Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

RAMP2-AS1 stabilized RAPM2 mRNA through TIA1 to inhibit the progression of non-small cell lung cancer

Siqin Wang[1#], Ce Xu[2#], Yuanyuan Shan[3*], Yi Zhang[2*]

[1] Emergency Department, The Third People's Hospital of Chengdu, The Affiliated Hospital of Southwest Jiaotong University, The Second Affiliated Hospital Chengdu Clinical College of Chongqing Medical University Chengdu, Sichuan, 610031, PR China
[2] Department of Oncology, Jimin Hospital, Shanghai, 200052, China
[3] Hangzhou Mushi Biotechnology Co., LTD. Hangzhou, Hangzhou, 310000, China

[#]These authors contributed equally to this work as co-first author

ARTICLE INFO

Original paper

Article history:
Received: February 15, 2023
Accepted: November 10, 2023
Published: December 20, 2023

Keywords:

Non-small cell lung cancer, RAMP2, RAMP2-AS1, TIA1

ABSTRACT

As the most common subtype of lung cancer, non-small cell lung cancer (NSCLC)is responsible for a large proportion of global cancer-caused deaths. The implication of long non-coding RNAs (lncRNAs) as tumor-suppressor or carcinogenic genes in NSCLC has been widely documented. Our study sought to investigate the performance of lncRNA RAMP2 antisense RNA1 (RAMP2-AS1) in NSCLC. GEPIA bioinformatics tool and RT-qPCR were applied for assessing the expression of RAMP2-AS1 and its neighboring gene receptor activity-modifying protein 2 (RAMP2) in NSCLC. Functional assays including CCK-8 assay, colony formation assay as well as caspase-3 activity analysis and Transwell invasion assays were applied for detecting the biological phenotypes of NSCLC cells. Interaction among RAMP2-AS1, RAMP2 and T-cell intracellular antigen 1cytotoxic granule associated RNA binding protein (TIA1) was evaluated by RNA immunoprecipitation and pulldown assays. We found that RAMP2-AS1 and RAMP2 were downregulated in NSCLC. Overexpression of RAMP2-AS1 hampered proliferation and invasion, whereas induced apoptosis of NSCLC cells. Mechanistically, RAMP2-AS1 interacted with TIA1 to stabilize the mRNA of RAMP2. In conclusion, we first uncovered that RAMP2-AS1 stabilized RAPM2 mRNA through TIA1 to inhibit the progression of NSCLC, providing new insight to improve the treatment efficacy of NSCLC.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.14.2

Introduction

Non-small cell lung cancer (NSCLC) comprises approximately 80% of lung cancer cases (1). NSCLC has long been the predominant reason for global cancer-related mortality, and its 5-year survival rate is pessimistic (2). Although progress has been achieved in the study of NSCLC during past decades, the molecular mechanism behind the initiation and development of NSCLC remains largely unknown (3).

Long non-coding RNAs (lncRNAs) are acknowledged as a cluster of non-coding RNAs (ncRNAs) with a length of over 200 bp (4). LncRNAs are multifunctional in cancer progression and can regulate proliferation, migration, invasion, and apoptosis (5-8). Also, several lncRNAs are identified to possess tumor-promoting or tumor-suppressing properties in NSCLC, such as SNHG1 (9), HOXD-AS1 (10), and PICART1 (11). RAMP2 antisense RNA 1 (RAMP2-AS1) is a novel lncRNA proven to present downregulation and inhibit cell proliferation in glioblastoma (12). However, no study has related RAMP2-AS1 to NSCLC.

Receptor activity-modifying protein 2 (RAMP2) belongs to the family of single-transmembrane-domain proteins named RAMP, which is responsible for the transport of calcitonin receptor-like receptors to the plasma membrane (13). RAMP family can determine the specificity of the calcitonin-receptor-like receptor (CLR), a G protein-coupled seven transmembrane domain receptor that is required for the function of Adrenomedullin (AM), a member of the calcitonin superfamily (13,14). Although several studies have stated that AM exerted oncogenic functions in certain types of diseases, such as colorectal cancer as well as pancreatic cancer (15,16). Some studies argued that AM-RAMP2 signaling possesses tumor-suppressive roles. For example, Abasolo et al. (17) uncovered that AMcurbedthe growth of prostate cancer cells both *in vitro* and *in vivo*. The endothelial adrenomedullin-RAMP2 system modulated vascular integrity and suppressed tumor metastasis (18). Importantly, a study pointed out that RAMP2 was low expressed in lung cancer and its overexpression inhibited the proliferation of lung cancer cells (19). Nonetheless, the specific mechanism of RAMP2 in NSCLC and its relation with RAMP2-AS1 remains elusive.

Hence, our study planned to figure out the role of RAMP2-AS1 in NSCLC.

* Corresponding author. Email: luren364118740@163.com

Materials and Methods

Cell lines and plasmid transfection

Human bronchial epithelial cell line (16HBE) and four accepted NSCLC cell lines (H23, H1299, SPC-A1, A549) were utilized in this study and kept in RPMI-1640 culture medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco). All cells were preserved in 5% CO_2 and 95% air at 37°C, and bought from American Type Culture Collection (ATCC, Rockville, MD, USA). Human RAMP2-AS1cDNA was amplified and cloned into the pcDNA3.1 vector, which was procured from Invitrogen (Carlsbad, CA, USA). The empty pcDNA3.1vector was taken as a negative control. For silencing RAMP2-AS1, TIA1 or RAMP2, the duplex-specific shRNAs (GenePharma, Shanghai, China) were used. Plasmid transfection into SPC-A1 or A549 cells was implemented using Lipofectamine 2000 reagent, which was procured from Invitrogen.

Quantitative real-time polymerase chain reaction (RT-qPCR)

Using RNAiso Plus (TaKaRaBioTechnology, Tokyo, Japan), total cellular RNAs were isolated from SPC-A1 and A549 cells and transcribed in strict accordance with the specification of the Prime ScriptTM RT Master Mix (TaKaRaBio Technology). SYBR Premix Ex Taq II procured from TaKaRaBio Technology was applied for real-time PCR with Applied Biosystems 7900 Real-Time PCR System, which was procured from Applied Biosystems (Foster City, CA, USA). Relative expressions of genes were determined via the threshold cycle. The equation $2^{-\Delta\Delta Ct}$ was used for the measurement of fold change.

Cell proliferation assays

SPC-A1 and A549 cells were cultivated with 10 μl of CCK8 reagent which was procured from Dojindo (Kumamoto, Japan) at different time points at 37°C in 96-well plates (3000 cells/well). After 2 hrs of incubation, cell viability was monitored by the microplate reader procured from Bio-Rad Laboratories (Hercules, CA, USA)via the absorbance at 450 nm. Furthermore, transfected cells were collected for colony formation assay in 6-well plates with 500 cells/well. 14 days later, visible colonies with more than 50 cells were subjected to fixation and the treatment of 0.1% crystal violet (Beyotime, Shanghai, China).

Cell invasion assay

The invasive ability of transfected NSCLC cells was measured using the Transwell chambers with Matrigel and the Corning Kit (Corning, Cambridge, MA, USA) in light of the instruction. 2×10^4 cells were placed into the upper well, and the complete culture medium with 10% FBS was added into the lower chamber. 48 hrs later, invasive cells were subjected to fixation in 4%paraformaldehyde and washed thrice, following the treatment of 0.1% crystal violet. The final image of cell invasion was observed and captured under a microscope (CX41, Olympus, Tokyo, Japan).

Cell apoptosis assay

Caspase-3 activity was determined in transfected cells to assess apoptotic changes by use of a Caspase-3 activity kit (Beyotime). Firstly, total cellular proteins were obtained and treated with caspase-3 substrate and buffer for 4 hrs. At length, caspase-3 activity was evaluated with the microplate reader at a wavelength of 405 nm.

Western blotting

Radioimmunoprecipitation assay buffer (Beyotime) was first applied for the extraction of total cellular protein. Protein samples were separated on 10% SDS-PAGE, followed by being transferred onto PVDF membranes, which were procured from Millipore (Billerica, MA, USA). Afterward, primary antibodies and HRP-conjugated secondary antibodies were subjected to incubation with the blocked membranes in 5% BSA. Anti-GAPDH (ab9484) and anti-RAMP2(ab96546) were procured from Abcam (Cambridge, MA, USA). Immune complexes were detected by chemiluminescence (Pierce Biotechnology, Waltham, MA, USA).

Luciferase reporter assay

Human 293T cells from ATCC were prepared and plated into 24-well culture plates. RAMP2 promoter was subcloned into pGL3 Basic vector, which was procured from Promega (Madison, WI, USA), and subjected to the co-transfection with pcDNA3.1/RAMP2-AS1 or control. At last, the Dual-LuciferaseReporter Assay System (Promega) was utilized as per the guidebook.

Actinomycin D assay

Actinomycin D (ActD, 2 mg/ml) procured from Sigma-Aldrich (St. Louis, MO, USA) was added into the culture medium for blocking mRNA transcription. After treatment, RT-qPCR analysis was conducted.

RNA-binding protein immunoprecipitation (RIP) assay

In strict accordance with the protocol of the EZ-Magna RIP kit procured from Millipore, RIP assay was performed in NSCLE cells. Cellular lysates were cultured in RIP buffer with magnetic beads, which were conjugated to TIA1 antibody, Ago2 antibody, IgG antibody (negative control) or SNRNP70 antibody (positive control). The precipitated RNAs were extracted for RT-qPCR analysis.

RNA pull-down assay

Purified total cellular RNAs from A549 or SPC-A1 cells were subjected to biotin labeling using Pierce RNA 3'EndDesthiobiotinylation Kit, which was procured from Thermo Fisher Scientific (Waltham, MA, USA). The cell lysates incubated with biotinylated RNAs were named as RAMP2 biotin, with RAMP2 non-biotin as the control group. Magnetic beads were added to each binding reaction, followed by western blotting. After treatment with Proteinase K or DMSO, the level of recovered RNA was determined via RT-qPCR.

Statistical analyses

In this study, triplicates were required for each assay. All statistical difference was assessed with the application of SPSS statistics 19.0 (SPSS, Chicago, IL, USA) as well as the GraphPad Prism 7.0 Software, which was procured from GraphPad Software (La Jolla, CA, USA). Data analyses were calculated statistically by one-way ANOVA with Tukey's multiple comparison test or sample-independent t-test. Data were exhibited as the mean ± standard

deviation and regarded as significant when p-value<0.05.

Results

RAMP2-AS1 served as a tumor suppressor in NSCLC

First of all, RAMP2-AS1 expression in NSCLC was analyzed through the bioinformatics tool GEPIA in order to evaluate the relation of RAMP2-AS1 with NSCLC. As presented in Figure 1A, RAMP2-AS1 was markedly low-expressed in lung adenocarcinoma (LUAD) specimens versus normal specimens. Concertedly, the reduced RAMP2-AS1 expression in NSCLC cell lines was confirmed throughRT-qPCR data (Figure 1B). Then, gain-of-function experiments were designed to find out the underlying biological performance of RAMP2-AS1 in NSCLC. A549 and SPC-A1 cells were applied for the overexpression of RAMP2-AS1 because they presented the lowest RAMP2-AS1 level among 4 NSCLC cell lines. The overt induction of RAMP2-AS1 level upon pcDNA3.1/RAMP2-AS1 transfection in both cell lines was confirmed by RT-qPCR (Figure 1C). Ectopic expression of RAMP2-AS1 reduced the proliferation of two NSCLC cell lines (Figures 1D and 1E). The caspase-3 activity of NSCLC cells transfected with pcDNA3.1/RAMP2-AS1 was increased (Figure 1F), indicating that RAMP2-AS1 prompted NSCLC cell apoptosis. Additionally, the invasive ability of NSCLC cells was attenuated by RAMP2-AS1 upregulation (Figure 1G). As abovementioned, RAMP2-AS1 was markedly downregulated in NSCLC and served as a tumor-suppressor by restraining cell proliferative and invasive phenotypes, whereas facilitating cell apoptosis in NSCLC.

RAMP2-AS1 post-transcriptionally upregulated RAMP2 by stabilizing its mRNA

Next, the downstream mechanism of RAMP2-AS1 was exploited. LncRNAs are postulated to possess the potential

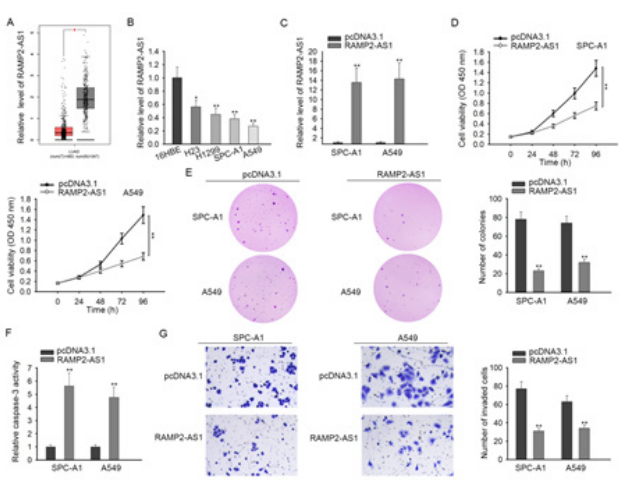

Figure 1. Role of RAMP2-AS1 in NSCLC. (A) Expression of RAMP2-AS1 in LUAD samples was assessed on GEPIA database. (B) RAMP2-AS1 level in NSCLC cell lines as well as normal cell line16HBE was assessed throughRT-qPCR. (C) RAMP2-AS1 overexpression efficiency in NSCLC cells was demonstrated by RT-qPCR. (D-E) Proliferation assays including CCK-8 assay as well as colony formation assay were applied to evaluate the proliferative phenotype of NSCLC cells. (F) Quantification of caspase-3 activity of NSCLC cells upon RAMP2-AS1 overexpression in NSCLC cells. (G) Representative pictures and quantification of invaded NSCLC cells with the overexpression of RAMP2-AS1. *P<0.05, **P<0.01.

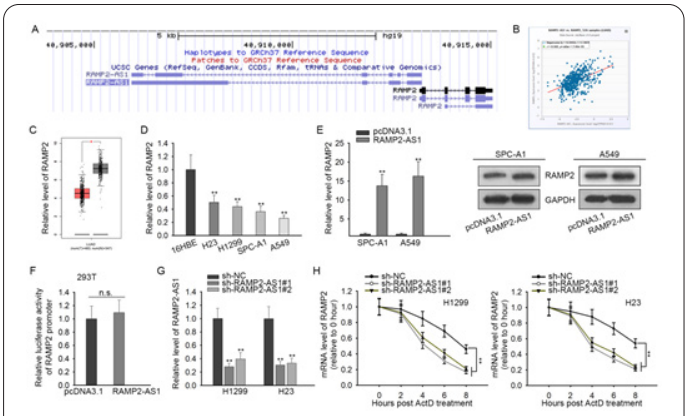

Figure 2. RAMP2-AS1 post-transcriptionally upregulated RAMP2 through stabilizing its mRNA. (A) Genomic location of RAMP2-AS1 and RAMP2 in UCSC genome browser. (B) Positive correlation between RAMP2-AS1 and RAMP2 level in LUAD samples from starBase pan-cancer. (C) Expression of RAMP2 in LUAD samples and normal samples analyzed by GEPIA. (D) RT-qPCR analysis of RAMP2 level in NSCLC cells as well as normal cells. (E) RAMP2 expression was detected by RT-qPCR as well as western blot analyses with the overexpression of RAMP2-AS1. (F) The impact of RAMP2-AS1 on RAMP2 transcription was evaluated by luciferase reporter assay. (G) The silence of RAMP2-AS1 in H1299 and H23 cells was evaluated by RT-qPCR. (H) ActD was applied for blocking mRNA generation. RT-qPCR results of remaining RAMP2 mRNA level after ActD addition. *P<0.05, **P<0.01.

to correlate with and regulate their nearby genes (20,21). Through UCSC (http://genome.ucsc.edu/), we found that the genomic position of RAMP2-AS1 was neighboring to RAMP2 (Figure 2A). RAMP2 has been documented to show downregulation in lung cancer tissues and play a tumor-suppressor role in lung cancer progression and the development of prostate cancer (17,19). The positive correlation of RAMP2-AS1 and RAMP2 levels in LUAD samples was revealed via the starBase pan-cancer tool (Figure 2B). Moreover, by analyzing the expression of RAMP2 through GEPIA, we uncovered the reduced level of RAMP2 in LUAD samples (Figure 2C). Accordingly, we validated the significantly low expression of RAMP2 in NSCLC cells (Figure 2D). Regulation of RAMP2-AS1 on RAMP2 expression was later evaluated. The levels of RAMP2 were increased in the presence of RAMP2-AS1 overexpression in NSCLC cells (Figure 2E). Furtherly, we detected at which level RAMP2-AS1 regulated RAMP2. It turned out that RAMP2-AS1 caused no pronounced change in the activity of the RAMP2 promoter (Figure 2F), suggesting that RAMP2-AS1 might regulate RAMP2post-transcriptionally. As largely reported, lncRNAs could regulate the mRNA stability of target genes at the post-transcriptional level (22,23). Hence, we detected the impact of RAMP2-AS1 on RAMP2 mRNA stability. RAMP2-AS1 was silenced in H1299 and H23 cells expressing relatively high RAMP2-AS1 and RAMP2 levels (Figure 2G). Consequently, the half-life of RAMP2 mRNA was curbed after the depletion of RAMP2-AS1 (Figure 2H). Collectively, RAMP2-AS1 post-transcriptionally upregulated RAMP2 by stabilizing its mRNA.

RAMP2-AS1 interacted with TIA1 to stabilize RAMP2 mRNA

Subsequently, we investigated how RAMP2-AS1 regu-

lated RAMP2 mRNA stability. To our knowledge, lncRNAs could regulate the stability of target mRNAs through the interplay with RNA binding proteins (RBPs) (22,23). Hence, we searched the starBase 3.0 database for the common RBPs of RAMP2-AS1 and RAMP2. As a result, we found 6 RBPs potentially interacting with both RAMP2-AS1 and RAMP2, which were CSTF2T, FBL, IGF2BP1, LIN28, PCBP2, and TIA1 (Figure 3A). To narrow the selection, we examined the levels of the 6 genes by RT-qPCR in cell lines. As presented in Figure 3B, only TIA1 was significantly downregulated in all NSCLC cell lines, indicating the involvement of TIA1 in NSCLC. TIA1 has been revealed to be implicated in the development of a variety of malignancies (24,25), and can regulate mRNA stability of tumor-suppressor genes such as PDCD4 (26). Hence, we conjecturedthat RAMP2-AS1 regulated RAMP2 mRNA stabilization via TIA1. RIP analysis depicted the abundance of RAMP2-AS1 and RAMP2 mRNA in the immunoprecipitated products of TIA1 antibody (Figure 3C). Pulldown assay showed that TIA1 protein was enriched by the pulldown of RAMP2 biotin probe group rather than RAMP2 non-biotin probe (Figure 3D). Additionally, we demonstratedthat RAMP2-AS1 could bepulled down by RAMP2 biotin probe instead of RAMP2 non-biotin probe, and the enrichment of RAMP2-AS1 in RAMP2 biotin pulldown was diminished by the addition of proteinase K (Figure 3E), indicating that TIA1 was required for the interplay between RAMP2-AS1 and RAMP2. Moreover, the knockdown efficiency of TIA1 in H1299 as well as H23 cells was verified (Figure 3F). After the addition of ActD to block mRNA transcription, we found through RT-qPCR that the decrease of remaining RAMP2 mRNA was sharpened by TIA silence (Figure 3G). Besides, the knockdown of TIA1 reversed the upregulation of RAMP2

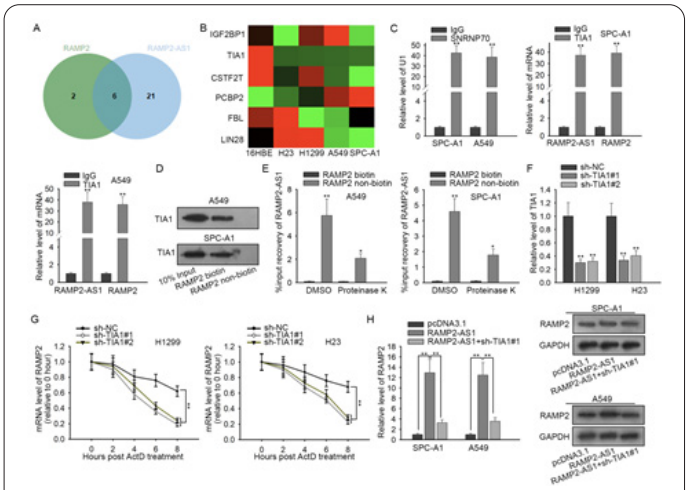

Figure 3. RAMP2-AS1 stabilized RAMP2 mRNA through TIA1. RAMP2-AS1 inhibited NSCLC progression through RAMP2. (A) Venn pattern of the shared RBPs for RAMP2-AS1 and RAMP2 from starBase 3.0. (B) Heat map of the level of 6 RBPs in NSCLC cells and normal cells. (C) The interaction of TIA1 with RAMP2-AS1 and RAMP2 mRNA was assessed by RIP assay. (D-E) The interaction of RAMP2 with TIA1 protein and RAMP2-AS1 was evaluated by pull-down assays. Proteinase K was added to digest TIA1 protein. (F) The efficiency of TIA1 silencing in H1299 and H23 cells was verified. (G) RT-qPCR results of remaining RAMP2 mRNA level after ActD addition at indicated time points. (H) RT-qPCR as well as western blot analyses of RAMP2 expression in A549 and SPC-A1 cells. $^{*}P<0.05$, $^{**}P<0.01$.

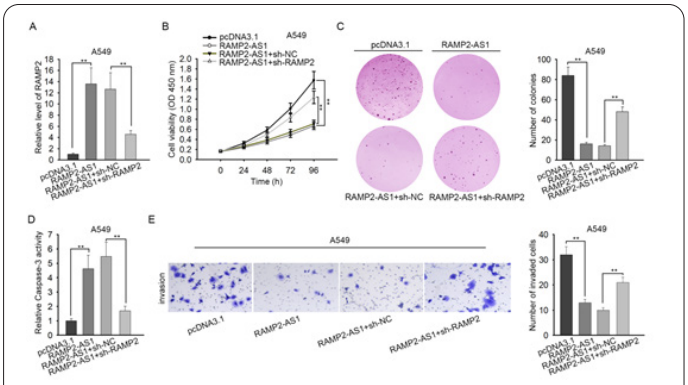

Figure 4. RAMP2-AS1 repressed NSCLC progression through RAMP2. (A) RT-qPCR data of RAMP2 expression in A549 cells with indicated transfections. (B-C) Proliferation assays revealed the proliferative phenotype of A549 cells with indicated transfections. (D) Caspase-3 level reflected the apoptosis of A549 cells under different transfections. (E) The invasive phenotype of A549 cells was depicted by Transwell-invasion assay under different transfections. $^{**}P<0.01$.

mRNA and protein caused by RAMP2-AS1 overexpression in NSCLC cells (Figure 3H). Altogether, RAMP2-AS1 interacted with TIA1 to stabilize RAMP2 mRNA.

RAMP2-AS1 reduced proliferation, increased apoptosis, and impeded invasion of NSCLC through RAMP2

Thereafter, to investigate whether RAMP2-AS1 repressed NSCLC progression through RAMP2, rescue assays were conducted in A549 cells. The expression of RAMP2 was elevated after RAMP2-AS1 overexpression, and the induction was abrogated by the transfection of sh-RAMP2 (Figure 4A). The decreased proliferation of A549 cells upon RAMP2-AS1 upregulation could be recovered by the silence of RAMP2 (Figures 4B and 4C). The apoptosis level of A549 cells was improved by forced expression of RAMP2-AS1, and silencing RAMP2 counteracted the effect (Figure 4D). The invasive capacity of A549 cells was hindered by RAMP2-AS1 overexpression and was restored by the co-transfection of pcDNA3.1/RAMP2 (Figure 4E). In sum, it was suggested that RAMP2-AS1 reduced proliferation, increased apoptosis, and impeded the invasion of NSCLC through RAMP2.

Discussion

During past decades, a great number of lncRNAs have been characterized to be functional in NSCLC initiation and development (27,28). A previous study stated that RAMP2-AS1 was low-expressed in glioblastoma and served as a tumor suppressor to suppress cell proliferative ability (12). The present study firstly showed that RAMP2-AS1 was downregulated in LUAD samples through bioinformatics analysis, and confirmed that RAPM2-AS1 level was significantly diminished in NSCLC cells, indicating the implication of RAMP2-AS1 in the progression of NSCLC. Functional experiments indicated that RAMP2-AS1 exhibited anti-tumor properties in NSCLC by hampering proliferation and invasion and facilitating apoptosis.

The role of RAMP2 in cancer progression is controversial as reported by previous studies. RAMP2 can determine the specificity of G protein-coupled seven transmembrane domain receptor CLR and CLR enables the function of AM (13,14). Some works suggested that AM-RAMP2

signaling could promote the progression of certain types of cancers by regulating proliferation, metastasis and angiogenesis (15,16), while others pointed out that RAMP2 can suppress tumor progression. For example, AM was proven to repress the growth of prostate cancer cells *in vivo* and *in vitro* (17). The endothelial adrenomedullin-RAMP2 system modulated vascular integrity and suppressed tumor metastasis (18). Notably, a study demonstrated that RAMP2 presented low expression in lung cancer specimens and overexpressing RAMP2 inhibited cell proliferation in lung cancer (19). Herein, we first found that RAMP2 was a neighbor to RAMP2-AS1, indicating the potential correlation between RAMP2-AS1 and RAMP2. We revealed the positive correlation of RAMP2-AS1 and RAMP2 in LUAD samples. Through TCGA data, we revealed the low expression of RAMP2 in LUAD samples. Mechanistically, we revealed that RAMP2-AS1 upregulated RAMP2 at the post-transcriptional level by stabilizing its mRNA. Former works have illustrated the regulation of lncRNA-RBP interaction on mRNA stability (22,23). Herein, we first identified that TIA1 interacted with both RAMP2-AS1 and RAMP2. TIA1 is reputed as an RBP participating in multiple biologic processes related to RNA metabolism in both cytoplasm and nucleus (29). Notably, TIA1 is suggested as a novel tumor suppressor gene by regulating genes responsible for tumor-related malignant biological activities, including apoptosis, proliferation, angiogenesis, metastasis as well as immune evasion (30-34). Also, the mRNA-stabilizing function of TIA1 has been revealed by previous studies (26,35). Accordingly, we first revealed that TIA1 was downregulated in NSCLC cells and that RAMP2-AS1 stabilized RAMP2 mRNA through recruiting TIA1. Finally, the rescue assays indicated that RAMP2-AS1 inhibited NSCLC progression through RAMP2-AS1.

In conclusion, we first suggested lncRNA RAMP2-AS1 as a tumor-suppressor in NSCLC by interacting with TIA1 to stabilize RAMP2, offering new insights for the therapy development of NSCLC.

Acknowledgements
Thanks to all the participants involved in this study.

Conflict of interest
The authors state that there are no conflicts of interest.

References

1. Pancewicz-Wojtkiewicz J, Bernatowicz PL. The effect of afatinib treatment in non-small cell lung cancer cells. Anticancer Res 2017 Jul; 37(7): 3543-3546. https://doi.org/10.21873/anticanres.11723
2. Junttila MR, Karnezis AN, Garcia D, Madriles F, Kortlever RM, Rostker F, Brown Swigart L, Pham DM, Seo Y, Evan GI, Martins CP. Selective activation of p53-mediated tumour suppression in high-grade tumours. Nature 2010 Nov 25; 468(7323): 567-571. https://doi.org/10.1038/nature09526
3. Mornex F, Girard N. Gemcitabine and radiation therapy in non-small cell lung cancer: state of the art. Ann Oncol 2006 Dec; 17(12): 1743-1747. https://doi.org/10.1093/annonc/mdl117
4. Clark MB, Mattick JS. Long noncoding RNAs in cell biology. Semin Cell Dev Biol 2011 Jun; 22(4): 366-376. https://doi.org/10.1016/j.semcdb.2011.01.001
5. Guo D, Wang Y, Ren K, Han X. Knockdown of LncRNA PVT1 inhibits tumorigenesis in non-small-cell lung cancer by regulating miR-497 expression. Exp. Cell Res 2018 Jan 1; 362(1): 172-179. https://doi.org/10.1016/j.yexcr.2017.11.014
6. Huang Y, Zhang J, Hou L, Wang G, Liu H, Zhang R, Chen X, Zhu J. LncRNA AK023391 promotes tumorigenesis and invasion of gastric cancer through activation of the PI3K/Akt signaling pathway. J Exp Clin Cancer Res 2017 Dec 28; 36(1): 194. https://doi.org/10.1186/s13046-017-0666-2
7. Jin X, Chen X, Hu Y, Ying F, Zou R, Lin F, Shi Z, Zhu X, Yan X, Li S, Zhu H. LncRNA-TCONS_00026907 is involved in the progression and prognosis of cervical cancer through inhibiting miR-143-5p. Cancer Med 2017 Jun; 6(6): 1409-1423. https://doi.org/10.1002/cam4.1084
8. Huang Y, Shi J, Xu Y. Long non-coding RNA NNT-AS1 contributes to cell proliferation, metastasis and apoptosis in human ovarian cancer. Oncol Lett 2018 Jun; 15(6): 9264-9270. https://doi.org/10.3892/ol.2018.8492
9. Cui Y, Zhang F, Zhu C, Geng L, Tian T, Liu H. Upregulated lncRNA SNHG1 contributes to progression of non-small cell lung cancer through inhibition of miR-101-3p and activation of Wnt/β-catenin signaling pathway. Oncotarget 2017 Mar 14; 8(11): 17785-17794. https://doi.org/10.18632/oncotarget.14854
10. Wang Q, Jiang S, Song A, Hou S, Wu Q, Qi L, Gao X. HOXD-AS1 functions as an oncogenic ceRNA to promote NSCLC cell progression by sequestering miR-147a. OncoTargets and therapy 2017 Sep 28; 10: 4753-4763. https://doi.org/10.2147/OTT.S143787
11. Zhang C, Su C, Song Q, Dong F, Yu S, Huo J. LncRNA PICART1 suppressed non-small cell lung cancer cells proliferation and invasion by targeting AKT1 signaling pathway. Am J Transl Res 2018 Dec 15; 10(12): 4193-4201.
12. Liu S, Mitra R, Zhao MM, Fan W, Eischen CM, Yin F, Zhao Z. The Potential Roles of Long Noncoding RNAs (lncRNA) in Glioblastoma Development. Mol Cancer Ther 2016 Dec; 15(12): 2977-2986. https://doi.org/10.1158/1535-7163.MCT-16-0320
13. McLatchie LM, Fraser NJ, Main MJ, Wise A, Brown J, Thompson N, Solari R, Lee MG, Foord SM. RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. Nature 1998 May 28; 393(6683):333-339. https://doi.org/10.1038/30666
14. Parameswaran N, Spielman WS. RAMPs: the past, present and future. Trends Biochem Sci 2006 Nov; 31(11): 631-638. https://doi.org/10.1016/j.tibs.2006.09.006
15. Nouguerède E, Berenguer C, Garcia S, Bennani B, Delfino C, Nanni I, Dahan L, Gasmi M, Seitz JF, Martin PM, Ouafik LH. Expression of adrenomedullin in human colorectal tumors and its role in cell growth and invasion in vitro and in xenograft growth in vivo. Cancer medicine 2013 Apr; 2(2): 196-207. https://doi.org/10.1002/cam4.51
16. Keleg S, Kayed H, Jiang X, Penzel R, Giese T, Büchler MW, Friess H, Kleeff J. Adrenomedullin is induced by hypoxia and enhances pancreatic cancer cell invasion. International Journal of Cancer 2007 Jul 1; 121(1): 21-32. https://doi.org/10.1002/ijc.22596
17. Abasolo I, Yang L, Haleem R, Xiao W, Pio R, Cuttitta F, Montuenga LM, Kozlowski JM, Calvo A, Wang Z. Overexpression of adrenomedullin gene markedly inhibits proliferation of PC3 prostate cancer cells in vitro and in vivo. Molecular and Cellular Endocrinology 2003 Jan 31; 199(1-2): 179-187. https://doi.org/10.1016/s0303-7207(02)00229-0
18. Tanaka M, Koyama T, Sakurai T, Kamiyoshi A, Ichikawa-Shindo Y, Kawate H, Liu T, Xian X, Imai A, Zhai L, Hirabayashi K, Owa S, Yamauchi A, Igarashi K, Taniguchi SI, Shindo T. The endothelial adrenomedullin-RAMP2 system regulates vascular integrity and suppresses tumour metastasis. Cardiovasc Res 2016 Sep; 111(4): 398-409. https://doi.org/10.1093/cvr/cvw166
19. Yue W, Dacic S, Sun Q, Landreneau R, Guo M, Zhou W, Siegfried

JM, Yu J, Zhang L. Frequent Inactivation of RAMP2, EFEMP1 and Dutt1 in Lung Cancer by Promoter Hypermethylation. Clinical Cancer Research 2007 Aug 1; 13(15 Pt 1): 4336-4344. https://doi.org/10.1158/1078-0432.CCR-07-0015

20. Gibbons HR, Shaginurova G, Kim LC, Chapman N, Spurlock CF, Aune TM. Divergent lncRNA GATA3-AS1 regulates GATA3 transcription in t-helper 2 cells. Front Immunol 2018 Oct 29; 9: 2512. https://doi.org/10.3389/fimmu.2018.02512
21. Zhang L, Li S, Choi YL, Lee J, Gong Z, Liu X, Pei Y, Jiang A, Ye M, Mao M, Zhang X, Kim J, Chen R. Systematic identification of cancer-related long noncoding RNAs and aberrant alternative splicing of quintuple-negative lung adenocarcinoma through RNA-Seq. Lung Cancer 2017 Jul; 109: 21-27. https://doi.org/10.1016/j.lungcan.2017.04.009
22. Li Z, Wang Y, Hu R, Xu R, Xu W. LncRNA B4GALT1-AS1 recruits HuR to promote osteosarcoma cells stemness and migration via enhancing YAP transcriptional activity. Cell Prolif 2018 Dec; 51(6): e12504. https://doi.org/10.1111/cpr.12504
23. Zhao Y, Liu Y, Lin L, Huang Q, He W, Zhang S, Dong S, Wen Z, Rao J, Liao W, Shi M. The lncRNA MACC1-AS1 promotes gastric cancer cell metabolic plasticity via AMPK/Lin28 mediated mRNA stability of MACC1. Mol Cancer 2018 Mar 6; 17(1): 69. https://doi.org/10.1186/s12943-018-0820-2
24. Yang X, Wang M, Lin B, Yao D, Li J, Tang X, Li S, Liu Y, Xie R, Yu S. miR-487a promotes progression of gastric cancer by targeting TIA1. Biochimie 2018 Nov; 154: 119-126. https://doi.org/10.1016/j.biochi.2018.08.006
25. Liu Y, Liu R, Yang F, Cheng R, Chen X, Cui S, Gu Y, Sun W, You C, Liu Z, Sun F, Wang Y, Fu Z, Ye C, Zhang C, Li J, Chen X. miR-19a promotes colorectal cancer proliferation and migration by targeting TIA1. Mol Cancer 2017 Mar 4; 16(1): 53. https://doi.org/10.1186/s12943-017-0625-8
26. Wigington CP, Jung J, Rye EA, Belauret SL, Philpot AM, Feng Y, Santangelo PJ, Corbett A.H. Post-transcriptional regulation of programmed cell death 4 (PDCD4) mRNA by the RNA-binding proteins human antigen R (HuR) and T-cell intracellular antigen 1 (TIA1). J Biol Chem 2015 Feb 6; 290(6): 3468-3487. https://doi.org/10.1074/jbc.M114.631937
27. Dong S, Qu X, Li W, Zhong X, Li P, Yang S, Chen X, Shao M, Zhang L. The long non-coding RNA, GAS5, enhances gefitinib-induced cell death in innate EGFR tyrosine kinase inhibitor-resistant lung adenocarcinoma cells with wide-type EGFR via downregulation of the IGF-1R expression. J Hematol Oncol 2015 Apr 29; 8: 43. https://doi.org/10.1186/s13045-015-0140-6
28. Han L, Zhang EB, Yin DD, Kong R, Xu TP, Chen WM, Xia R, Shu YQ, De W. Low expression of long noncoding RNA PANDAR predicts a poor prognosis of non-small cell lung cancer and affects cell apoptosis by regulating Bcl-2. Cell Death Dis 2015 Feb 26; 6(2): e1665. https://doi.org/10.1038/cddis.2015.30
29. Sánchez-Jiménez C, Izquierdo JM. T-cell intracellular antigens in health and disease. Cell cycle (Georgetown, Tex.) 2015; 14(13): 2033-2043. https://doi.org/10.1080/15384101.2015.1053668
30. Tian Q, Streuli M, Saito H, Schlossman SF, Anderson P. A polyadenylate binding protein localized to the granules of cytolytic lymphocytes induces DNA fragmentation in target cells. Cell 1991 Nov 1; 67(3): 629-639. https://doi.org/10.1016/0092-8674(91)90536-8
31. Zlobec I, Karamitopoulou E, Terracciano L, Piscuoglio S, Iezzi G, Muraro MG, Spagnoli G, Baker K, Tzankov A, Lugli A. TIA-1 cytotoxic granule-associated RNA binding protein improves the prognostic performance of CD8 in mismatch repair-proficient colorectal cancer. PloS one 2010 Dec 10; 5(12): e14282. https://doi.org/10.1371/journal.pone.0014282
32. Hamdollah Zadeh MA, Amin EM, Hoareau-Aveilla C, Domingo E, Symonds KE, Ye X, Heesom KJ, Salmon A, D'Silva O, Betteridge KB, Williams AC, Kerr DJ, Salmon AH, Oltean S, Midgley RS, Ladomery MR, Harper SJ, Varey AH, Bates DO. Alternative splicing of TIA-1 in human colon cancer regulates VEGF isoform expression, angiogenesis, tumour growth and bevacizumab resistance. Mol Oncol 2015 Jan; 9(1): 167-178. https://doi.org/10.1016/j.molonc.2014.07.017
33. Reyes R, Alcalde J, Izquierdo JM, Depletion of T-cell intracellular antigen proteins promotes cell proliferation. Genome Biology 2009; 10(8): R87. https://doi.org/10.1186/gb-2009-10-8-r87
34. Gold MH, Adelglass J. Evaluation of safety and efficacy of the TriFractional RF technology for treatment of facial wrinkles. Journal of Cosmetic and Laser Therapy 2014 Jan; 16(1): 2-7. https://doi.org/10.3109/14764172.2013.854624
35. Motohashi H, Mukudai Y, Ito C, Kato K, Shimane T, Kondo S, Shirota T. Tumor protein D52 expression is post-transcriptionally regulated by T-cell intercellular antigen (TIA) 1 and TIA-related protein via mRNA stability. Biochem J 2017 May 4; 474(10): 1669-1687. https://doi.org/10.1042/BCJ20160942