integrin inhibitor ATN-161 combined with Jinfukang to treatment with CTC-TJH-01 and H1975 cells. As shown in Fig. 5A and B, when compared with Jinfukang alone group, the combined group had more significant inhibition on p-Src protein expression. In addition, cell migration assay was also observed the same results. ATN-161 treatment can promote the inhibitory effect of Jinfukang on the migration of CTC-TJH-01 and H1975 cells (Fig. 5C and D). Furthermore, in the anoikis assays, we also observed that ATN-161 can promote the effect of Jinfukang on anoikis of CTC-TJH-01 and H1975 cells (Fig. 6A and B). Collectively, the above results suggest that Jinfukang may inhibit the occurrence of lung cancer metastasis by inhibiting integrin/Src signaling pathway to induce lung cancer cells anoikis.

4. Discussion

Metastasis is considered to be a lethal hallmark of cancer (Steeg, 2016). At present, however, there is still no ideal intervention for metastasis prevention. The main reason is that researchers in this field usually apply primary cancer cell lines to underlying anti-metastasis study. Circulating tumor cells in the peripheral blood have not attracted much attention. Recent studies have shown that CTCs can be applied

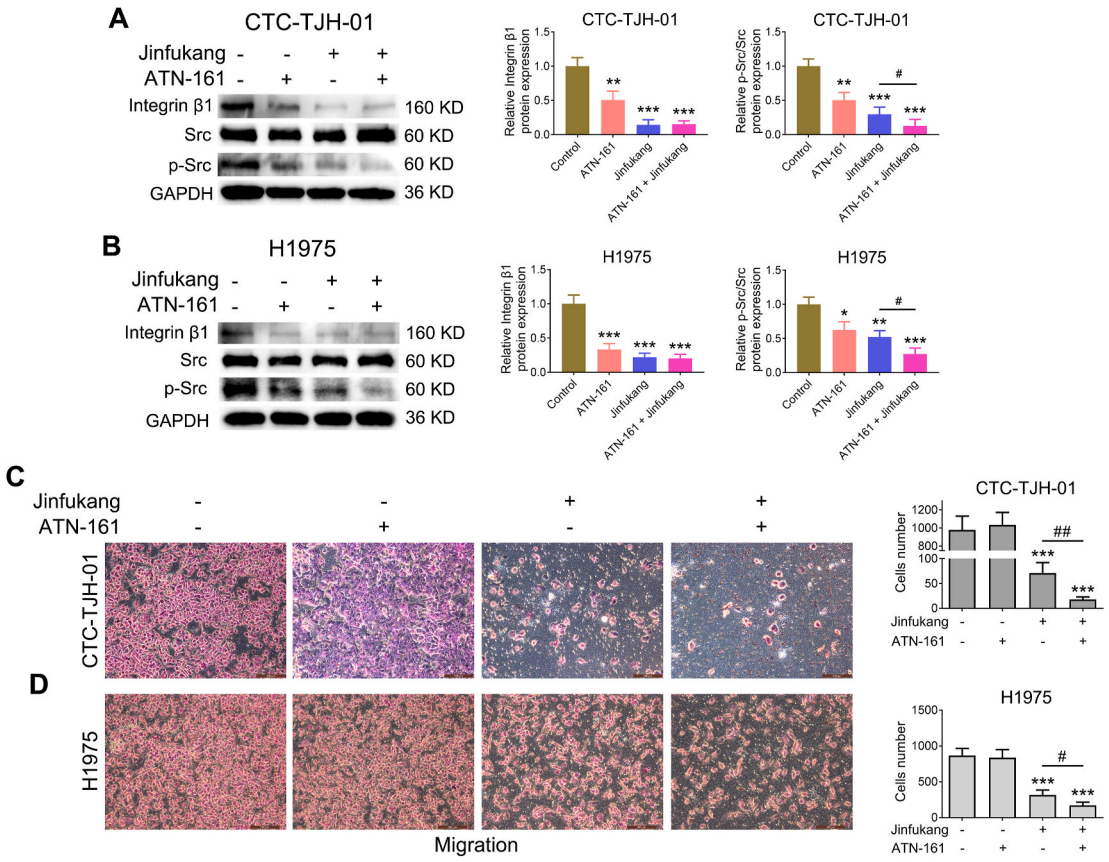

Fig. 5. Jinfukang inhibited the cell migration via Integrin/Src axis in lung cancer cells. Cells were incubated with Jinfukang (100 μg/mL), ATN-161 (10 μmol/L) or a combination of both for 24 h. A Western blot assay was used to measure the protein expression levels of Integrin β1, Src, and p-Src in CTC-TJH-01 (A) and H1975 (B) cells. GAPDH was used as an internal standard. The migration capacity of CTC-TJH-01 (C) and H1975 (D) cells was measured using transwell assays after treatment with Jinfukang, ATN-161 or a combination of both for 16 h. Each bar represents the mean ± SD of three separate experiments. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, combination vs Jinfukang $\#P < 0.05$; $\#\#P < 0.01$.

to predict disease progression and survival (Alama et al., 2014; Zhou et al., 2017). Multiple lines of evidence indicate that CTCs are already present even before the primary cancer can be detected (Hu et al., 2019; Ilie et al., 2014). Therefore, it is thought that stable CTC cell lines are optimal tool for studying tumor metastasis. Currently, *in vitro* established CTCs include breast cancer, colorectal cancer, small cell lung cancer, non-small cell lung cancer and so on (Cayrefourcq et al., 2015; Hodgkinson et al., 2014; Wang et al., 2016; Yu et al., 2014). We previously established a permanent CTC cell line named CTC-TJH-01, which was able to induce tumorigenesis, lung organ colonization and metastasis (Que et al., 2019a). Thus, in this study, we used CTC-TJH-01 cells to study the anti-metastasis effect of Jinfukang.

The traditional Chinese medicine prescription, Jinfukang, which consists of 12 Chinese herbal medicines, has been administered in clinical practice for treating NSCLC patients and was approved for clinical application by the China Food and Drug Administration (Cassileth et al., 2009; Que et al., 2020). A clinical study found that Jinfukang has the advantage of inhibiting metastasis in lung cancer and stabilizing tumour lesions (Liu et al., 2001). Although many studies have confirmed that the anti-tumour mechanisms of Jinfukang may included induction of cell cycle arrest, apoptosis, and epigenetic regulation (Kou et al., 2017; Lu et al., 2018; Lu, J. et al., 2016a; Lu, J. et al., 2016b). However, the mechanism underlying the effect of Jinfukang on lung cancer metastasis especially for CTCs is still unknown. Only a study by Hailang et al. reported that Jinfukang could inhibit the formation and migration of lymphatic endothelial cells (He et al., 2016). In this regard, our data clearly demonstrated that Jinfukang could significantly inhibit the invasion and migration of lung CTCs in a concentration dependent manner. At the same time, the expression of MMP-2 and MMP-9 proteins was suppressed by Jinfukang.

A key factor in metastasis is that CTCs are shed from primary tumors and enter peripheral blood to acquire anoikis resistance and to avoid immune killing and peripheral blood shear stress (Pantel and Speicher, 2016). Anoikis is a special programmed cell death form induced by the loss of cell contact with extracellular matrix and other cells. Apoptosis refers to the autonomous and orderly death of cells controlled by genes. The ability and mechanism of tumor cells to obtain anoikis resistance has caused widespread concern in the scientific community. Many studies have shown that induction of anoikis in the primary lung cancer can inhibit metastasis (Busaranon et al., 2016; Wongpankam et al., 2012). Our results show that Jinfukang could significantly induce anoikis in lung CTCs *in vitro,* and the proportion of anoikis increases with the increase of drug concentration. In addition, we also found that Jinfukang decreased the expression of p-MEK, and p-ERK in lung CTCs. Besides, we confirmed that Polyphyllin VII has the same effect, and it is the major constituent of Jinfukang (Data not shown).

Cancer cells achieved anoikis resistance mainly through regulating integrin switch, epithelial-mesenchymal transition, and activation of pro-survival signaling (Wang, W.C. et al., 2018). Among them, existing studies have found that PI3K/Akt, Ras/Raf-MEK/ERK, integrin/Src and other signaling pathways are involved in anoikis resistance (Meng et al., 2019). Wang and colleagues find that inhibiting integrin/Src signaling can reverse anoikis resistance in human gastric cancer cells (Wang, K. et al., 2018). Another study found that activation of integrin β1/FAK/ERK pathway with Crabp2 can promote lung cancer cells to obtain anoikis resistance and metastasis (Wu et al., 2019). Our study

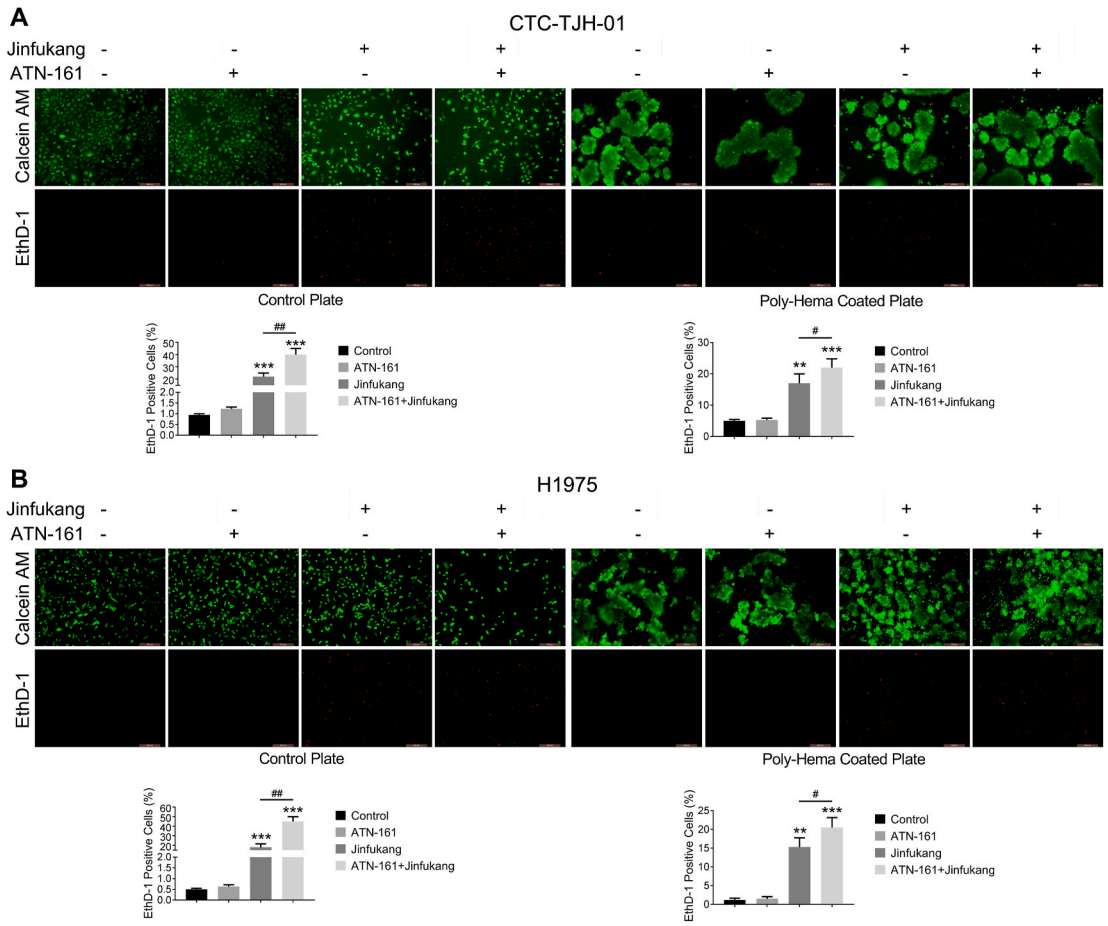

Fig. 6. Downregulation of integrin promotes the anoikis of lung cancer cells induced by Jinfukang. An anoikis assay was used to measure the anoikis levels in CTC-TJH-01 (A) and H1975 (B) cells after incubation with Jinfukang (100 μg/mL), ATN-161 (10 μmol/L) or a combination of both for 24 h. Each bar represents the mean ± SD of three separate experiments. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, combination vs Jinfukang #P < 0.05; ##P < 0.01.

found that Jinfukang can induce the anoikis of lung cancer cells by inhibiting integrin/Src and MEK/ERK signaling pathway. Although Jinfukang can down-regulate the expression of integrin on CTC-TJH-01 and H1975 cells, CTC-TJH-01 cells are more sensitive to Jinfukang-induced anoikis. This results show that there are some cell biological differences between CTCs in peripheral blood and tumor cells from primary tumors. In addition, we also found that Jinfukang inhibited the metastasis of lung cancer cells by inducing anoikis of CTCs. However, the inhibition of Jinfukang on lung cancer cell metastasis is related to the regulation of anoikis have been only confirmed *in vitro,* and further *in vivo* studies are needed.

5. Conclusion

In summary, our studies show that Jinfukang inhibit the migration and invasion of lung CTCs by suppress integrin/Src cascade. In addition, we affirmed that Jinfukang inhibit the metastasis of lung cancer cells by inducing anoikis of CTCs. Furthermore, when ATN-161 is combined with Jinfukang, it can promote Jinfukang to induce anoikis of lung cancer cells and inhibit cell migration. These results provide new insights for understanding how Jinfukang exerts effects against lung cancer metastasis.

Author contributions

Jian-Hui Tian, Huai-Min Liu and He-Gen Li designed and supervised the study. Zu-Jun Que, Yun Yang, Hai-Tao Liu, Wen-Ji Shang-Guang and Pan Yu performed experiments and were involved with data collection, analysis. Zujun Que, Yun Yang, and Lihua Zhu wrote the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2020.113473.

References

Alama, A., Truini, A., Coco, S., Genova, C., Grossi, F., 2014. Prognostic and predictive relevance of circulating tumor cells in patients with non-small-cell lung cancer. Drug Discov. Today 19 (10), 1671–1676.

Allemani, C., Matsuda, T., Di Carlo, V., Harewood, R., Matz, M., Niksic, M., Bonaventure, A., Valkov, M., Johnson, C.J., Esteve, J., Ogunbiyi, O.J., Azevedo, E.S. G., Chen, W.Q., Eser, S., Engholm, G., Stiller, C.A., Monnereau, A., Woods, R.R., Visser, O., Lim, G.H., Aitken, J., Weir, H.K., Coleman, M.P., Group, C.W., 2018. Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet 391 (10125), 1023–1075.

Anderson, R.L., Balasas, T., Callaghan, J., Coombes, R.C., Evans, J., Hall, J., Kinrade, S., Jones, D., Jones, P.S., Jones, R., Marshall, J.F., Panico, M.B., Shaw, J.A., Steeg, P.S., Sullivan, M., Tong, W., Westwell, A.D., Ritchie, J., Uk, C.R., 2019. Cancer therapeutics CRC Australia metastasis working group. A framework for the development of effective anti-metastatic agents. Nat Rev Clin Oncol 16 (3), 185–204.

Bi, L., Xie, C., Jiao, L., Jin, S., Hnit, S.S.T., Mu, Y., Wang, Y., Wang, Q., Ge, G., Wang, Y., Zhao, X., Shi, X., Kang, Y., De Souza, P., Liu, T., Zhou, J., Xu, L., Dong, Q., 2020. CPF impedes cell cycle re-entry of quiescent lung cancer cells through transcriptional suppression of FACT and c-MYC. J. Cell Mol. Med. 24 (3), 2229–2239.

Busaranon, K., Plaimee, P., Sritularak, B., Chanvorachote, P., 2016. Moscatilin inhibits epithelial-to-mesenchymal transition and sensitizes anoikis in human lung cancer H460 cells. J. Nat. Med. 70 (1), 18–27.

Cassileth, B.R., Rizvi, N., Deng, G., Yeung, K.S., Vickers, A., Guillen, S., Woo, D., Coleton, M., Kris, M.G., 2009. Safety and pharmacokinetic trial of docetaxel plus an Astragalus -based herbal formula for non-small cell lung cancer patients. Canc. Chemother. Pharmacol. 65 (1), 67–71.

Cayrefourcq, L., Mazard, T., Joosse, S., Solassol, J., Ramos, J., Assenat, E., Schumacher, U., Costes, V., Maudelonde, T., Pantel, K., Alix-Panabieres, C., 2015. Establishment and characterization of a cell line from human circulating colon cancer cells. Canc. Res. 75 (5), 892–901.

Gkountela, S., Castro-Giner, F., Szczerba, B.M., Vetter, M., Landin, J., Scherrer, R., Krol, I., Scheidmann, M.C., Beisel, C., Stirnimann, C.U., Kurzeder, C., Heinzelmann-Schwarz, V., Rochlitz, C., Weber, W.P., Aceto, N., 2019. Circulating tumor cell clustering shapes DNA methylation to enable metastasis seeding. Cell 176, 98–112 e114.

He, H.L., Dan, W., Jie, T., Zhou, X.M., Li, J.X., Ling, X., 2016. Jin Fu Kang oral liquid inhibits lymphatic endothelial cells formation and migration. Evid Based Complement Alternat Med 2016 (3), 1–10.

Hnit, S.S.T., Yao, M., Xie, C., Ge, G., Bi, L., Jin, S., Jiao, L., Xu, L., Long, L., Nie, H., Jin, Y., Rogers, L., Suchowerska, N., Wong, M., Liu, T., De Souza, P., Li, Z., Dong, Q., 2020. Transcriptional regulation of G2/M regulatory proteins and perturbation of G2/M Cell cycle transition by a traditional Chinese medicine recipe. J. Ethnopharmacol. 251, 112526.

Hodgkinson, C.L., Morrow, C.J., Li, Y., Metcalf, R.L., Rothwell, D.G., Trapani, F., Polanski, R., Burt, D.J., Simpson, K.L., Morris, K., 2014. Tumorigenicity and genetic profiling of circulating tumor cells in small-cell lung cancer. Nat. Med. 20 (8), 897–903.

Hu, Z., Ding, J., Ma, Z.C., Sun, R.P., Seoane, J.A., Scott, S.J., Suarez, C.J., Berghoff, A.S., Cremolini, C., Falcone, A., Loupakis, F., Birner, P., Preusser, M., Lenz, H.-J., Curtis, C., 2019. Quantitative evidence for early metastatic seeding in colorectal cancer. Nat. Genet. 51 (7), 1113–1122.

Ilie, M., Hofman, V., Long-Mira, E., Selva, E., Vignaud, J.M., Padovani, B., Mouroux, J., Marquette, C.H., Hofman, P., 2014. Sentinel" circulating tumor cells allow early diagnosis of lung cancer in patients with chronic obstructive pulmonary disease. PloS One 9 (10), e111597.

Joosse, S.A., Gorges, T.M., Pantel, K., 2015. Biology, detection, and clinical implications of circulating tumor cells. EMBO Mol. Med. 7 (1), 1–11.

Kou, Y., Li, G., Shao, J., Liu, C., Wu, J., Lu, J., Zhao, X., Tian, J., 2017. Genome-wide profiling reveals that herbal medicine jinfukang-induced polyadenylation alteration is involved in anti-lung cancer activity. Evid Based Complement Alternat Med 2017, 5326909.

Liang, X., Chen, W.X., Shi, H.Y., Gu, X.Y., Li, Y.Q., Qi, Y.X., Xu, K., Zhao, A.G., Liu, J.W., 2018. PTBP3 contributes to the metastasis of gastric cancer by mediating CAV1 alternative splicing. Cell Death Dis. 9, 569.

Liu, J.X., Shi, Z.M., Li, H.G., Xu, Z.Y., Zhu, Y.W., Zhao, L.H., Hong, G., Liu, L.S., Zhu, H. R., Hui, Z., 2001. Clinical observation on 271 cases of non-Small cell lung cancer treated with yifei kangliu yin. Chin. J. Integr. Med. 7, 247–250.

Lu, J., Chen, J., Kang, Y., Wu, J., Shi, H., Fu, Y., Jiao, L., Dong, C., Li, X., Jin, Y., Zhao, W., Xu, L., Zhao, X., 2018. Jinfukang induces cellular apoptosis through activation of Fas and DR4 in A549 cells. Oncol Lett 16 (4), 4343–4352.

Lu, J., Chen, J., Xu, N.J., Wu, J., Kang, Y.N., Shen, T.T., Kong, H.L., Ma, C., Cheng, M., Shao, Z.F., Xu, L., Zhao, X.D., 2016a. Activation of AIFM2 enhances apoptosis of human lung cancer cells undergoing toxicological stress. Oncology letters 258, 227–236.

Lu, J., Zhang, X.L., Shen, T.T., Ma, C., Wu, J., Kong, H.L., Tian, J., Shao, Z.F., Zhao, X.D., Xu, L., 2016b. Epigenetic profiling of H3K4Me3 reveals herbal medicine jinfukang-induced epigenetic alteration is involved in anti-lung cancer activity. Evidence-Based Complementray and Alternative Medicine : eCAM 2016 (4), 7276161.

Lu, S.M., Ma, Y.M., Sun, T., Ren, R., Zhang, X.N., Ma, W.S., 2016. Expression of α-fetoprotein in gastric cancer AGS cells contributes to invasion and metastasis by influencing anoikis sensitivity. Oncol. Rep. 35 (5), 2984–2990.

Massague, J., Obenauf, A.C., 2016. Metastatic colonization by circulating tumour cells. Nature 529 (7586), 298–306.

Meng, L.B., Liu, B.Q., Ji, R., Jiang, X., Yan, X.B., Xin, Y., 2019. Targeting the BDNF/TrkB pathway for the treatment of tumors. Oncol Lett 17 (2), 2031–2039.

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.

Pantel, K., Speicher, M., 2016. The biology of circulating tumor cells. Oncogene 35 (10), 1216–1224.

Que, Z.J., Luo, B., Wang, C.T., Qian, F.F., Jiang, Y., Li, Y., Han, X.H., Li, H.G., Liu, J.X., Tian, J.H., 2020. Proteomics analysis of tumor exosomes reveals vital pathways of Jinfukang inhibiting circulating tumor cells metastasis in lung cancer. J. Ethnopharmacol. 256, 112802.

Que, Z.J., Luo, B., Zhou, Z.Y., Dong, C.S., Jiang, Y., Wang, L., Shi, Q.H., Tian, J.H., 2019a. Establishment and characterization of a patient-derived circulating lung tumor cell line in vitro and in vivo. Canc. Cell Int. 19, 21.

Que, Z.J., Zhou, Z.Y., Luo, B., Dong, C.S., Jiang, Y., Li, H.G., Tian, J.H., 2019b. Jingfukang induces anti-cancer activity through oxidative stress-mediated DNA damage in circulating human lung cancer cells. BMC Compl. Alternative Med. 19 (1), 204.

Reinhold, W.C., Sunshine, M., Varma, S., Doroshow, J.H., Pommier, Y., 2015. Using CellMiner 1.6 for systems pharmacology and genomic analysis of the NCI-60. Clin. Canc. Res. 21 (17), 3841–3852.

Siegel, R.L., Miller, K.D., Jemal, A., 2019. Cancer statistics. Ca - Cancer J. Clin. 69 (1), 7–34, 2019.

Steeg, P.S., 2016. Targeting metastasis. Nat. Rev. Canc. 16 (4), 201–218.

Wang, K., Zhu, X., Mei, D., Ding, Z., 2018. Caveolin-1 contributes to anoikis resistance in human gastric cancer SGC-7901 cells via regulating Src-dependent EGFR-ITGB1 signaling. J. Biochem. Mol. Toxicol. 32 (10), e22202.

Wang, W.C., Zhang, X.F., Peng, J., Li, X.F., Wang, A.L., Bie, Y.Q., Shi, L.H., Lin, M.B., Zhang, X.F., 2018. Survival mechanisms and influence factors of circulating tumor cells. BioMed Res. Int. 6304701.

Wang, Z.H., Wu, W.J., Wang, Z., Tang, Y., Deng, Y.L., Xu, L., Tian, J.H., Shi, Q.H., 2016. Ex vivo expansion of circulating lung tumor cells based on one-step microfluidics-based immunomagnetic isolation. Analyst 141 (12), 3621–3625.

Weber, G.F., 2013. Why does cancer therapy lack effective anti-metastasis drugs? Canc. Lett. 328 (2), 207–211.

Wongpankam, E., Chunhacha, P., Pongrakhananon, V., Sritularak, B., Chanvorachote, P., 2012. Artonin E mediates MCL1 down-regulation and sensitizes lung cancer cells to anoikis. Anticancer Res. 32 (12), 5343–5351.

Wu, J.-I., Lin, Y.-P., Tseng, C.-W., Chen, H.-J., Wang, L.-H., 2019. Crabp2 promotes metastasis of lung cancer cells via HuR and integrin β1/FAK/ERK signaling. Sci. Rep. 9 (1), 845.

Yu, M., Bardia, A., Aceto, N., Bersani, F., Madden, M.W., Donaldson, M.C., Desai, R., Zhu, H., Comaills, V., Zheng, Z., Wittner, B.S., Stojanov, P., Brachtel, E., Sgroi, D., Kapur, R., Shioda, T., Ting, D.T., Ramaswamy, S., Getz, G., Iafrate, A.J., Benes, C., Toner, M., Maheswaran, S., Haber, D.A., 2014. Cancer therapy. Ex vivo culture of circulating breast tumor cells for individualized testing of drug susceptibility. Science 345 (6193), 216–220.

Zhou, J., Dong, F., Cui, F., Xu, R., Tang, X.K., 2017. The role of circulating tumor cells in evaluation of prognosis and treatment response in advanced non-small-cell lung cancer. Canc. Chemother. Pharmacol. 79 (4), 825–833.