LncRNA HOTAIR regulates HIF-1α/AXL signaling through inhibition of miR-217 in renal cell carcinoma

Quan Hong, Ou Li, Wei Zheng, Wen-zhen Xiao, Lu Zhang, Di Wu, Guang yan Cai, John Cijiang He and Xiang mei Chen*

Long non-coding RNA HOTAIR was regarded as an oncogene in multiple cancers. Previous studies have shown that HOTAIR is involved in the proliferation and tumorigenesis of renal carcinoma cells, while microRNA (miR)-217 functions as a tumor suppressor in renal cell carcinoma (Rcc). However, the underlying molecular mechanism of HOTAIR in Rcc, especially in association with miR-217, has not been studied. In this study, we first demonstrated that HOTAIR expression was upregulated, which was correlated with tumor progression, and miR-217 downregulated in Rcc tissues and cells. Importantly, HOTAIR expression was negatively correlated with miR-217 expression in Rcc tissues. Gain- and loss-of-function of HOTAIR revealed that HOTAIR functioned as a ceRNA for miR-217 to facilitate HIF-1α expression and then upregulated AXL level promoting Rcc proliferation, migration, and EMT process, and inhibiting apoptosis. Furthermore, HOTAIR knockdown suppressed tumor growth and reduced the expression of proliferation antigen ki-67, HIF-1α, and AXL, but upregulated the expression of miR-217 in vivo. Finally, with AXL inhibitor BGB324, we confirmed that HOTAIR promoted Rcc activity through AXL signaling both in vitro and in vivo. In conclusion, these results suggest that HOTAIR promotes Rcc tumorigenesis via miR-217/HIF-1α/AXL signaling, which may provide a new target for the diagnosis and therapy of Rcc disease.

Cell Death and Disease (2017) 8, e2772; doi:10.1038/cddis.2017.181; published online 11 May 2017

Renal cell carcinoma (Rcc) represents more than 90% of cases of kidney cancer, which is currently the 9th most common cancer in men and the 14th in women all over the world. Rcc is mainly classified into three histological subtypes: clear cell (70–80%), papillary (10–15%), and chromophobe (5–10%) Rcc. The incidence of Rcc varies between countries and is still increased worldwide. Despite advances in imaging techniques, the diagnosis of early stage kidney cancer is very poor. In addition, the resistance to traditional therapies results in poor prognosis in Rcc patients. Therefore, it is urgent to find an effective method for early diagnosis and treatment of Rcc.

Long non-coding RNAs (lncRNAs), a heterogeneous class of transcripts longer than 200 nucleotides, are pervasively transcribed in the genome and associated with physiological and pathological processes. IncRNAs cannot code protein but can regulate gene expression at the transcriptional and post-transcriptional levels. Increasing evidence indicates that IncRNAs are involved in the modulation of cancer cell behavior, such as proliferation, metastasis, epithelial-mesenchymal transition (EMT), apoptosis, and drug resistance. HOX transcript antisense intergenic RNA (HOTAIR) is a lncRNA encoded by the human HOXC locus on chromosome 12q13. Increased expression of HOTAIR has been involved in the increased metastasis of breast, cervical, and colorectal cancer. Furthermore, HOTAIR is associated with the poor prognosis of pancreatic, breast, liver, and cervical cancer. Recent studies reported that HOTAIR expression is elevated in Rcc cells, and silencing HOTAIR expression inhibits the proliferation and tumorigenicity of Rcc cells. However, the underlying molecular mechanism of HOTAIR in Rcc has not been fully understood.

Recent reports have suggested that lncRNAs function as competing endogenous RNAs (ceRNAs) for microRNA (miRNA) to mediate mRNAs expression at post-transcriptional level. HOTAIR regulates cyclin J expression via inhibition of miR-205 expression in bladder cancer. HOTAIR modulates HER2 expression through sponging miR-331-3p to promote the tumorigenesis of gastric cancer. miR-217 is a tumor suppressor in many cancers including Rcc. Recently, studies showed that the interaction between HOTAIR and PIK3R3 is mediated through miR-217 in ovarian cancer cells, but the interaction between HOTAIR and miR-217 in Rcc has not been reported before.

In this study, we hypothesized that HOTAIR might promote Rcc progression through inhibition of miR-217 expression. In the present study, we first detected the expression of HOTAIR and miR-217 in tumor tissues from clear cell Rcc patients as well as in Rcc cells. Furthermore, the underlying mechanism of HOTAIR in the development of Rcc was analyzed both in vitro and in vivo. This study may provide a new target for Rcc treatment.

Results

HOTAIR expression is negatively correlated with miR-217 in human renal cancer tissues. To verify whether HOTAIR is differentially expressed in Rcc tissues, HOTAIR expression...
was determined in 86 paired Rcc samples and adjacent histological normal tissues. As shown in Figure 1a, compared with normal control, HOTAIR expression was notably upregulated in Rcc tissues. Interestingly, the elevated HOTAIR expression was positively correlated with the TNM stage (Table 1). Also, we detected miR-217 expression and found it was reduced in Rcc tissues (Figure 1b). Surprisingly, miR-217 expression was inversely correlated with HOTAIR levels in 86 Rcc tissue samples \( (r^2 = 0.4155, P < 0.0001) \) (Figure 1e). Moreover, HOTAIR level was higher and miR-217 was lower in Rcc cell lines, including 769-P and ACHN cells, than in normal renal cells (HK-2; Figures 1c and d).

**HOTAIR as a target of miR-217.** To clarify the underlying relationship between HOTAIR and miR-217, we performed bioinformatics analysis by miRcode (http://www.mircode.org). The data showed that HOTAIR contains one conserved target site of miR-217 (Figure 1f), which is consistent with previous study.\(^{16}\) Moreover, miR-217 reduced the luciferase activity of pGL3-HOTAIR but not pGL3-HOTAIR-MUTANT (Figure 1g). These results indicate that miR-217 could bind directly to HOTAIR at the miRNA recognition sites.

**HOTAIR modulates Rcc proliferation, migration, apoptosis, and EMT via negative regulation of miR-217.** To further investigate whether HOTAIR functions through miR-217, HOTAIR expression was upregulated/downregulated and intervened with miR-217 mimic or anti-217 in Rcc cells. HOTAIR overexpression increased the proliferation, migration, and the expression of Vimentin and Snail, but reduced E-cadherin expression in ACHN cells; however, this effect could be ablated by miR-217 mimic (Figures 2a, c, f, and h). In addition, HOTAIR downregulation significantly reduced the proliferation, migration, and the expression of Vimentin and Snail induced by TGF-\(\beta\)1, but facilitated...
E-cadherin expression and the apoptosis of 769-P cells, which could be counteracted by anti-217 (Figures 2b, d, e, g, and h). These data suggest that HOTAIR promotes RCC cell proliferation, migration, EMT, and inhibits apoptosis via downregulation of miR-217.

**HOTAIR functions as a ceRNA for miR-217 to facilitate HIF-1α expression.** Through searching TargetScan (http://www.targetscan.org), we found that miR-217 could bind to the 3'UTR of HIF-1α (Figure 3a). Moreover, miR-217 mimic significantly suppressed the mRNA and protein level of HIF-1α in ACHN cells, while downregulation of miR-217 increased HIF-1α expression in 769-P cells (Figures 3b and c). In addition, miR-217 remarkably dampened the luciferase activity of cells transfected with pGL3-HIF-1α but not pGL3-HIF-1α-MUTANT, suggesting a direct interaction between miR-217 and the 3'UTR of HIF-1α (Figure 3d).

HOTAIR and HIF-1α share the same response elements of miR-217 (Figures 1f and 3a). Hence, HOTAIR may act as a ceRNA for miR-217 to mediate HIF-1α expression in RCC development. To test this hypothesis, we first determined miR-217 expression after downregulation of HOTAIR and found si-HOTAIR significantly upregulated miR-217 levels in RCC cells (Figure 3e). Then, we performed an RIP assay on Ago2, which is the vital component of the RNA-induced silencing complex (RISC).17 As shown in Figure 3f, overexpression of HOTAIR led to the increased enrichment of Ago2 on HOTAIR but substantially decreased enrichment on HIF-1α transcripts. In parallel, knockdown of HOTAIR had the contrary effects. These results indicate that HOTAIR could compete with HIF-1α transcripts for the Ago2-based RISC.

Moreover, we evaluated whether the HOTAIR-mediated sequestration of miR-217 was responsible for the upregulation
of HIF-1α. The luciferase activity of HIF-1α wild-type reporters but not the mutant one was significantly increased after HOTAIR upregulation, whereas miR-217 mimic abolished this effect. And HOTAIR siRNA showed the reversed effect on the luciferase activity of HIF-1α, which was rescued by anti-217 (Figure 3g). In addition, these results were further confirmed at HIF-1α mRNA level (Figure 3h). Collectively, these results suggest that HOTAIR functions as a molecular sponge for miR-217 to facilitate HIF-1α expression.

HOTAIR promotes Rcc tumorigenesis through HIF-1α/AXL signaling both in vitro and in vivo. Studies have indicated that HIF-1 could bind directly to AXL and activate its expression;¹⁸ hence, to further investigate the mechanisms of HOTAIR, we detected AXL expression in Rcc cells. HOTAIR overexpression increased the mRNA and protein levels of AXL, whereas downregulation of HOTAIR notably decreased their expression (Figures 4a and b). To determine whether HOTAIR promotes Rcc activity through AXL signaling, an
AXL inhibitor BGB324 was used. The results showed that BGB324 suppressed the effect of HOTAIR upregulation on the proliferation and EMT process of ACHN cells in a dose-dependent manner (Figures 4c and d). Also, the mRNA levels of HIF-1α and AXL were detected in 86 Rcc samples and adjacent histological normal tissues. As shown in Figures 4e and f, the mRNA expression of HIF-1α and AXL in Rcc tissues was dramatically upregulated compared with normal control tissues. Furthermore, the role of HOTAIR in Rcc tumorigenesis was analyzed in vivo and the results showed that knockdown of HOTAIR significantly inhibited tumor growth and the staining intensity of proliferation antigen ki-67 (Figures 5a and b). HOTAIR downregulation reduced the expression of HIF-1α and AXL, but elevated miR-217 levels (Figures 5c–f). In addition, the role of AXL in HOTAIR-mediated Rcc tumorigenesis was further confirmed by oral administration of BGB324. BGB324 notably decreased HOTAIR-induced tumor growth and EMT process (Figures 5g and h). Collectively, these data show that HOTAIR promotes Rcc tumorigenesis via activating HIF-1/AXL signaling.

Discussion

Studies have revealed that lncRNAs play vital roles in Rcc pathogenesis. However, the role and mechanism of HOTAIR in Rcc has not been fully understood. In this study, we found that HOTAIR expression was significantly increased in Rcc tissue samples compared with their corresponding non-tumor
tissues, and the elevated expression was positively correlated with the TNM stage (Figure 1). Also, HOTAIR expression was higher in Rcc cells. Studies have shown that HOTAIR acts as a ceRNA to sponge miRNAs to modulate the de-repression of miRNA targets in bladder and gastric cancer. Hence, we speculated that HOTAIR acts as a ceRNA in Rcc. miR-217 plays a suppression role in the growth of various types of tumors including Rcc. We further investigated miR-217 expression and found it was remarkably downregulated in Rcc tissues and cells. Furthermore, miR-217 expression was negatively correlated with HOTAIR level in Rcc tissues (Figure 1).

The results of bioinformatics analysis showed HOTAIR possesses one conserved target site for miR-217 binding. And dual-luciferase reporter assay demonstrated that miR-217 could reduce the luciferase activity of pGL3-HOTAIR (Figure 1). In addition, HOTAIR overexpression markedly increased the proliferation, migration, and EMT process, which were ablated by miR-217 mimic. Contrarily, knockdown of HOTAIR remarkably reduced the proliferation, migration, EMT, and promoted the apoptosis of 769-P cells, which could be counteracted by anti-217 (Figure 2). Taken together, these results indicate that HOTAIR promotes Rcc development via negative regulation of miR-217.
HIF-1α is a transcription factor that is frequently stabilized and active in human clear cell Rcc. It is largely responsible for activating the transcription of target genes that are widely involved in the malignant features of tumors. High levels of HIF-1α are associated with the poor prognosis of clear cell Rcc. It has previously been reported that miR-217 increased HIF-1α levels by targeting SIRT1 in rat glomerular mesangial cells cultured with high glucose. However, in our study, the result of bioinformatics analysis revealed HIF-1α is a potential target of miR-217. And miR-217 decreased HIF-1α expression, which could be upregulated by anti-217. Also, miR-217 notably dampened the luciferase activity of pGL3-HIF-1α. These results indicated that HIF-1α was a direct target of miR-217. An RIP assay on Ago2 showed HOTAIR overexpression increased the enrichment on HOTAIR but significantly decreased the enrichment on HIF-1α transcripts and knockdown of HOTAIR had the reversed effect. These results indicated that HOTAIR could compete with HIF-1α transcripts for the Ago2-based RISC. Furthermore, the luciferase activity of HIF-1α reporters and HIF-1α mRNA expression were increased after HOTAIR overexpression, but reversed by miR-217 mimic; however, they were reduced when HOTAIR was downregulated, which could be rescued by anti-217 (Figure 3). Taken together, these results suggest that HOTAIR functions as a ceRNA for miR-217 to facilitate the expression of HIF-1α.

Rankin et al. demonstrated that HIF-1 bound directly to AXL to activate its expression. AXL, a member of the TAM family of receptor tyrosine kinases (RTKs), has been identified as an essential mediator of cancer metastasis including Rcc. Binding of its ligand, growth arrest-specific protein 6 (Gas6), AXL receptor is dimerized and autophosphorylated at its tyrosine residues, which subsequently triggers a cascade of intracellular signaling, involving PI3K/Akt, ERK, NF-κB, p38, Rho family proteins, JAK-STAT, and Src family kinases. AXL oncogenic signaling promotes cancer cell survival, proliferation, migration, invasion, and the EMT phenotype, as well as resisting tumor cell apoptosis. In addition, AXL mediates a resistance to sunitinib treatment in Rcc. However, in our study, the result of bioinformatics analysis revealed HIF-1α is a potential target of miR-217. And miR-217 decreased HIF-1α expression, which could be upregulated by anti-217. Also, miR-217 notably dampened the luciferase activity of pGL3-HIF-1α. These results indicated that HIF-1α was a direct target of miR-217. An RIP assay on Ago2 showed HOTAIR overexpression increased the enrichment on HOTAIR but significantly decreased the enrichment on HIF-1α transcripts and knockdown of HOTAIR had the reversed effect. These results indicated that HOTAIR could compete with HIF-1α transcripts for the Ago2-based RISC. Furthermore, the luciferase activity of HIF-1α reporters and HIF-1α mRNA expression were increased after HOTAIR overexpression, but reversed by miR-217 mimic; however, they were reduced when HOTAIR was downregulated, which could be rescued by anti-217 (Figure 3). Taken together, these results suggest that HOTAIR functions as a ceRNA for miR-217 to facilitate the expression of HIF-1α.

In conclusion, our study revealed that HOTAIR is an oncogene in Rcc. Elevated level of HOTAIR is positively correlated with tumor progression. HOTAIR functions as a ceRNA for miR-217 to facilitate Rcc progression partly via HIF-1α/AXL signaling (Figure 3i). Thus, our study provides further insight into the molecular mechanism of HOTAIR in Rcc tumorigenesis, which may promote the development of lncRNA-directed diagnosis and therapy for this disease.
FBS for inducing cell migration. After 48 h incubation, the cells attached to the lower surface were fixed with 4% paraformaldehyde, stained with crystal violet, and then examined under a microscope.

Flow cytometry. Apoptosis was assayed through dual staining with annexin V-FITC and propidium iodide (PI; BD Biosciences, Franklin Lakes, NJ, USA). In brief, cells were harvested 48 h after transfection; Annexin V-FITC and PI were added to the cellular suspension according to the manufacturer's instructions. Then, samples were analyzed by a FACSCalibur flow cytometer (BD, San Jose, CA, USA).

Luciferase assay. 0.3 μg of pGL3-HOTAIR (HOT AIR-WT), pGL3-HIF-1α–MUTANT (HOTAIR-MUT), or pGL3-HIF-1α–MUTANT (HIF-1α-MUT) was cotransfected with 40 nM miR-217 mimic or NC-mimic into HEK293T cells using Lipofectamine 2000. The luciferase activities were measured 48 h after transfection using the Dual-luciferase assay kit (Promega) according to the manufacturer's protocol.

RNA immunoprecipitation assay. RNA immunoprecipitation (RIP) was implemented by the EZ-Magna RIP RNA-binding protein immunoprecipitation kit (Millipore, Billerica, MA, USA). Cells were lysed into complete RIP lysis buffer. 100 μl of cell lysate was incubated with RIP buffer containing magnetic beads conjugated with human anti-Ago2 antibody (1:50 dilution, Millipore) and negative control normal mouse IgG. Samples were incubated with Proteinase K buffer and then target RNA was extracted for further study.

Establishment of tumorigenicity models. Five-week-old female BALB/c athymic nude mice were obtained from the National Laboratory Animal Center (Beijing, China). The animals were acclimated for 7 days before the experiment. All animal protocols were approved by the Animal Ethics Committee of the Chinese PLA General Hospital and Military Medical College. To establish Rcc xenograft models, 4 x 10⁵ 769P cells stably transfected with s-HOTAIR or s-NC were inoculated subcutaneously into the dorsal right flank of each mouse. To validate the mechanism of HOTAIR in vivo, 4 x 10⁵ ACHN cells with stable HOTAIR upregulation were injected into the mice. Tumor diameters (mm) were measured every 2 days starting the 12th day after inoculation. Tumour volume (mm³) was calculated using the formula V= (shortest diameter)² x (longest diameter)/2. *P < 0.05. Tumor tissues were excised for further study in day 30.

Real-time PCR (qRT-PCR). Total RNA was extracted with TRizol (Invitrogen, Carlsbad, CA, USA) and reversely transcribed into cDNA. Then, qRT-PCR was performed with Power SYBR Green (Takara, Kusatsu, Shiga, Japan). Data were normalized to GAPDH expression. The amplification and detection of miR-217 were normalized to GAPDH expression. The amplification and detection of miR-217 were performed using the Dual-luciferase assay kit (Promega) according to the manufacturer's protocol.

Immunohistochemistry. The Rcc tissues were fixed with 10% neutral formalin, embedded in paraffin, and sectioned into 4-μm thick slices. After dewaxing, rehydration, and antigen retrieval, the slides were then incubated with anti-Ki-67 antibody (Abcam, Cambridge, MA, USA), Vimentin (Abcam), Snail (Abcam), AXL (Abcam), and HIF-1α (Cell signaling, Danvers, MA, USA) at 4 °C overnight. The membranes were blocked with 5% fat-free milk powder in TBST buffer and then exposed to antibodies against E-cadherin (Abcam, Cambridge, MA, USA), and then labeled with a secondary antibody conjugated to horseradish peroxidase (HRP) for 1 h. The membranes were then washed with TBST buffer, and finally, the proteins were visualized using DAB/NiCl₂ solution. The protein level was determined by the intensity of its brown coloration. The immunohistochemical images were analyzed by a digital imaging system (Olympus, Tokyo, Japan).

Conflict of Interest
The authors declare no conflict of interest.

Acknowledgements. This work was supported by Chinese National Natural Sciences Foundation (No. 84709494), Major State Basic Research Development Program of China (No. 2013CB308000 and No. 2014CB900205).

1. Znaor A, Loriet-Tieulent J, Laversanne M, Jemal A, Bray F. International variations and trends in renal cell carcinoma incidence and mortality. Eur Urol 2015; 67: 519–530.
2. Silva-Santos RM, Costa-Pinheiro P, Luis A, Antunes L, Lobo F, Oliveira J et al. MicroRNA profile: a promising ancillary tool for accurate renal cell tumour diagnosis. Br J Cancer 2013; 109: 2646–2653.
3. Ljungberg B, Campbell SC, Choi HY, Jacqmin D, Lee JE, Weikert S et al. The epidemiology of renal cell carcinoma. Eur Urol 2011; 66: 615–621.
4. Glen H. Lenvatinib therapy for the treatment of patients with advanced renal cell carcinoma. Front Oncol 2016; 12: 2195–2204.
5. Qu L, Ding J, Chen C, Wu ZJ, Li B, Gao Y et al. Exosome-transmitted IncARSR promotes stemness resistance in renal cancer by acting as a competing endogenous RNA. Cancer Cell 2016; 29: 653–668.
6. Yang L, Lin C, Jin C, Yang JC, Tanasa B, Li W et al. IncRNA-dependent mechanisms of androgen-receptor-regulated gene activation programs. Nature 2013; 500: 598–602.
7. Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu K, Brugmann SA et al. Functional demarcation of active and silent chromatin domains in human Hox loci by noncoding RNAs. Cell 2007; 129: 1311–1323.
8. Kim HJ, Lee DW, Yim GW, Nam EJ, Kim S, Kim SW et al. Long non-coding RNA-HOTAIR is associated with human cervical cancer progression. Int J Oncol 2015; 46: 621–630.
9. Wu ZH, Wang XL, Tang HM, Jiang T, Chen J, Lu S et al. Long non-coding RNA-HOTAIR is a powerful predictor of metastasis and poor prognosis and is associated with epithelial-mesenchymal transition in colon cancer. Oncol Rep 2014; 32: 395–402.
10. Kim K, Juturub I, Chaladapia G, Johnson G, Frank J, Burghardt R et al. HOTAIR is a negative prognostic factor and exhibits pro-oncogenic activity in pancreatic cancer. Oncogene 2013; 32: 1616–1625.
11. Wu Y, Liu J, Zheng Y, You L, Ku D, Liu T. Suppressed expression of long non-coding RNA-HOTAIR inhibits proliferation and tumorigenicity of renal carcinoma cells. Tumor Biol 2014; 35: 11887–11894.
12. Sun C, Li S, Zhang F, Xi Y, Wang L, Bi Y et al. Long non-coding RNA NEAT1 promotes non-small cell lung cancer progression through regulation of miR-377-3p-EP30F3 pathway. Oncotarget 2016; 7: 51784–51818.
13. Sun X, Du P, Yuan W, Du Z, Yu M, Xu Y et al. Long non-coding RNA HOTAIR regulates cyclin J via inhibition of microRNA-205 expression in bladder cancer. Cell Death Dis 2015; 6: e1907.
14. Liu XH, Sun M, Nie FQ, Ge YB, Zhang EB, Yin DD et al. Lnc RNA-HOTAIR functions as a competing endogenous RNA to regulate HER2 expression by sponging miR-331-3p in gastric cancer. Mol Cancer 2014; 13: 92.
15. Li H, Zhao J, Zhang JW, Huang QY, Huang JZ, Chi LS et al. MicroRNA-217, down-regulated in clear cell renal cell carcinoma and associated with lower survival, suppresses cell proliferation and migration. Nephrology 2013; 60: 511–515.
16. Dong L, Hui L. HOTAIR promotes proliferation, migration, and invasion of ovarian cancer SKOV3 cells through regulating PIK3R3. Med Sci Monit 2016; 22: 325–332.
17. Kreil J, Stebbing J, Carissimi C, Dobrowolska AF, de Giorgio A, Frampton AE et al. TP53 regulates miRNA association with AGO2 to remove the miRNA-miRNA interaction network. Genome Res 2016; 26: 331–341.
18. Rankin EB, Fuh KC, Castellini L, Viswanathan K, Finger EC, Diep AN et al. Direct regulation of GAS6/AXL signaling by HIF promotes renal metastasis through SRC and MET. Proc Natl Acad Sci USA 2014; 111: 13373–13378.
19. Hirata H, Hinoda Y, Brahimy V, Deng G, Nakajima K, Tabatabai ZL et al. Long noncoding RNA MALAT1 promotes aggressive renal cell carcinoma through Ezh2 and interacts with miR-205. Cancer Res 2015; 75: 1322–1331.
20. Minton DR, Fu L, Chen Q, Robinson BD, Gross SS, Nanus DM et al. Analysis of the transcriptome and metabolome demonstrate that HIF1alpha mediates altered tumor metabolism in clear cell renal cell carcinoma. PLoS ONE 2015; 10: e0126049.
21. Chen G, Xu JY, Chen J, Zhang JX, Zhou J, Liang Y et al. Loss of PIG3 increases HIF-1alpha level by promoting protein synthesis via mTOR pathway in renal cell carcinoma cells. Oncotarget 2016; 7: 27176–27184.
22. Kiette T, Seligson DB, Riggs SB, Lepport JT, Berkman MK, Kleid MD et al. Hypoxia-inducible factor 1 alpha in clear cell renal cell carcinoma. Clin Cancer Res 2007; 13: 7388–7393.
23. Shao Y, Lu C, Wu C, Zhou Y, Wang Q, Mi-217 promotes inflammation and fibrosis in high glucose cultured rat glomerular mesangial cells via Sirt1/HIF-1alpha signaling pathway. Diabetes Metab Res Rev 2016; 32: 534–543.
24. Wang C, Jin H, Wang N, Fan S, Wang Y, Zhang Y et al. Gas6/Adx axis contributes to chemoresistance and metastasis in breast cancer through Akt/GSK-3beta/catenin signaling. Theranostics 2016; 6: 1205–1219.
25. Wu X, Liu X, Koui S, Lee CY, Zhang Z, Halmos B. AXL kinase as a novel target for cancer therapy. Oncotarget 2014; 5: 9546–9563.
26. Han J, Tian R, Yong B, Luo C, Tan P, Shen J et al. Gas6/Axl mediates tumor cell apoptosis, migration and invasion and predicts the clinical outcome of osteosarcoma patients. Biochem Biophys Res Commun 2013; 435: 493–500.

27. van der Mijn JC, Broxterman HJ, Knol JC, Piersma SR, De Haas RR, Dekker H et al. Sunitinib activates Axl signaling in renal cell cancer. Int J Cancer 2016; 138: 3002–3010.

28. Yu H, Liu R, Ma B, Li X, Yen HY, Zhou Y et al. Axl receptor tyrosine kinase is a potential therapeutic target in renal cell carcinoma. Br J Cancer 2015; 113: 616–625.

29. Kato M, Putta S, Wang M, Yuan H, Lanting L, Nair I et al. TGF-beta activates Akt kinase through a microRNA-dependent amplifying circuit targeting PTEN. Nat Cell Biology 2009; 11: 881–889.

30. Liu Q, Jin J, Ying J, Cui Y, Sun M, Zhang L et al. Epigenetic inactivation of the candidate tumor suppressor gene ASCMTS1 in human renal cell carcinoma and its role as a potential therapeutic target. Oncotarget 2015; 6: 22706–22723.

Supplementary Information accompanies this paper on Cell Death and Disease website (http://www.nature.com/cddis).