Transthyretin (TTR) Suppressed Tumor Progression in Non-Small Cell Lung Cancer by Inactivating MAPK/ERK Pathway

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Research

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Abstract

**Background:** Lung cancer is a leading cause of cancer death around the world, while the Transthyretin (TTR) is a specific biomarker for clinical diagnosis. However, its role in lung cancer remains to be unknown.

**Methods:** In the present study, we made attempt to investigate effect of abnormal expression of TTR on Non-small-cell lung carcinoma (NSCLC) by overexpression or knockdown of TTR. To further investigate the mechanisms underlying the potential role of TTR in NSCLC, we searched and verified several signal pathways. In vivo experiments, to verify the effect of TTR overexpression on tumors.

**Results:** We found that up-regulated TTR obviously suppressed cell proliferation, migration, invasion and increased apoptosis. Significant suppression of phosphor-MAPK/ERK was observed in TTR-treated NSCLC cells, implying that TTR was important for cellular progress by regulating MAPK/ERK signaling pathway. In vivo experiment, overexpression of TTR promoted cell apoptosis and inhibited tumor growth.

**Conclusions:** Overall, our results suggest that TTR has a potential anti-tumor effect in human NSCLC progression, which provides theoretical basis for the diagnosis and treatment of NSCLC. Above all, further understanding of TTR was useful for clinical care.

1. Introduction

Lung cancer is the most common malignant tumor in the world and is primary reason of cancer-related death, thus posing a great threat to human health[1]. In China, the morbidity and mortality of lung cancer rank first in malignant tumors[2]. Of note, non-small-cell lung carcinoma (NSCLC) is main type of lung cancer[3]. NSCLC can be further divided into two main subtypes: adenocarcinoma and squamous cell carcinoma[3]. Although great progresses have been obtained in early detection and treatment strategies over the past few decades, the survival rate still remains low level[4]. Therefore, further investigation on the molecular mechanisms on NSCLC will be essential for diagnosis and therapy.

Transthyretin is a 55-kDa homotetrameric protein, which participates in the transport of thyroid hormones in blood [5, 6] and plays an important role in retinol metabolism. It is partly produced by the liver and extrahepatic tissues, such as retinal pigment, epithelium cells, choroids plexus and islet A and B cells [7–9]. TTR is a highly abundant protein that binds to circulating RBP to prevent low molecular mass RBP [10–14]. The levels of TTR could be decreased in severe liver disease, malnutrition and inflammation[15–17]. In addition, TTR level was decreased in the serum of patients with ovarian cancer, advanced cervical and endometrial carcinomas [18]. Therefore, TTR has been proved to be a functional biomarker for the identification of lung cancer. However, the effect and mechanism of TTR on the lung cancer cells still remain elusive.

In this study, we investigated the mechanism underlying abnormal expression of TTR on NSCLC cells, and try to search for efficient signaling pathway in TTR-mediated NSCLC cells. By further investigation
2. Materials And Methods

2.1 Clinical tissue specimens

The tumor tissues and paired adjacent non-cancer tissues were collected from a total of 25 patients diagnosed with NSCLC at Tianjin Hospital, Tianjin Medical University. The protocol of this study was approved by the Ethics Committee of Tianjin Hospital, Tianjin Medical University, and written informed consent was acquired from all recruited patients. None of the participants received anti-tumor treatment prior to surgical resection.

2.2 Cell culture and cell transfection

Human NSCLC cell lines (A549 and H1975) and Human normal lung cell lines (16HBE) were achieved from the Chinese Academy of Science Cell Bank (Shanghai, China). Cells were cultured in RPMI-1640 medium (HyClone, South Logan, UT, USA) supplied with 10% fetal bovine serum (FBS, Biowest, Barcelona, Catalonia, Spain) at 37 °C and 5% CO₂. TTR overexpression and inhibitor were achieved from GenePharma (Shanghai, China). The shRNA vectors against TTR (TTR inhibitor) or were utilized for knockdown of TTR with scrambled shRNA (NC) as negative control. For TTR overexpression, the full length of TTR CDS (coding domain sequence) was inserted into pcDNA3.1 vectors (Invitrogen, USA) and the empty plasmids served as negative control. Cell transfection was performed with Lipofectamine 2000 (Invitrogen, USA) according to the product manuals.

2.3 RNA isolation and qRT-PCR

Total RNA was extracted using TRIzol reagent (Invitrogen, USA). Equal amounts of RNA were transcribed into cDNA using the cDNA First Strand Synthesis kit. Total cDNA was used as a starting material for real-time PCR using the Step One real-time PCR System (Life Technologies Corp), and each sample was measured in triplicate. Total reaction system as follows (20 µL): cDNA 1 µL, SYBR Premix EX Taq 10 µL, each of the primers (10 µM) 1 µL, and ddH₂O 7 µL. The procedure of PCR was as follows: 95 °C for 3 min followed by 40 cycles of 95 °C for 10 s, and 60 °C for 30 s. All fold changes were calculated using the 2−ΔΔCT comparative method using GAPDH for normalization.

2.4 Western blotting

50 µg total protein per lane were separated by 10% SDS-PAGE gel and blotted onto polyvinylidene fluoride (PVDF) membranes. The membrane was blocked in PBST and probed with primary antibodies for Bcl-2 (1: 1, 000, ab32124; Abcam, Cambridge, MA, USA), Bax(1: 1, 000, ab32503; Abcam, Cambridge, MA, USA), caspase-3 (1: 1, 000, ab13847; Abcam, Cambridge, MA, USA), caspase-9 (1: 1, 000, ab13847; Abcam, Cambridge, MA, USA), MMP-2 (1: 1, 000, ab37150; Abcam, Cambridge, MA, USA), MMP-9 (1: 1, 000, ab73734; Abcam, Cambridge, MA, USA), phosphor-MAPK/ERK (1:2,000, #9106, Danvers, MA, USA), MAPK/ERK (1:1,000, #9102, Danvers, MA, USA ), and GAPDH (1: 5, 000, G8795; Sigma-Aldrich, St. Louis, MO, USA) at 4 °C overnight. Subsequently, application of appropriate secondary antibodies to incubate
the membranes (1: 5, 000, ab6858; Abcam, Cambridge, MA, USA), followed by visualized using an ECL kit (Beyotime Institute of Biotechnology, Beijing, China). The bands were scanned using adensitometer, and the gray value of the bands were calculated automatically by the ImageJ software version k 1.45 (NIH, Bethesda, MD, USA).

2.5 Cell proliferation assay
EdU assay were applied to detect cell proliferation according to the manufacturers’ protocol. For colony formation assay, cells were seeded in a 6-well plate at a density of 1, 000 cells/well for two weeks, after which clones were fixed with methanol and stained with 0.1% crystal violet.

2.6 Cell apoptosis assay
FITC-Annexin V/PI apoptotic detection kit (BD ingen, San Diego, CA, USA) was used to detect apoptotic cells by flow cytometry. The cells (1 × 10^5 cells) were stained with propidium iodide (5 mL) and Annexin V-FITC (5 mL), followed by fluorescence determination by Flow cytometer (Beckman, Miami, FL, USA). Tunel assay was also applied to detect the apoptosis in cells with the In Situ Cell Death Detection Kit (Roche, USA). The cells were washed with PBS and fixed in 500 µl 4% paraformaldehyde solution for 15 min at 37 °C. All analyses were performed in triplicate.

2.7 Scratch assay
Cells were seeded in 6-well plates. Approximate 80% of the cells were put into the wall and rinsed in PBS for twice to remove floating cells. Wounds were obtained from sterile pipette tips. Afterwards, cells were washed with PBS twice and 2 mL RPMI-1640 culture medium containing 10% FBS was added into the culture plate. Photographs were taken at 24 h and 48 h after wound generation.

2.8 Transwell invasion assay
Transwell invasion assay was measured according to the manufacturer's instructions. In brief, a total of 4 × 10^4 cells/well in 100µL DMEM (0.5% FBS) were placed in the upper Transwell chamber (Corning Incorporated, NY, USA), which was pre-coated with matrigel (Growth factor reduced, BD Biosciences, MD, USA). The bottom chambers were filled with DMEM containing 20% FBS. Once passed through the membranes, the cells were fixed and stained after 24 h. Cells in nine randomly selected fields were counted under an inverted microscope (Carl ZEISS, Jena, German) at 200 × magnification and an average value was used as the number of invaded cells. For the cell transmembrane migration assay, all the steps were carried out similarly to those in the invasion assay except for the Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) coating.

2.9 Tumor xenograft model in nude mice
Male BALB/c nude mice (5 weeks old; 18–20 g) were purchased from the Beijing Vital River Laboratory Animal Technology (Beijing, China). The xenograft model of human A549 and H1975 cells were established. A549 and H1975 cells transfected with sh-NEAT1 or sh-NC were injected subcutaneously into the posterior fossa of nude mice (six per group). After 5d, when average tumor volume was approximately 50 mm^3, mice were intraperitoneally injected with paclitaxel (3.5 mg/kg body weight) or PBS every three days. The tumor volumes were measured every week by using calipers: Volume (cm^3) = width^2 (cm^2) × length (cm)/2. Mice were sacrificed at 32th day after injection and tumors were removed, weighed and analyzed by RT-qPCR.
2.10 Statistical analysis
All results are performed at least three independent replicates and reported as means ± standard deviation. All statistical analyses were performed using GraphPad software 5.0. Differences between two groups were calculated by Student’s t-test. Differences between three or more groups were calculated by one-way analysis of variance (ANOVA) test followed by Tukey’s multiple comparison test. A $P$ value < 0.05 was considered statistically significant.

3. Results

3.1 The expression level of TTR is decreased in NSCLC tissues and NSCLC cells

By examining 25 pairs of human NSCLC tissues and normal tissues, we found a prominent decrease in TTR expression in NSCLC tissues compared to that in the normal tissues (Figure 1A). At the same time, we observed that compared with human bronchial epithelial cells, the expression level of TTR in two NSCLC cell lines was significantly reduced (Figure 1B). These results indicate that TTR may negatively correlated with NSCLC progression.

3.2 TTR represses NSCLC cell proliferation and promotes its apoptosis \textit{in vitro}

To dissect the role of TTR in cell proliferation, we over-expressed TTR (TTR overexpression) or transfected with TTR inhibitors in A549 and H1975 cells, respectively (Figure 2A). EdU assay indicated that proliferation capacity of both A549 and H1975 cells were reduced in TTR-overexpressed group, while were increased in the TTR inhibitor-treated group (Figure 2B).

Consistently, these results were further confirmed by colony formation assay (Figure 2C).

In addition, we measured cell apoptosis by flow cytometry and TUNEL assays, suggesting that proliferation capacity of both A549 and H1975 cells was decreased in TTR-overexpressed group (Figure 3A, B). At the molecular level, Western blot analysis was performed to detect the expression levels of apoptosis-related proteins. We found that pro-apoptotic protein Bcl2 was down-regulated and anti-apoptosis proteins (Bax, caspase-3, and caspase-9) was up-regulated in TTR overexpression group (Figure 3C). The results indicated that TTR overexpression group promoted cell apoptosis in A549 and H1975 cells.

3.3 TTR inhibits NSCLC cell migration and invasion \textit{in vitro}

The effects of TTR on cell migration and invasion were studied by Transwell chamber and scratch methods. Transwell chamber assay indicated that less cells were migrated and invaded to the lower medium in TTR overexpression group compared with TTR inhibitor group (Figure 4A, B). As shown in Figure 4C, scratch assay indicated that there was significant difference on migrated distance between control group and TTR inhibitor group. Then, we performed western blotting to detect Matrix metal proteinases (MMP2/9). The expression level of MMP-2 and MMP-9 were down-regulated in TTR overexpression group (Figure 4D). According to these results, up-regulated TTR suppressed NSCLC cell migration and invasion \textit{in vitro}. 
3.4 TTR inhibits the activation of MAPK/ERK pathway in vitro

To further gain insights into the downstream signaling pathways regulation by TTR in NSCLC cells, we examined the functional effects of TTR in several important cancer pathways including Wnt, TGF-β, NF-κB and MAPK/ERK by RT-PCR. TTR overexpression significantly inhibited the expression of MAPK/ERK in A549 and H1975 cells (Figure 5A). Western blot results showed phosphor-MAPK/ERK was observed to be significant suppressed in the TTR overexpression group (Figure 5B), indicating that the MAPK/ERK signaling pathway was essential for tumor growth in NSCLC cells. TTR overexpression inhibited MAPK/ERK phosphorylation, thereby stimulating cell proliferation, cell survival.

3.5 TTR ameliorates NSCLC progression in vivo

Based on the function of TTR in vitro, we further investigated whether overexpression of TTR could suppressed NSCLC progression in vivo. The expression of TTR was detected by RT-PCR (Figure 6A). As a result, tumors from TTR overexpression grew obviously slower than the tumors from other groups. 5 weeks after injection, the mice were killed and the tumor was removed. The tumor size and weight were obviously smaller than other groups (Figure 6B). These results implied that TTR could inhibited tumor progression. TUNEL assay showed that increased apoptosis in TTR overexpression group compared with NC group. Conversely, inhibiting the expression level of TTR can significantly reduce cell apoptosis (Figure 6C).

4. Discussion

Lung cancer is explored and reported to be the most common cause of cancer death in humans, with a 5-year survival rate of less than 15% [19, 20]. Many explorations about prevention, diagnosis and therapy improvement of lung cancer, the prognosis of lung cancer patients still pessimistic. In this research, we demonstrated that TTR suppressed tumor progression in NSCLC by inactivating MAPK/ERK pathway.

Abnormal expressions of TTR have been reported to play an important role in tumor formation and development[21, 22]. For example, Shimura T et al. found that transthyretin levels showed statistically significant inverse correlations with tumor size and the numbers of involved lymph nodes which is found contributed to predicting the prognosis of patients with gastric cancer[23].However, there is still limited understanding of effect of TTR abnormal expressions on tumor progression. In this study, we found that human NSCLC tissues had lower expression level of NSCLC than normal tissue, and its expression level was correlated with clinic pathological features. Inhibiting the expression level of TTR in NSCLC cells cultured in vitro can significantly increase cell proliferation, migration and invasion, and reduce cell apoptosis. Conversely, overexpression of TTR is sufficient to inhibit cell proliferation and invasive ability exhibited by NSCLC cells, and to increase apoptosis of NSCLC cells. To further investigate mechanisms of TTR on tumor progress in NSCLC, we searched for several signaling pathways, and found that MAPK/ERK signals were responded to the TTR-manipulation in NSCLC cells. In vivo experiment, overexpression of TTR obviously inhibited tumor growth and promoted cell apoptosis, implying that TTR was important for regular cellular progress.
The tumor associated pathways, such as Wnt, PI3K/AKT or NF-kB, may play an important role in the development of tumorigenesis [24–30]. Further, there is increasing evidence that typical MPAK/ERK signaling pathway is a pivotal signaling pathway involved in the development of several cancers [31–36]. In order to further explore the potential molecular mechanism of TTR in A549 and H1975 cells, we focused on the MAPK/ERK signaling pathway. Previous studies have reported that some genes affected the MAPK/ERK signal pathway [37]. Aberrant activation of the MAPK/ERK pathway promoted cell proliferation, migration and differentiation, and induced cell cycle arrest [38–41]. Our results suggested that the expression of phosphor-MAPK/ERK was markedly suppressed in the TTR overexpression group, indicating that the MAPK/ERK signaling pathway was essential for tumor growth in NSCLC cells.

Taken together, our work firstly demonstrated that TTR played a vital role in NSCLC tumorigenesis. TTR suppressed cell proliferation, migration, and invasion, and promoted apoptosis at least partly through inhibiting MPAK/ERK signaling pathway, which was demonstrated to be an effective diagnostic and predictive biomarker for diagnosis and treatment in NSCLC.

**Declarations**

**Compliance with Ethical Standards**

**Ethics approval and consent to participate**

The protocol of this study was approved by the Ethics Committee of Tianjin Hospital, Tianjin Medical University, and written informed consent was acquired from all recruited patients. None of the participants received anti-tumor treatment prior to surgical resection. The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Animal Ethics Committee of Tianjin Hospital.

**Availability of data and material**

The datasets used during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors' contributions**

Dong-bin Wang conceived and designed the study. Xuan Li and Lan Ma performed the literature search and data extraction. Xi-ke Lu and Hong-gang Xia drafted the manuscript. Zhong-yi Sun investigates for
the experiment. Xun Zhang and Xia Chen verified the feasibility of the experiment. All authors read and approved the final manuscript.

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References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J. Jemal A: Global cancer statistics. CA Cancer J Clin. 2012;65(2):87–108.

2. Wood SL, Pernemalm M, Crosbie PA, Whetton AD. Molecular histology of lung cancer: from targets to treatments. Cancer Treat Rev. 2015;41(4):361–75.

3. Zhou Q, Huang SX, Zhang F, Li SJ, Liu C, Xi YY, Wang L, Wang X, He QQ, Sun CC, Li DJ. MicroRNAs: A novel potential biomarker for diagnosis and therapy with non-small cell lung cancer. Cell Prolif 50(6), 2017.

4. Siegel RL, Miller KD, Jemal A. Cancer statistics. CA Cancer J Clin. 2018;68(1):7–30.

5. Power DM, Elias NP, Richardson SJ, et al. Evolution of the thyroid hormone binding protein, transthyretin. Gen Comp Endocrinol, 119: 241 – 55, 2000.

6. Fung ET, Yip TT, Lomas L, et al. Classification of cancer types by measuring variants of host response proteins using SELDI serum assays. Int J Cancer. 2005;115:783–9.

7. Jacobsson B, Collins VP, Grimelius L, et al. Transthyretin immunoreactivity in human and porcine liver, choroid plexus, and pancreatic islets. J Histochem Cytochem. 1989;37:31–7.

8. Itoh N, Hanafusa T, Miyagawa J, et al. Transthyretin (prealbumin) in the pancreas and sera of newly diagnosed type I (insulin-dependent) diabetic patients. J Clin Endocrinol Metab. 1992;74:1372–7.

9. Thomas JGL, Jeffrey M, Victoria M, et al. Transthyretin (prealbumin) immunoreactivity in renal cell carcinoma and other neoplasms. Int J Surg Pathol. 1996;4::1–7.

10. Menard C, Johann D, Lowenthal M, et al. Discovering clinical biomarkers of ionizing radiation exposure with serum proteomic analysis. Cancer Res. 2006;66:1844–50.

11. Lowenthal MS, Mehta AI, Frogale K, et al. Analysis of albumin-associated peptides and proteins from ovarian cancer patients. Clin Chem. 2005;51:1933–45.

12. Liotta LA, Petricoin EF. Serum peptidome for cancer detection: spinning biologic trash into diagnostic gold. J Clin Invest. 2006;116:26–30.

13. Geho DH, Liotta LA, Petricoin EF, et al. The amplified peptidome: the new treasure chest of candidate biomarkers. Curr Opin Chem Biol. 2006;10:50–5.

14. Raghu P, Sivakumar B. Interactions amongst plasma retinol-binding protein, transthyretin and their ligands. Implications in vitamin A homeostasis and transthyretin amyloidosis. Biochim Biophys Acta. 2004;1703:1–9.
15. Imanishi T. Clinical and experimental studies on the profiles of serum proteins in acute hepatic injury. Gastroenterol Jpn. 1981;16:493–505.

16. Marten NW, Sladek FM, Straus DS. Effect of dietary protein restriction on liver transcription factors. Biochem J. 1996;317:361–70.

17. Ritchie RF, Palomaki GE, Neveux LM, et al. Reference distributions for the negative acute-phase serum proteins, albumin, transferrin and transthyretin: a practical, simple and clinically relevant approach in a large cohort. Clin Lab Anal. 1999;13:273–9.

18. Kozak KR, Su F, Whitelegge JP, et al. Characterization of serum biomarkers for detection of early stage ovarian cancer. Proteomics. 2005;5:4589–96.

19. Zhao L, Zhang Y, Liu J, et al. MiR-185 inhibits cell proliferation and invasion of non-small cell lung cancer by targeting KLF7.[J]. Oncology Research, 2018.

20. Masters GA, Johnson DH, Temin S. Systemic Therapy for Stage IV Non-Small-Cell Lung Cancer: American Society of Clinical Oncology Clinical Practice Guideline Update.[J]. J Oncol Pract. 2017;33(30):832–7.

21. Raveche ES, Salerno E, Scaglione BJ, Manohar V, Abbasi F, Lin YC, Fredrickson T, Landgraf P, Ramachandra S, Huppi K, Toro JR, Zenger VE, Metcalf RA, Marti GE. AbnormmicroRNA-16 locus with synteny to human 13q14 linked to CLLin NZB mice. Blood. 2007;109:5079–86.

22. Hatley ME, Patrick DM, Garcia MR, Richardson JA, Bassel-Duby R, van Rooij E, Olson EN. Modulation of K-Rasdependentlung tumorigenesis by MicroRNA-21. Cancer Cell. 2010;18:282–93.

23. Shimura T, Shibata M, Gonda K, et al. Serum transthyretin level is associated with prognosis of patients with gastric cancer[J]. J Surg Res. 2018;227:145–50.

24. Liu W, Chen Y, Xie H, et al. TIPE1 suppresses invasion and migration through down-regulating Wnt/β-catenin pathway in gastric cancer[J]. J Cell Mol Med. 2017;22(2)(10):1103–17. 22,2(2017-10-).

25. Qiao B, He BX, Cai JH, et al. MicroRNA-27a-3p Modulates the Wnt/β-Catenin Signaling Pathway to Promote Epithelial-Mesenchymal Transition in Oral Squamous Carcinoma Stem Cells by Targeting SFRP1[J]. Sci Rep. 2017;7:44688.

26. Fu X, Liu M, Qu S, et al. Exosomal microRNA-32-5p induces multidrug resistance in hepatocellular carcinoma via the PI3K/Akt pathway.[J]. Journal of Experimental Clinical Cancer Research. 2018;37(1):52.

27. Sun D, Tang B, Li Z, et al. MiR-29c reduces the cisplatin resistance of non-small cell lung cancer cells by negatively regulating the PI3K/Akt pathway[J]. Sci Rep. 2018;8(1):8007.

28. Hui L, Huang ZP, Jiao L, et al. MiR-494-3p promotes PI3K/AKT pathway hyperactivation and human hepatocellular carcinoma progression by targeting PTEN[J]. Sci Rep. 2018;8(1):10461-.

29. Liao J, Wu F, Wen L, et al. Taraxerol exerts potent anticancer effects via induction of apoptosis and inhibition of Nf-kB signalling pathway in human middle ear epithelial cholesteatoma cells[J]. Tropical Journal of Pharmaceutical Research, 2018, 17(6).
30. Gao X, Chen, Guangming, Gao C, et al. MAP4K4 is a novel MAPK/ERK pathway regulator required for lung adenocarcinoma maintenance[J]. Mol Oncol. 2017;11(6):628–39.

31. Wagner EF, Nebreda AR. Signal integration by JNK and p38 MAPK pathways in cancer development[J]. Nat Rev Cancer. 2009;9(8):537.

32. Zhu C, Qi X, Chen Y, et al. PI3K/Akt and MAPK/ERK1/2 signaling pathways are involved in IGF-1-induced VEGF-C upregulation in breast cancer[J]. Journal of Cancer Research Clinical Oncology. 2011;137(11):1587–94.

33. Chang MC, Chen CA, Chiang YC, et al. Abstract 1405: Mesothelin enhances ovarian cancer invasion of by inducing MMP-7 through MAPK/ERK and JNK pathways[J]. Can Res. 2011;71(8 Supplement):1405–5.

34. Dahllmann M, Okhrimenko A, Marcinkowski P, et al. RAGE mediates S100A4-induced cell motility via MAPK/ERK and hypoxia signaling and is a prognostic biomarker for human colorectal cancer metastasis[J]. Oncotarget, 2014, 5(10).

35. Han HB, Gu J, Ji DB, et al. PBX3 promotes migration and invasion of colorectal cancer cells via activation of MAPK/ERK signaling pathway[J]. World J Gastroenterol. 2014;20(48):18260–70.

36. Specific alterations of the microRNA transcriptome and global network structure in colorectal cancer after treatment with MAPK/ERK inhibitors[J]. J Mol Med. 2012;90(12):1421–38.

37. Paroo Z, Ye X, Chen S, et al. Phosphorylation of the Human MicroRNA-Generating Complex Mediates MAPK/Erk Signaling[J]. Cell. 2009;139(1):0–122.

38. Reddy KB, Nabha SM, Atanaskova N. Role of MAP kinase in tumor progression and invasion[J]. Cancer Metastasis Rev. 2003;22(4):395–403.

39. Nele Van Der Steen, RJ, Honeywell H, Dekker J, Van Meerloo J, Kole R, Musters R, Ruijtenbeek C, Rolfo P, Pauwels, Godefridus J, Peters. Elisa Giovannetti. Resistance to crizotinib in a cMET gene amplified tumor cell line is associated with impaired sequestration of crizotinib in lysosomes. Journal of Molecular Clinical Medicine. 2018;1(2):99–106.

40. Liu H, (Liu H, Zhao J (Zhao J, Lv J (Lv, J.)). Inhibitory effects of miR-101 overexpression on cervical cancer SiHa cells. European Journal of Gynaecological Oncology 38(2): 236–240, 2017.

41. Hu H (Hu, He); Zhang GW (Zhang, Guowei); Tian, G (Tian, Geng); Lv, G (Lv, Guang); Jin YL (Jin, Yongli). miRNA profiling reveals the upregulation of osteogenesis-associated miRNAs in ovariectomy osteoporosis mice. Clinical and experimental obstetrics & gynecology. 2018;45(6): 817–822.

Figures
Figure 1

Expression of TTR in tissues and cell lines of NSCLC. (A) Expression of TTR was decreased in tissues of NSCLC tissues compared to normal tissues as determined by quantitative real-time reverse transcriptase PCR. **P < 0.01 vs. Normal tissues. (B) Expression of TTR was decreased in A549 and H1975 cell lines compared to 16HBE cell line as determined by quantitative real-time reverse transcriptase PCR. **P < 0.01 vs. 16HBE.
Figure 2

Effect of TTR on cell proliferation of NSCLC in vitro. (A) RT-qPCR showed expression level of TTR in A549 and H1975 cell lines with different treatment. (B) EdU positive nuclei indicated proliferating cells in A549 and H1975 cell lines in different group. (C) Colony formation assay showed a higher proliferative capacity in A549 and H299 cell lines in TTR inhibitor group. ***P < 0.001 vs. NC.
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Figure 3

Effect of TTR on cell apoptosis of NSCLC in vitro. (A) and (B) Cell apoptosis ability of A549 and H1975 cells were detected by flow cytometry and Tunel analysis. (C) Western blot assay was used to explore the expression level of apoptosis-related proteins in A549 and H1975 cells. **P < 0.01 vs. NC. ***P < 0.001 vs. NC.
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Figure 4

TTR suppressed NSCLC cell migration and invasion in vitro. Transwell migration(A) and invasion(B), wound healing(C) migration assays were applied to detected cell migration and invasion ability of A549 and H1975 cells. (D) The protein levels of MMP-2 and MMP-9 were explored using western blot assay. **P < 0.01 vs. NC. ***P < 0.001 vs. NC.
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Figure 5

TR suppressed tumor progression via MAPK/ERK pathway in vitro. (A) TTR overexpression significantly suppressed MAPK/ERK in A549 and H1975 cells. (B) MAPK/ERK signaling pathway related proteins were explored by western bolt.
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TR suppressed tumor progression via MAPK/ERK pathway in vitro. (A) TTR overexpression significantly suppressed MAPK/ERK in A549 and H1975 cells. (B) MAPK/ERK signaling pathway related proteins were explored by western bolt.
Figure 6

Up-regulated TTR suppressed NSCLC progression in vivo. (A) The expression of TTR was detected by RT-PCR. (B) Tumor size and tumor weight of different group. (C) TUNEL staining in the xenograft tissues of nude mice was used to explore the apoptosis ability in different group. **P < 0.01 vs. NC. ***P < 0.001 vs. NC.
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Up-regulated TTR suppressed NSCLC progression in vivo. (A) The expression of TTR was detected by RT-PCR (B) Tumor size and tumor weight of different group. (C) TUNEL staining in the xenograft tissues of nude mice was used to explore the apoptosis ability in different group. **P < 0.01 vs. NC. ***P < 0.001 vs. NC.