Cellular Physiology and Biochemistry

Cell Physiol Biochem 2020;54:53-70
DOI: 10.33594/000000205
Published online: 22 January 2020
Accepted: 18 December 2019

Original Paper

Genistein Represses HOTAIR/Chromatin Remodeling Pathways to Suppress Kidney Cancer

Mitsuho Imai-Sumida Pritha Dasgupta Priyanka Kulkarni Marisa Shiina Yutaka Hashimoto Varahram Shahryari Shahana Majid Yuichiro Tanaka Rajvir Dahiya Soichiro Yamamura

Department of Urology, San Francisco Veterans Affairs Medical Center and University of California San Francisco, San Francisco, CA, USA

Key Words
Genistein • Renal cancer • HOTAIR • PRC2 • SMARCB1

Abstract
Background/Aims: Genistein, a soy isoflavone, has been shown to have anti-cancer effects in various cancers including renal cancer. Long non-coding RNA, HOX transcript antisense RNA (HOTAIR), is involved in cancer progression and metastasis, such as renal cancer. Our aim was to investigate the effects of genistein on HOTAIR chromatin remodeling functions. ***Methods:*** We used MTS assays and Transwell migration assays to study the effects of genistein on cell proliferation and migration respectively in human renal cell carcinoma (RCC) cell lines. We used Western blots to analyze SNAIL and ZO-1 expression. We performed chromatin immunoprecipitation (ChIP) assays to study recruitment of the polycomb repressive complex 2 (PRC2) to the ZO-1 promoter. We performed RNA immunoprecipitation (RIP) assays to study interaction between HOTAIR and PRC2, SMARCB1 or ARID1A. We also performed transfection experiments to overexpress EED, HOTAIR and knockdown SMARCB1. ***Results:*** Genistein reduced cell proliferation and migration of human renal cell carcinoma cell lines. ChIP assays indicated that genistein reduces recruitment of the PRC2 to the ZO-1 promoter and increased its expression. RIP assays showed that genistein inhibits HOTAIR interaction with PRC2, leading to tumor suppression. Immunoprecipitation also revealed that genistein reduced EED levels in PRC2, suggesting that decreased EED levels suppress HOTAIR interaction with PRC2. EED overexpression in the presence of genistein restored PRC2 interaction with HOTAIR and reduced ZO-1 transcription, suggesting genistein activates ZO-1 by inhibiting HOTAIR/PRC2 functions. RIP assays also showed that HOTAIR interacts with SMARCB1 and ARID1A, subunits of the human SWI/SNF chromatin remodeling complex and genistein reduces this interaction. Combination of HOTAIR overexpression and SMARCB1 knockdown in the presence of genistein revealed that genistein inhibits SNAIL transcription via the HOTAIR/

Soichiro Yamamura
Department of Urology, San Francisco Veterans Affairs Medical Center and University of California San Francisco, San Francisco, CA 94121 (USA)
E-Mail soichiro.yamamura@ucsf.edu

SMARCB1 pathway. ***Conclusion:*** Genistein suppresses EED levels in PRC2 and inhibits HOTAIR/PRC2 interaction. Genistein suppresses HOTAIR/PRC2 recruitment to the ZO-1 promoter and enhances ZO-1 transcription. Genistein also inhibits SNAIL transcription via reducing HOTAIR/SMARCB1 interaction. We demonstrate that the reduction of HOTAIR interaction with chromatin remodeling factors by genistein represses HOTAIR/chromatin remodeling pathways to suppress RCC malignancy.

Introduction

Renal cell carcinoma (RCC) is a common cancer with more than 60, 000 new cases in the United States in 2017 [1]. Despite the development of therapeutic regimens, RCC prognosis is poor. The overall 5-year survival of patients with metastatic RCC is less than 10%, possibly due to its low response to chemo- and/or radiotherapy, delayed diagnosis, and relatively high incidence of metastasis [2]. Patients with advanced RCC generally respond poorly to standard therapies [3]. Therefore, there is an urgent need for early diagnosis and identification of novel therapeutic targets for RCC.

Genistein [4′,5,7-trihydroxyisoflavone or 5,7-dihydroxy-3-(4-hydroxyphenyl) chromen-4-one($C_{15}H_{10}O_5$)] is a naturally occurring isoflavone found in high amounts in soy products. Genistein has been specifically evaluated in various studies and has been shown to have anti-cancer effects in a variety of cancer types, including breast, ovarian and hepatocellular cancers. The anti-cancer activity of genistein is associated with suppression of cell proliferation and/or induction of apoptosis [4]. In other studies, genistein was found to have anti-cancer effects through epigenetic modulation, including DNA promoter methylation and histone modification [5]. Although several studies have reported that genistein inhibits kidney cancer progression in *in vitro* and *in vivo* models, the molecular mechanisms of genistein action in kidney cancer are not fully understood.

Long non-coding RNAs (lncRNAs) are transcribed RNA molecules over 200 nucleotides in length and known to be associated with various malignancies [6]. Long non-coding RNA, HOX transcript antisense RNA (HOTAIR) is located on chromosome 12 inside the Homeobox C (HOXC) locus and encodes a 2.2 kb lncRNA molecule [7]. HOTAIR is highly expressed in a variety of cancers and has been implicated in cancer development and progression [8-14]. HOTAIR expression has been shown to promote cancer cell invasion [9, 10, 15], increase cell proliferation, and reduce apoptosis [11, 15]. Many lncRNAs can regulate chromatin states and play biological roles in epigenetic modification [16]. For instance, HOTAIR has been reported to be required for targeting polycomb repressive complex 2 (PRC2) in trans to the HOXD locus [7, 17] and plays a critical role in cancer metastasis through its effect on genome-wide PRC2 reprogramming [10]. The PRC2 is involved in diverse cellular processes through histone modification and consists of four core subunits: EZH2 (the catalytic subunit enhancer of zeste homolog 2), EED (embryonic ectoderm development), SUZ12 (suppressor of zeste 12), and retinoblastoma-associated proteins 46/48. Additionally, JARID2, a member of the JmjC domain-containing protein family, has been characterized as a novel component of PRC2 [18-20].

The human SWI/SNF (SWItch/Sucrose Non-Fermentable) chromatin remodeling complex is crucial in regulating gene expression and known to be involved in a variety of cellular processes, including differentiation and proliferation. Impaired and/or defective activity of this complex may affect tumor development [21]. The complex contains AT-rich interactive domain-containing protein 1A (ARID1A; also known as BAF250A and SMARCF1), SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1 (SMARCB1, also known as BAF47 and INI1), and subfamily A, Member 4 (SMARCA4; also known as BRG1). PBRM1 (also known as BAF180) and BRM (also known as SMARCA2) are also subunits of the SWI/SNF complex [22]. Loss of SMARCB1 expression has been described in malignant tumors including RCC and has been implicated in RCC aggressiveness [23]. Mutations in SMARCA4 have been reported for various cancers including clear cell renal

cell carcinoma (ccRCC) [24]. ARID1A is also frequently mutated in cancer including ccRCC [24, 25]. Lower ARID1A levels are associated with worse ccRCC prognosis [26, 27].

In this study, we document anti-cancer effects of genistein in renal cancer. We demonstrate that suppression of HOTAIR interaction with PRC2 by genistein results in activation of ZO-1 transcription. We also show that genistein treatment reduces HOTAIR interaction with SMARCB1 and ARID1A, subunits of the SWI/SNF chromatin remodeling complex and represses SNAIL transcription. These results suggest that genistein inhibits HOTAIR/chromatin remodeling pathways to suppress kidney cancer.

Materials and Methods

Materials

Genistein (>98% purity) and dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich. RIPA buffer was purchased from Cell Signaling Technology, protease inhibitor and phosphatase inhibitor were from Roche. SNAIL (3879), ZO-1 (8193), EZH2 (5246) and SMARCB1 (8745) antibodies were purchased from Cell Signaling Technology. EZH2 (39876), EED (61204) and SUZ12 (39878) antibodies were purchased from Active Motif. EED antibody (09-774) was purchased from (EMD Millipore). INI1 (SMARCB1) (sc-166165), Brg-1 (SMARCA4) (sc-17796), ARID1A (sc-32761) and Actin (sc-47778) antibodies were purchased from Santa Cruz Biotechnology. Human EED expression vector (HG11307-CF) was purchased from Sino Biological. SMARCB1 Pre-design Chimera RNAi (H00006598) was purchased from Abnova. Control siRNA (6568) was purchased from Cell Signaling Technology.

Transfection

Human EED expression vector and HOTAIR expression vector [15] were transfected using X-tremeGENE HP DNA Transfection Reagent (Sigma-Aldrich) according to the manufacture's protocol. SMARCB1 Pre-design Chimera RNAi was transfected using Lipofectamine RNAiMAX Transfection Reagent according to the manufacture's protocol.

Cell culture and Treatment

Human clear cell renal carcinoma cell lines (ccRCC), 786-O and ACHN were purchased from The American Type Culture Collection. 786-O cells were cultivated in RPMI medium and ACHN cells were cultivated in Eagle's Minimum Essential medium. Both were supplemented with 10% fetal bovine serum (FBS, Corning) and 1% penicillin/streptomycin (Gibco). The cell lines were cultured at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Genistein was dissolved in DMSO and cells were treated with 25 μM genistein for the indicated time. The final concentration of DMSO in the culture did not exceed 0.1% (v/v). The medium was changed every day with fresh media containing genistein or DMSO as a control.

Cell proliferation assay

Cell proliferation/viability was assessed using a CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega), as described previously [28]. Briefly, cells were seeded at a density of 1×10^3 cells per well in flat bottomed 96-well plates. At the indicated times, CellTiter 96 Aqueous One reagent was added to each well according to the manufacturer's instructions. Cell viability was determined by measuring the absorbance at 490 nm using a kinetic microplate reader (Spectra MAX 190; Molecular Devices). Data are the mean ± standard deviation (SD) of three independent experiments.

Migration assay

786-O and ACHN cells were treated with 25 μM genistein or DMSO as a control for 96 hours. For migration assay, culture inserts of 8-μm pore size (Transwell; Corning) were placed into the wells of 24-well culture plates. In the lower chamber, 500 μl of culture medium containing 10% FBS was added and 1×10^5 cells diluted in culture media not including FBS were seeded to the upper chamber. After 48 hours of incubation the cells which attached to the lower surface were stained with 0.05% crystal Violet. Crystal Violet was solubilized with methanol and absorbance (540 nm) of the solution was measured by a kinetic

microplate reader (Spectra MAX 190; Molecular Devices Co.). Data are the mean ± S.D. of three independent experiments.

Total RNA and protein extraction

Total RNA was extracted from cultured bladder cell lines using a miRNeasy mini kit (Qiagen) according to the manufacturer's instructions. For protein extraction, cells were lysed with RIPA buffer (Thermo Scientific) containing Protease and Phosphatase inhibitor cocktail (Thermo Scientific).

Reverse transcription and real-time PCR

cDNA was synthesized using an iScript Synthesis Kit (Bio-Rad). Quantitative real-time PCR (qPCR) analysis was performed in triplicate with an Applied Biosystems Prism 7500 fast Sequence Detection System using TaqMan Fast Universal PCR Master Mix according to the manufacturer's protocol (Applied Biosystems). The PCR program was as follows: denaturation at 95 °C for 15 min and 45 cycles of amplification consisting of denaturation at 95 °C for 15 s, annealing at 58 °C for 30 s, and extension at 72 °C for 30 s. TaqMan probes and primers were purchased from Applied Biosystems. Human GAPDH were used as an internal control. Levels of RNA expression were determined by using the 7500 Fast System SDS software version 1.3.1 (Applied Biosystems).

Western blot analysis

Protein extracts were resolved by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride membranes (Hybond-P; GE Healthcare), followed by incubation with the indicated primary and secondary antibodies conjugated to horseradish peroxidase (GE Healthcare). Signals were detected using the ECL detection system (Amersham ECL plus Western Blotting detection system).

Promoter activity assay

786-O and ACHN cells were co-transfected with a Renilla luciferase plasmid containing the promoter region of ZO-1 (Active Motif) and pCMVHA hEZH2 (Addgene) or pCMV6-Entry (Origene). Promoter activity was determined using a LightSwitch Luciferase Assay Kit (Active Motif) according to the manufacturer's instructions. pCMVHA hEZH2 was a gift from Kristian Helin (Addgene plasmid # 24230; http://n2t.net/addgene:24230 ; RRID:Addgene_24230) [29].

Chromatin immunoprecipitation (ChIP) assay

ChIP assays were performed using a Simple ChIP Plus Enzymatic Chromatin IP kit (Cell Signaling Technology) according to the procedures provided by the manufacturer. Cells were incubated with 1% formaldehyde for 10 min at room to cross-link DNA, and the chromatin fraction prepared from the cells was digested with micrococcal nuclease and immunoprecipitated with anti-EZH2 antibody (5246; Cell Signaling Technology) or normal rabbit IgG (sc-2027, Santa Cruz). The DNA was recovered from the immunoprecipitants and subjected to PCR targeting the ZO-1 promoter with primers (Table 1). Precipitated genomic DNA was amplified by real-time PCR. PCR was performed using SYBR Green PCR Master Mix according to the manufacturer's suggestions (Qiagen).

RNA Immunoprecipitation (RIP) assay

After cells were cultured with genistein or carrier for 4 days, RIP buffer, including RNase inhibitor (SUPERase-In, Ambion) and Halt protease and phosphatase inhibitor cocktail (Thermo Scientific), were used to collect protein. This was followed by immunoprecipitation overnight at 4 °C with either anti-EZH2, ARID1A, SMARCB1, or normal rabbit IgG (Santa Cruz Biotechnology) and Protein A/G Mag Beads (GenScript). After immunoprecipitation, the bead suspensions were washed five times with RIP wash buffer, and the immunoprecipitated proteins were detected by Western blotting. The RNA from the bead samples was purified using a miRNeasy mini kit (Qiagen) according to the manufacturer's instructions. cDNAs were synthesized from the purified RNA using an iScript Synthesis Kit (Bio-Rad), and analyzed by qPCR using TaqMan Fast Universal PCR Master Mix for HOTAIR.

Table 1. Primers used for ChIP qPCR

Primer name	Forward	Reverse
ZO-1-1	GAGGTCTAATGTGGGGTGTGG	CAGAAGCGTTGCTCTCGTTC
ZO-1-2	ACCAGTTTCAGCCTTGGCA	TCCTTCACTGGGCAAACAGC
ZO-1-3	GACCATGCTGTTTGCCCAGT	AAGCCGGGTAACCCAAGTAA
ZO-1-4	TTACCCGGCTTTTCCTTCCG	TTCGCCACGTAACTTCCCG
ZO-1-5	CGGGAAGTTACGTGGCGAA	CTCGGACAAAAGTCCGGGAA
ZO-1-6	CCCGGACTTTTGTCCGAGTT	AAACATCTCCCGAGAGCG

RNA pull-down assay

HOTAIR cDNA was amplified from 786-O cells by using primers carrying NheI and BamHI restriction enzyme sites at the flanking ends. The amplified cDNA was sequenced and subcloned into the NheI and BamHI restriction site of the pcDNA3.1(+) plasmid (Thermo Fisher Scientific).

The MS2 stem loops (12X) in pSL-MS2-12X was sub-cloned into the BamHI and NotI restriction site of the HOTAIR expression vector above. pSL-MS2-12X was a gift from Robert Singer (Addgene plasmid # 27119; http://n2t.net/addgene:27119 ; RRID:Addgene_27119) [29].

Maltose-binding protein (MBP)-affinity purification was used for RNA pull-down assays to study interaction of HOTAIR with ARID1A and SMARC1B proteins. 786-O or ACHN cells were transfected with MS2-tagged HOTAIR constructs. At 48 hours after the transfection, the cells were lysed with buffer (150 mM KCl, 25 mM Tris pH 7.4, 2 mM EDTA, 1.0 % NP40) and subjected to RNA pull-down assays. pMBP-MS2 was a gift from Josep Vilardell (Addgene plasmid # 65104; http://n2t.net/addgene:65104; RRID:Addgene_65104) [30]. The MS2-MBP protein was expressed and purified from NEB® Express Competent E. coli (New England Biolabs) using amylose resin (New England Biolabs) according to the manufacturer's instructions. The lysate was incubated with amylose resin which harbored MS2-MBP protein at 4 °C for 4 hours and the interacted proteins were analyzed by SDS-PAGE.

Molecular profiling datasets

Normalized gene expression datasets of RCC subtypes were downloaded from NCBI GEO website (Gene Expression Omnibus; http://www.ncbi.nlm.nih.gov/geo). Accession number: GSE15641, which provides transcriptional profiling with oligonucleotide microarrays (22, 283 genes) on 49 RCC tumors, 20 non-RCC renal tumors, and 23 normal kidney samples. The data was analyzed by Student's t-test.

Statistical analysis

Data are shown as mean values ± standard deviation (SD). The Student's t-test was used to compare the two distinct groups. One–way ANOVA followed by *Dunnett's* post hoc test was used to compare more than three distinct groups (Fig. 1b). P values of less than 0.05 were regarded as statistically significant.

Results

Genistein inhibits RCC cell proliferation and cell migration

We evaluated the effect of genistein (Fig. 1a) on the growth of 786-O and ACHN renal cancer cells using the MTS cell proliferation assay. Cells were incubated at different concentrations of genistein (0, 10 and 25 μM) for the indicated time. The results show that genistein significantly inhibited RCC cell proliferation compared to controls. The inhibitory effects of genistein on cell viability were dose-dependent (Fig. 1b). We next studied the effects of genistein on cell migration. 786-O and ACHN cells were treated with 25 μM genistein and subjected to Transwell migration assay. The migration assay shows that genistein reduced the migration capacities of 786-O and ACHN cells (Fig. 1c). After treatment with genistein, the cell shapes of 786-O and ACHN were found to be enlarged compared to controls (data not shown). These results indicate that genistein suppresses 786-O and ACHN cell proliferation, migration and alters the cell shape from a fibroblast shape to an epithelial-like shape, a feature of epithelial–mesenchymal transition (EMT).

Genistein suppresses EZH2 recruitment to the ZO-1 *promoter*

Through the action of EMT-inducing transcription factors, cells lose epithelial characteristics, such as Tight Junction Protein ZO-1 (ZO-1) expression [31]. We found that genistein treatment upregulated ZO-1 mRNA and protein levels (Fig. 2a and b). PRC2 mediates silencing of epithelial genes such as CDH1 (E-cadherin), and thus involves the initial recruitment of an EMT-inducing transcription factor (for example, SNAIL) to the gene promoter [32]. We assayed ZO-1 promoter activity using luciferase reporter assay. EZH2 activated ZO-1 promoter activity (Supplementary Fig. 1 – for all supplemental material see www.cellphysiolbiochem.com). We then performed ChIP (Chromatin immunoprecipitation) assays to examine whether genistein affects EZH2 recruitment to the ZO-1 promoter region in 786-O and ACHN cells. Six PCR primer sets for the ZO-1 promoter were designed (Fig. 2c)

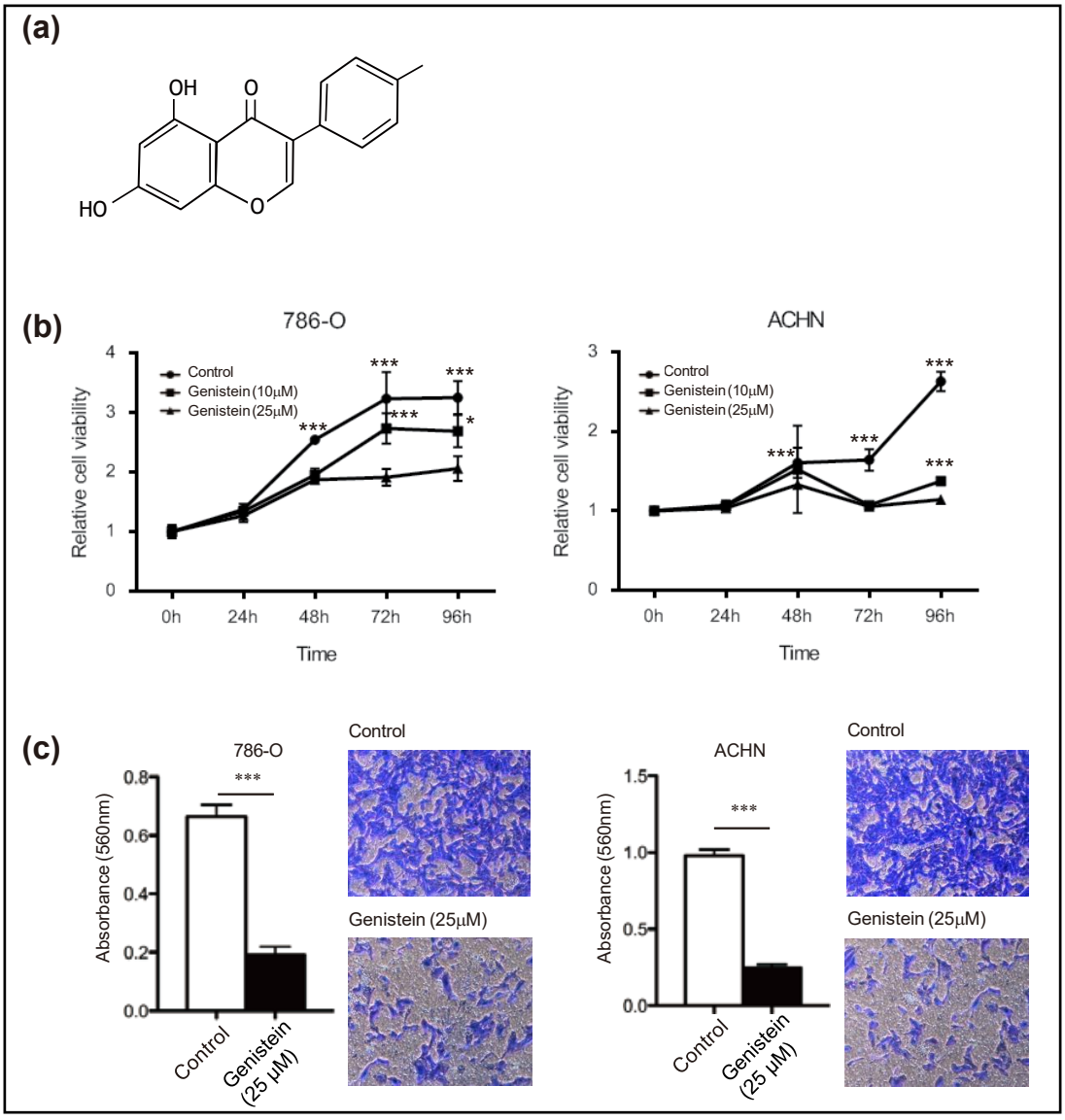

Fig. 1. Genistein inhibits cell proliferation and migration. (a) Chemical structure of genistein. (b) Renal cancer cell lines (786-O and ACHN) were treated with control (DMSO), and genistein (10 and 25 μM) for the indicated hours and cell proliferation assays were performed. (c) 786-O and ACHN were treated with 25μM genistein or control (DMSO) for 96 hours and then used for Transwell assay. The graphs show absorbance of crystal violet staining eluted from migrated cells (right panel). The left panel shows typical images of the migrated cells. *p<0.05, **p<0.01, ***p<0.001.

and the ribosomal protein L30 (RPL30) was used as a positive control to validate ChIP assays. ChIP assays show EZH2 recruitment to the ZO-1 promoter region in 786-O and ACHN cells with and without genistein (25 μM) treatment (Fig. 2d). EZH2 recruitment to the promoter was down-regulated by genistein treatment with all primer sets except for primer 4 for in 786-O cells (Fig. 2e). EZH2 recruitment to the promoter was down-regulated by genistein treatment specifically with primer 3 set in ACHN cells (Fig. 2e).

Genistein inhibits interaction between the PRC2 and HOTAIR

HOTAIR has been reported to bind to PRC2 and to inactivate tumor suppressors by recruiting PRC2 to the tumor suppressor promoters [9]. 786-O and ACHN cells were treated with genistein for 96 hours and HOTAIR expression was measured by quantitative RT-PCR. Genistein suppressed HOTAIR expression as we previously reported [15] (Fig. 3a). PRC2 subunits (EZH2, SUZ12 and EED) expression was also analyzed by Western blot in 786-O and ACHN cells treated with genistein (25 μM). Genistein had no significance effects on these protein expression (Fig. 3b). Given that genistein attenuates cell proliferation, cell migration and EMT, to test the impact of genistein on HOTAIR interaction with PRC2, we treated renal cancer cells with genistein and performed RNA immunoprecipitation (RIP) assays using EZH2 antibody. Immunoprecipitation (IP) revealed that EZH2 pulled down EED and Suz12, core components of PRC2, showing that PRC2 was pulled down with and without genistein treatment (Fig. 3d). It is noted that the amount of Suz12 in PRC2 is small in these renal cancer cells (Fig.3d). RIP assays also revealed that HOTAIR bound to PRC2, and that this binding was inhibited by genistein treatment (Fig. 3c). IP (Fig. 3d) shows that genistein significantly reduced the amount of immunoprecipitated EED, suggesting that reduced EED accounted for the inhibition of HOTAIR interaction with PRC2. These results suggest that genistein plays an antitumor role by inhibiting interaction between HOTAIR and PRC2. The attenuated PRC2 recruitment to the ZO-1 promoter region by genistein (Fig. 2e) is assumed to be partly mediated by HOTAIR.

Overexpression of EED restores interaction of HOTAIR with PRC2 and reduces ZO-1 expression with genistein treatment

Since the amount of EED was significantly reduced in the EZH2 immunoprecpitated complex in genistein treated cells (Fig. 3d), we overexpressed EED to see if EED overexpression restored HOTAIR binding to PRC2 with genistein treatment. Fig. 4b shows that EED overexpression (Fig. 4c) restored the amount of EED in the immunoprecipitated complex and rescued HOTAIR binding (Fig. 4a). EED overexpression also reduced ZO-1 mRNA expression (Fig. 4d). These results indicate that EED plays a pivotal role in HOTAIR interaction with PRC2 Taken together with the ChIP assay results (Fig. 2e), genistein suppresses HOTAIR/PRC2 recruitment to the ZO-1 promoter and enhances ZO-1 transcription.

Genistein reduces interaction of HOTAIR with SMARCB1

Exome sequencing studies have revealed that SMARCB1, a core component of the human SWI/SNF complex, is mutated in ccRCC, indicating that SMARCB1 also plays a vital role in ccRCC [33]. SMARCB1 expression was analyzed by Western blot in 786-O and ACHN cells treated with genistein (25 μM). Genistein slightly reduced SMARCB1 protein expression (Fig. 5a). To study interaction of HOTAIR with SMARCB1 protein, we performed RNA pull-down assays with transfection of MS2-tagged HOTAIR vector. The results show that HOTAIR interacts with SMARCB1 protein in 786-O and ACHN cells (Fig. 5b). We treated 786-O and ACHN cells with genistein and investigated the effects of genistein on the interaction between SMARCB1 and HOTAIR by RIP assays. IP shows that SMARCB1 and ARID1A which are subunits of the SWI/SNF complex, were pulled down (Fig. 5d). The RIP assays revealed that genistein reduced the interaction of HOTAIR with SMARCB1 in both cell lines (Fig. 5c). Taken together, these results show that SMARCB1 interacts with HOTAIR and that genistein reduces the interaction with HOTAIR, thereby playing an antitumor role in ccRCC by inhibiting HOTAIR oncogenic remodeling functions.

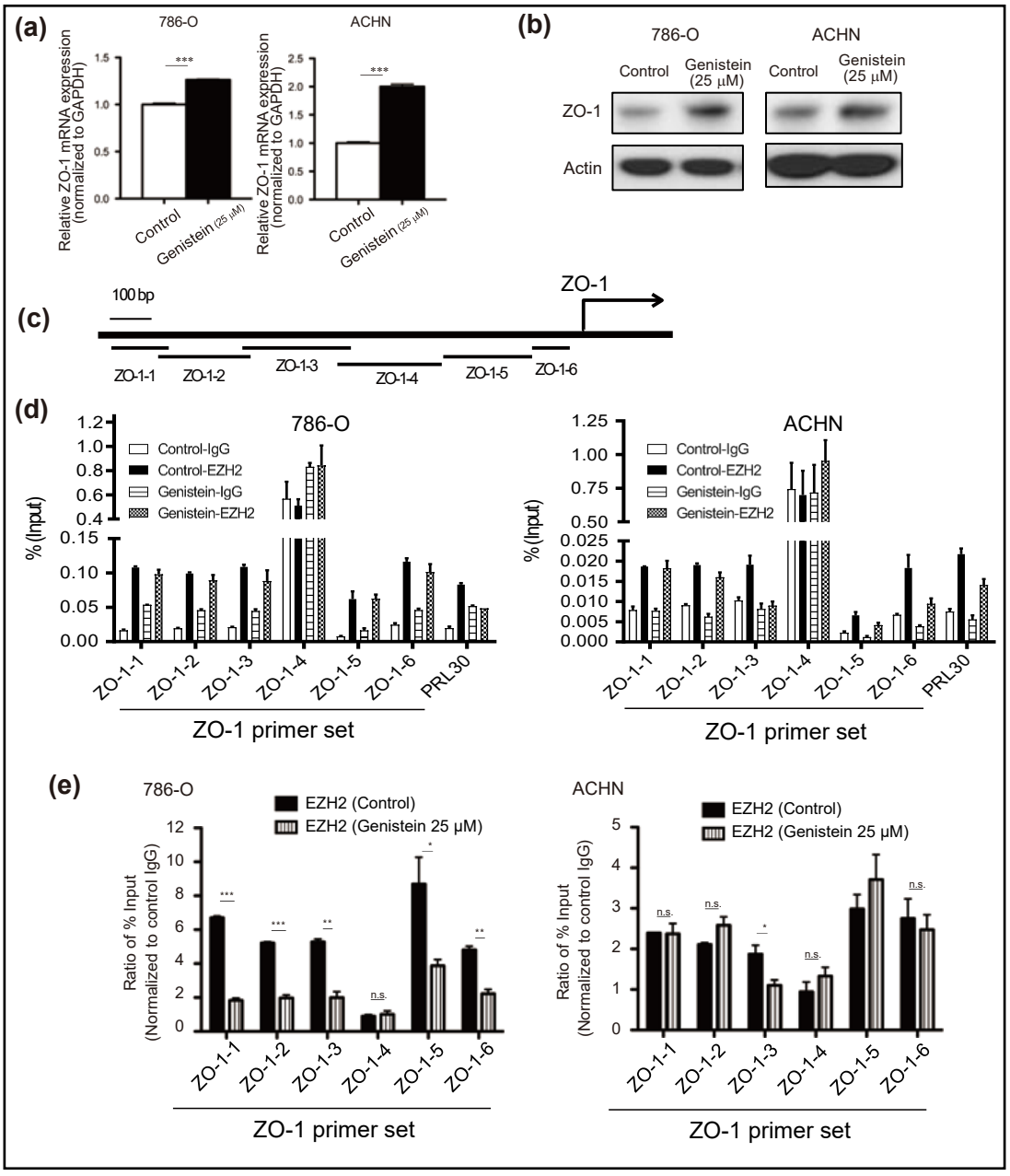

Fig. 2. Genistein suppresses EZH2 recruitment to the ZO-1 promoter region. (a) 786-O and ACHN cells were treated with genistein (25 μM) or control (DMSO) for 96 hours. Then, quantitative RT-PCR was performed for ZO-1. (b) Expression of ZO-1 protein was analyzed by Western blot in 786-O and ACHN cells treated with genistein (25 μM) or control (DMSO) for 96 hours. (c) Schematic representation of the promoter region of the human ZO-1 gene and primer set location. Primer sequences are listed in Table 1. (d) ChIP-quantitative RT-PCR analyses of EZH2 binding at selected multiple ZO-1 promoter targets with and without genistein. The ribosomal protein L30 (RPL30) was used as a positive control to validate ChIP assay. Y axis shows percent of input recovery. (e) Relative change of EZH2 at elected multiple ZO-1 promoter targets after genistein (25 μM) treatment for 96 hours. Y axis shows the ratio of percentage of input normalized to control IgG. Expression levels are normalized to GAPDH and those in control-treated cells were defined as 1. *p<0.05, **p<0.01, ***p<0.001, 'n.s.' p>0.05.

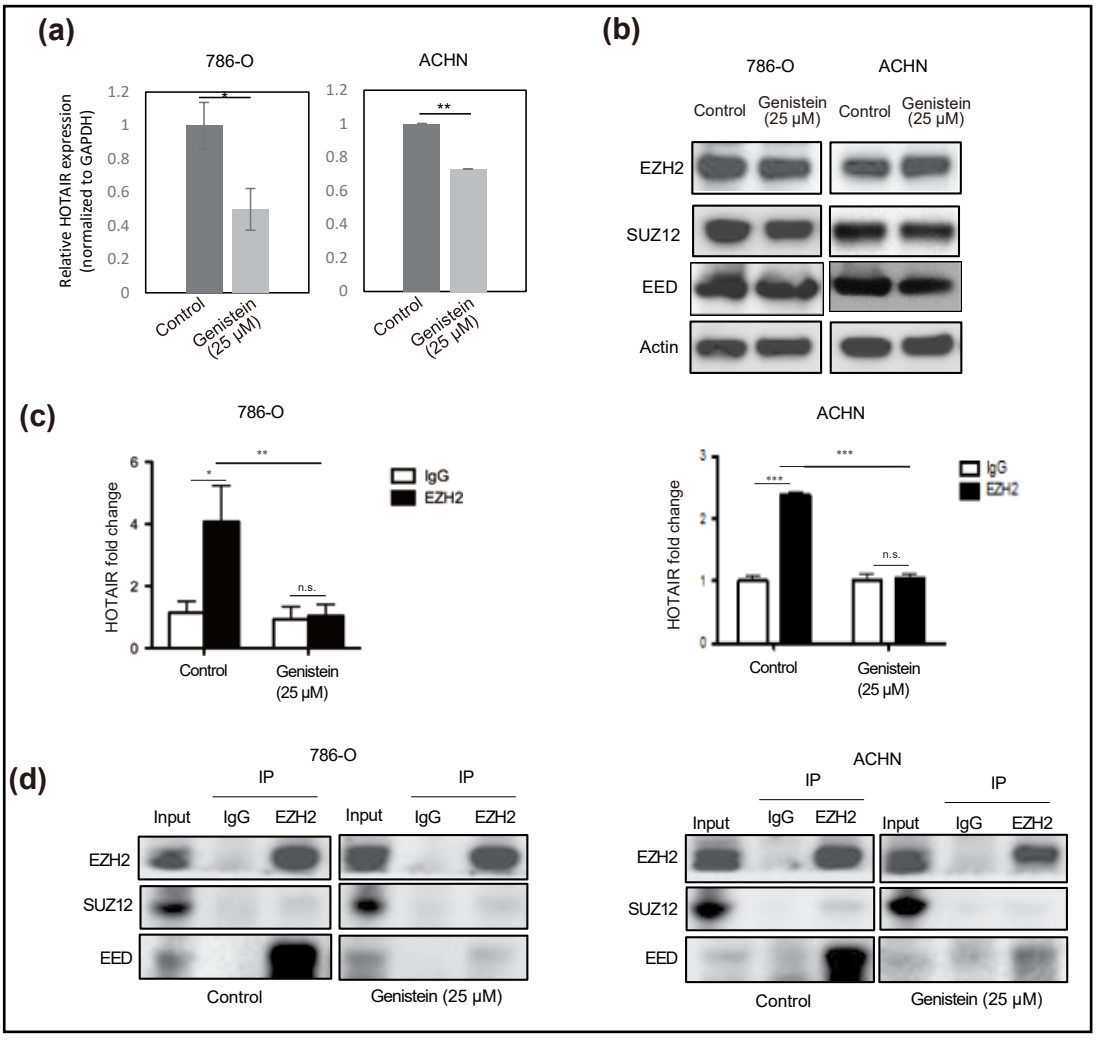

Fig. 3. Genistein inhibits interaction of HOTAIR with PRC2. (a) 786-O and ACHN cells were treated with genistein (25 μM) or control (DMSO) for 96 hours. Then, quantitative RT-PCR was performed for HOTAIR. (b) PRC2 subunit expression was analyzed by Western blot in 786-O and ACHN cells treated with genistein (25 μM) or control (DMSO) for 96 hours. (c) PRC2 from 786-O and ACHN cells cultured with genistein (25 μM) or control (DMSO) for 96 hours were pulled down using anti-EZH2 antibody or control IgG. HOTAIR associated with the PRC2 was detected by quantitative RT-PCR. Data from quantitative RT-PCR are presented with respect to control IgG that is set to a value of 1. (d) Immunoprecipitated PRC2 was detected by Western blot. *p<0.05, **p<0.01, ***p<0.001, 'n.s.' p>0.05.

Genistein reduces interaction of HOTAIR with ARID1A

Loss-of-function mutations in ARID1A, one of the subunits in the human SWI/SNF complex, has been reported in various cancers [22]. ARID1A expression was analyzed by Western blot in 786-O and ACHN cells treated with genistein (25 μM). Genistein slightly reduced ARIDIA protein expression in 786-O cells and had no effect in ACHN cells (Fig. 6a). Fig. 5d also shows SMARCB1 pulled down ARID1A. Thus, we examined the interaction of HOTAIR with ARID1A and the effects of genistein on this interaction and confirm the results of HOTAIR interaction in Fig. 5c and d since ARID1A plays a vital role in ccRCC. To study the interaction of HOTAIR with ARID1A protein, we performed RNA pull-down assays with transfection of MS2-tagged HOTAIR vector. RNA pull-down assays show that HOTAIR interacts with ARID1A protein in 786-O and ACHN cells. (Fig. 6b). Protein RNA-binding site

prediction software, OmiXcore [34], indicated that ARID1A may directly bind to HOTAIR. The software shows high-scoring HOTAIR nucleotide for ARID1A binding in red, and low-scoring nucleotide in white (Fig. 6c). The other Protein RNA-binding site prediction software

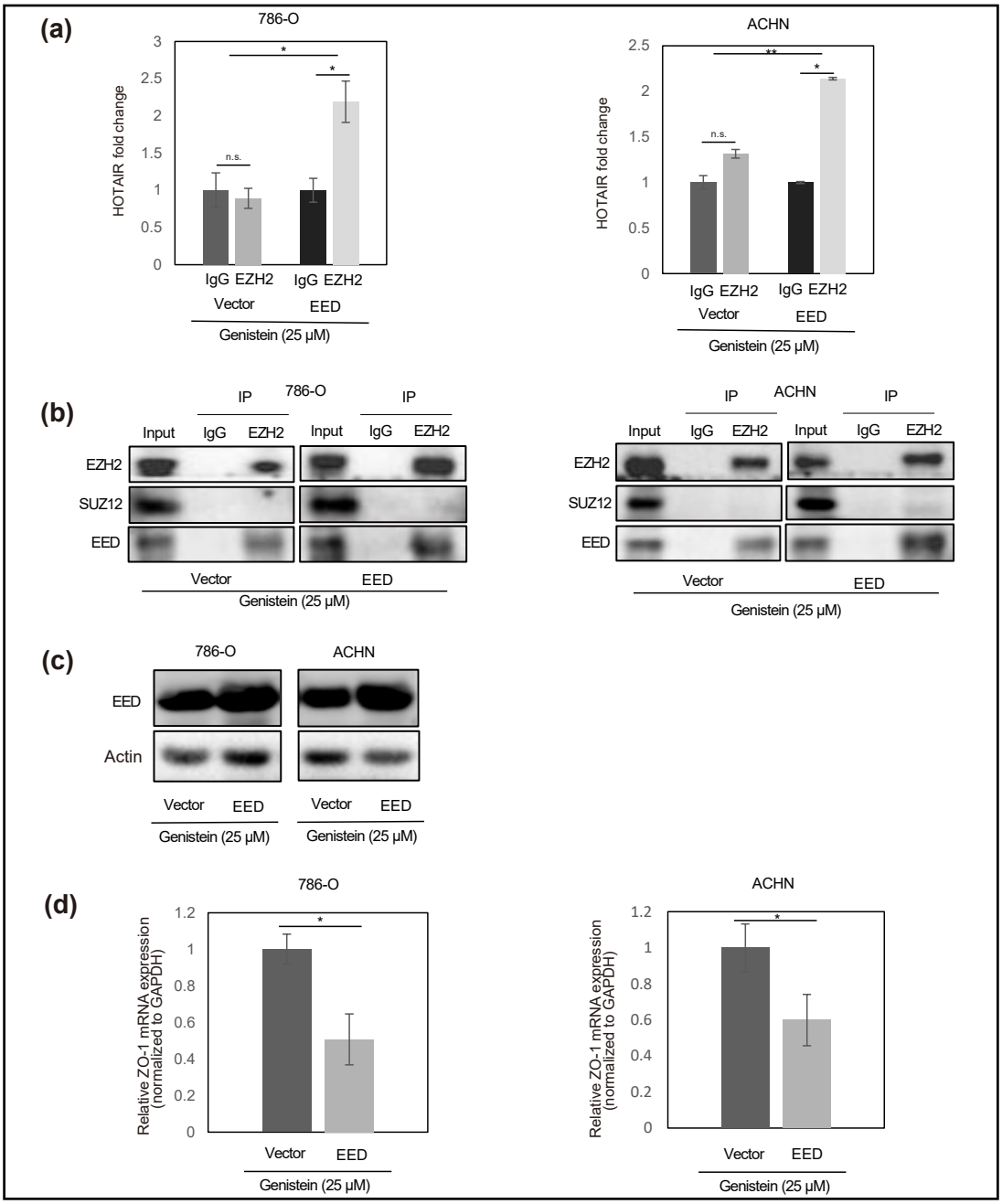

Fig. 4. EED overexpression restores interaction of HOTAIR with PRC2 and reduces ZO-1 mRNA with genistein treatment. (a) 786-O and ACHN cells in 6-well plates were treated with 25 uM genistein for 24 hours and transfected with 100 ng of control vector (pcMV6-ENTRY) or EED expression vector and incubated for 72 hours in the presence of genistein. PRC2 from 786-O and ACHN cells cultured with genistein (25 μM) or control for 96 hours were pulled down using anti-EZH2 antibody or control IgG. HOTAIR associated with the PRC2 was detected by quantitative RT-PCR. Data from quantitative RT-PCR are presented with respect to control IgG that is set to a value of 1 *p<0.05, **p<0.01. (b) Immunoprecipitated PRC2 subunits were detected by Western blot. (c) EED expression was analyzed by Western blot. D. ZO-1 mRNA expression level was measured by quantitative RT-PCR.

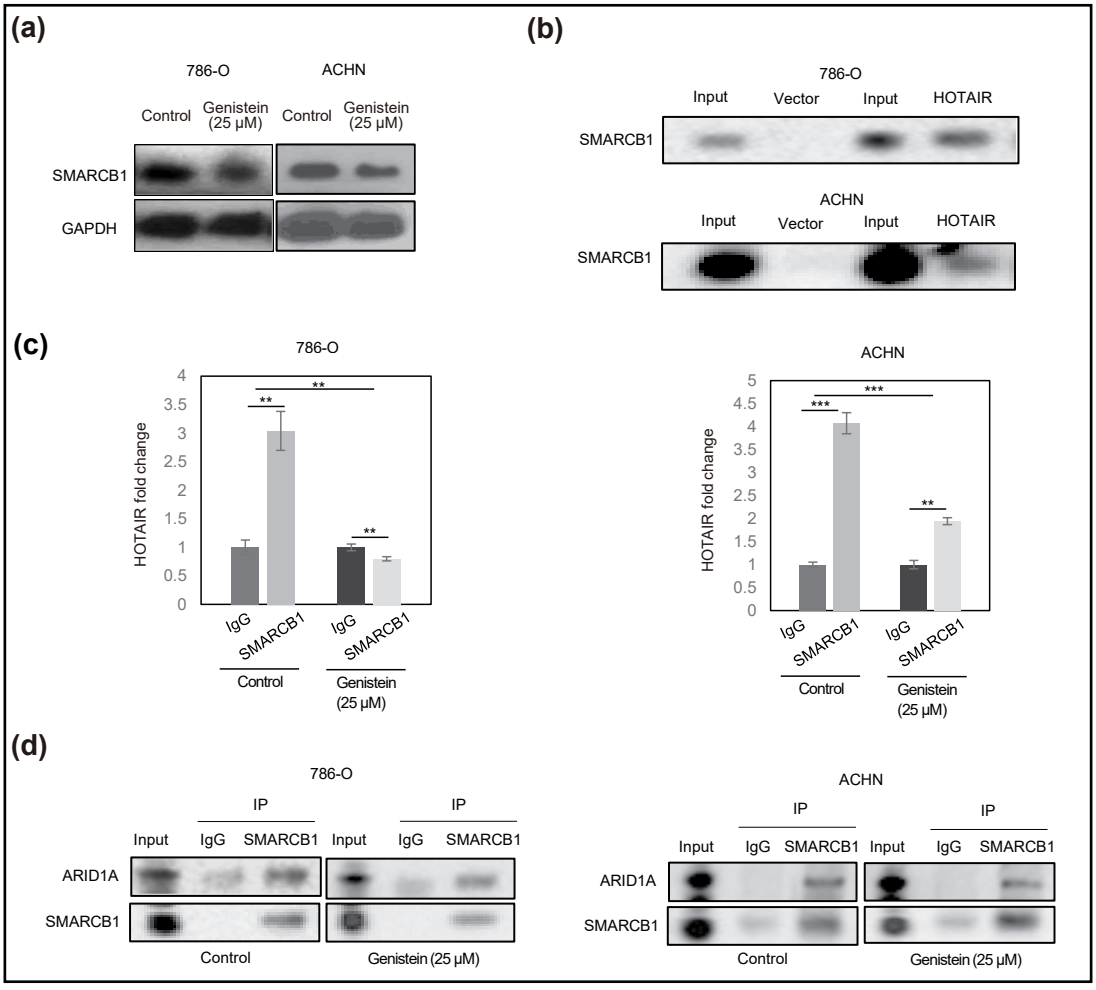

Fig. 5. Genistein reduces interaction of HOTAIR with SMARCB1. (a) SMARCB1 expression was analyzed by Western blot in 786-O and ACHN cells treated with genistein (25 μM) or control (DMSO) for 96 hours. (b) 786-O and ACHN cells were transfected with control vector (pcDNA3.1(+)) or MS2-tagged HOTAIR vector and RNA pull-down assays were performed. Pulled-down SMARCB1 was detected by Western blot. (c) SMARCB1 from 786-O and ACHN cells cultured with genistein (25 μM) or control (DMSO) for 48 hours were pulled down using anti-SMARCB1 antibody. HOTAIR associated with SMARCB1 proteins was detected by quantitative RT-PCR. Data from quantitative RT-PCR are presented with respect to IgG that is set to a value of 1. (d) Immunoprecipitated proteins were analyzed by Western blot. **p<0.01, ***p<0.001.

(https://sysimm.ifrec.osaka-u.ac.jp/aarna/index.php [35]), indicated that there are potential ARID1A binding sites with RNA. The software shows high-scoring ARID1A residues (1005 to 1118) in red, and low-scoring residues in blue (Fig. 6d). We treated 786-O and ACHN cells with genistein and investigated the effects of genistein on the interaction between ARID1A and HOTAIR by RNA immunoprecipitation (RIP) assays. IP shows that ARID1A was pulled down (Fig. 6f) and we observed that genistein reduced the interaction of HOTAIR with ARID1A in these cells (Fig. 6e). These results show that ARID1A interacts with HOTAIR and that genistein reduces the interaction with HOTAIR, suppressing ccRCC malignancy.

SMARCB1 and HOTAIR overexpression restore SNAIL expression with genistein treatment

We evaluated whether genistein suppresses the EMT-related gene Snail Family Transcriptional Repressor 1 (SNAIL) that plays a vital role in EMT induction using quantitative RT-PCR and Western blot. 786-O and ACHN cells were treated with genistein and

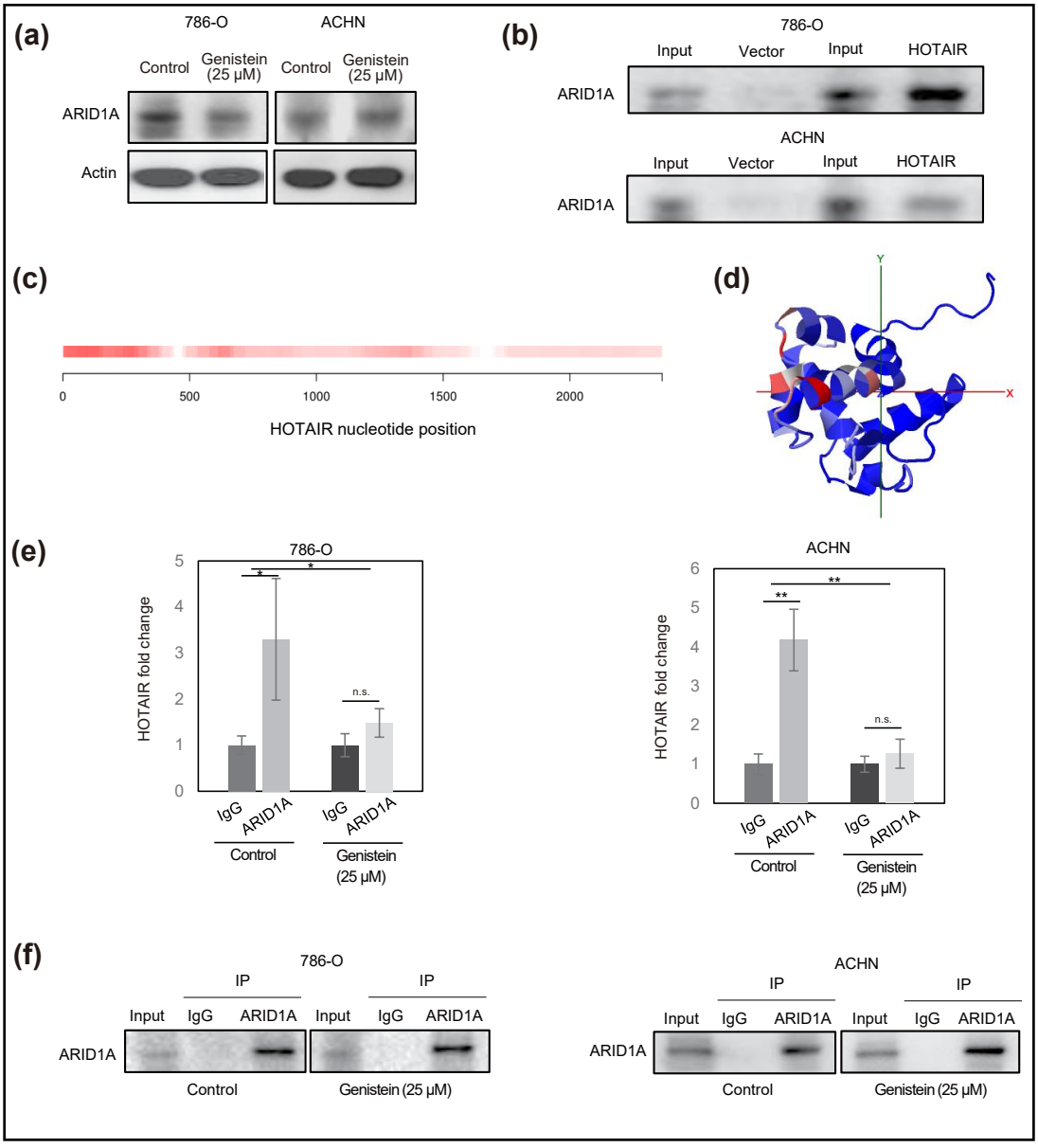

Fig. 6. Genistein reduces interaction of HOTAIR with ARID1A. (a) ARID1A expression was analyzed by Western blot in 786-O and ACHN cells treated with genistein (25 μM) or control (DMSO) for 96 hours. (b) 786-O and ACHN cells were transfected with control vector (pcDNA3.1(+)) or MS2-tagged HOTAIR vector and RNA pull-down assays were performed. Pulled-down ARID1A was detected by Western blot. (c) Protein RNA-binding site prediction software, OmiXcore, indicated that ARID1A may directly bind to HOTAIR. High-scoring HOTAIR nucleotides for ARID1A binding are in red, and low-scoring nucleotides are in white. (d) Potential biding sites of ARID1A with HOTAIR. RNA-binding site prediction software (https://sysimm.ifrec.osaka-u.ac.jp/aarna/index.php) shows high-scoring residues in red, and low-scoring residues in blue. (e) ARID1A from 786-O and ACHN cells cultured with genistein (25 μM) or control (DMSO) for 96 hours were pulled down using anti-ARID1A antibody. HOTAIR associated with ARID1A was detected by quantitative RT-PCR. Data from quantitative RT-PCR are presented with respect to IgG that is set to a value of 1. F, Immunoprecipitated ARID1A was analyzed by Western blot. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

we found that genistein down-regulated SNAIL mRNA (Fig. 7a) and protein expression (Fig. 7b). To examine whether HOTAIR/SMARCB1 regulates SNAIL expression in the presence of genistein, we overexpressed HOTAIR, knocked down SMARCB1 in the presence of 25 μM genistein and performed RIP assays using SMARCB1 antibody. SMARCB1 protein expression after knockdown was analyzed by Western blot (Fig. 7e and Supplementary Fig. 2) and overexpressed HOTAIR was assayed by quantitative RT-PCR (Supplementary Fig. 3). HOTAIR overexpression increased SNAIL mRNA expression (Fig. 7f) as reported previously [9]. IP shows that SMARCB1 knockdown reduced SMARCB1 levels in the immunoprecipitated complexes (Fig. 7d). RIP assays show that HOTAIR overexpression restored HOTAIR interaction with SMARCB1 in the presence of genistein (Fig. 7c). SMARCB1 knockdown suppressed HOTAIR interaction with SMARCB1 (Fig. 7c). Consequently, SMARCB1 knockdown reduced SNAIL expression (Fig. 7f). These results suggest that HOTAIR interaction with SMARCB1 partly induces SNAIL mRNA in the presence of genistein.

Discussion

The anti-cancer effects of genistein have been previously reported in various cancer cells, but the detailed mechanisms are not currently understood. Genistein functionally inhibits cell proliferation, invasion and apoptosis in renal cancer cells [36]. HOTAIR interacts with PRC2, specifically EZH2, an epigenetic modifier that is frequently perturbed in cancer [9, 16]. In this study, we examined whether genistein affects HOTAIR/PRC2 complex and found that genistein suppressed HOTAIR interaction with PRC2, resulting in induction of ZO-1 expression. We have also demonstrated that HOTAIR interacts SMARCB1 and ARID1A, subunits of the human SWI/SNF complex and that genistein reduced these interactions, leading to suppression of SNAIL expression.

PRC2 binds to approximate 20% of lincRNAs in human cells and other chromatin-modifying complexes binds to other lncRNAs [37]. lncRNAs have been proposed to play roles in PRC2 recruitment to specific chromatin regions [38]. The well-known example of PRC2 targeting by a lncRNA is trans-acting lncRNA HOTAIR [7, 17]. HOTAIR is necessary for PRC2 occupancy and histone H3 lysine-27 trimethylation of genes in different chromosomes [39]. HOTAIR reprograms the chromatin state to promote cancer metastasis [9]. PRC2 has histone methyltransferase activity and trimethylates histone H3 on lysine 27 (H3K27me3) which transcriptionally silences chromatin.

ZO-1 is required for tight junction formation and loss of ZO-1 function leads to EMT [40, 41]. We found that genistein increased ZO-1 expression levels in 786-O and ACHN cells. ZO-1 promoter activity assays (Supplementary Fig. 1) and ChIP assays revealed that EZH2 was recruited to the ZO-1 promoter, suggesting that PRC2 was recruited to ZO-1 promoter (Fig. 2d). Genistein reduces the recruitment, indicating that genistein increases ZO-1 levels by reducing the recruitment of PRC2 to the ZO-1 promoter as shown in Fig. 2d and e.

We have found that genistein inhibits the interaction of HOTAIR with PRC2 in 786-O and ACHN cells. Immunoprecipitation (IP) showed that genistein significantly decreased EED in both cell lines (Fig. 4b), indicating a dramatic change in PRC2 structure. Since it has been reported that the EZH2/EED heterodimer is necessary and sufficient for binding to HOTAIR [42], reducing EED in PRC2 may result in the suppression of interaction between HOTAIR and PRC2. Overexpression of EED restores the interaction between HOTAIR and PRC2 and suppresses ZO-1 mRNA expression. These results indicate that EED reduction in PRC2 by genistein inhibits the interaction between HOTAIR and PRC2. ChIP assay shows recruitment of PRC2 to the ZO-1 promoter and genistein reduces the recruitment (Fig. 2d and e). Taken together, genistein inhibits HOTAIR/PRC2 recruitment to the ZO-1 promoter and activates ZO-1 transcription. The mechanisms by which genistein reduces EED in PRC2 remains to be elucidated.

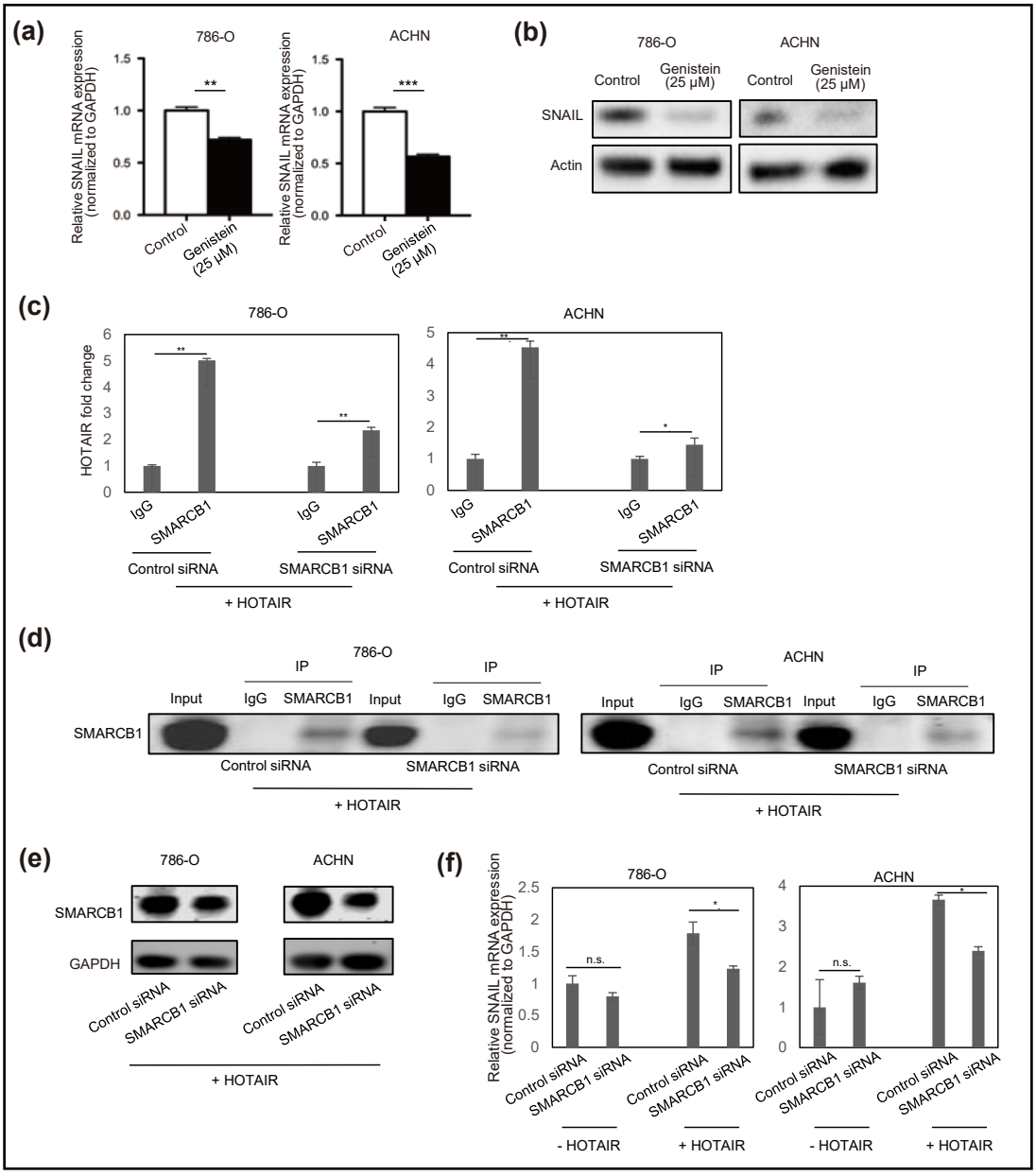

Fig. 7. SMARCB1 and HOTAIR overexpression restore SNAIL expression with genistein treatment. (a) 786-O and ACHN cells were treated with genistein (25 μM) or control (DMSO) for 96 hours and quantitative RT-PCR was performed for SNAIL and (b) expression of SNAIL protein was analyzed by Western blot. 786-O and ACHN cells in 12-well plates were treated with 25 uM genistein. After 24 hours, the cells were transfected with 25 pmol control siRNA or SMARCB1 siRNA and incubated for 72 hours in the presence of genistein. After 48 hours, the cells were transfected with 2.5 ng of HOTAIR expression vector for 48 hours in the presence of genistein. SMARCB1 proteins from 786-O and ACHN cells were pulled down using anti-SMARCB1 antibody or control IgG. (c) HOTAIR associated with SMARCB1 was detected by quantitative RT-PCR. Data from quantitative RT-PCR are presented with respect to IgG that is set to a value of 1. (d) Immunoprecipitated SMARCB1 was analyzed by Western blot. (e) SMARCB1 protein expression was analyzed by Western blot. (f) SNAIL mRNA expression was analyzed by quantitative RT-PCR. *p<0.05, **p<0.01, ***p<0.001. 'n.s.' p>0.05.

The human SWI/SNF, a nucleosome remodeling complex, promotes a variety of human cancers due to mutations of its components. Exome sequencing studies have revealed that SMARCB1, a core component of the human SWI/SNF complex, is mutated in ccRCC [33, 43]. c-MYC has been reported to interact with SMARCB1 and the SWI/SNF complex is necessary for c-MYC-mediated transactivation [44, 45]. Loss-of-function mutations in ARID1A, one of the components in the human SWI/SNF complex have been also reported in various cancers, indicating that ARID1A is a tumor suppressor [22]. Recent exome sequencing studies have revealed that ARID1A is mutated in clear ccRCC [26]. Since several lncRNAs have been reported to interact with the SWI/SNF complex and regulate gene expression or cancer development [46-50] and SMARCB1 and ARID1A have been reported to have significant roles in ccRCC development, we examined HOTAIR interaction with SMARCB1 and ARID1A. We have shown that HOTAIR interacts with SMARCB1 and ARID1A and genistein reduces these interactions (Fig. 5b and Fig. 6e).

Since HOTAIR has been reported to induce SNAIL mRNA [9], we examined whether the HOTAIR/SMARCB1 activates SNAIL transcription. Our experiments using HOTAIR overexpression with SMARCB1 knockdown in the presence of genistein revealed that the HOTAIR/SMARCB1 pathway activates SNAIL transcription in the presence of genistein (Fig. 7) thus our results suggest that the reduced interaction of SMARCB1 with HOTAIR by genistein treatment suppresses SNAIL activation by HOTAIR. SNAIL promoter activity using luciferase reporter assay revealed that SMARCB1 did not activate SNAIL promoter (data not shown), indicating that SMARCB1 indirectly regulates SNAIL transcription.

Conclusion

Genistein inhibits HOTAIR/PRC2 interaction by reducing EED levels in PRC2 and suppresses HOTAIR/PRC2 recruitment to the ZO-1 promoter, thereby enhancing ZO-1 transcription. Genistein reduces HOTAIR/SMARCB1 interaction and inhibit SNAIL transcription. Our results demonstrate that genistein reduces HOTAIR interaction with chromatin remodeling factors, resulting in repression of HOTAIR/chromatin remodeling pathways and kidney cancer suppression. The mechanisms by which genistein inhibits interaction of HOTAIR with PRC2 or the human SWI/SNF subunits await further investigation. Given the diverse functions of genistein in cancers other than RCC, these insights may be extrapolated to a broad spectrum of cancers and aid in the rational design of strategies aimed at overcoming and preventing cancers.

Acknowledgements

We thank Dr. Roger Erickson for support and assistance with the preparation of the manuscript.

This work was supported by National Institutes of Health Grant R01CA196848, RO1CA199694 and UO1CA184966. This study was also supported by Veterans Affairs Program Project BX001604 and Veterans Affairs Merit Review BX001123 grants. Rajvir Dahiya is a Senior Research Career Scientist (BX004473), Department of Veterans Affairs. Mitsuho Imai-Sumida was supported by the Astellas Foundation for Research on Metabolic Disorders fellowship funded by Astellas Pharma, Inc.

Disclosure Statement

The authors declare to have no competing interests.

References

1 Siegel RL, Miller KD, Jemal A: Cancer statistics, 2015. CA Cancer J Clin 2015;65:5-29.
2 Siegel R, Ma J, Zou Z, Jemal A: Cancer statistics, 2014. CA Cancer J Clin 2014;64:9-29.
3 Rouviere O, Bouvier R, Negrier S, Badet L, Lyonnet D: Nonmetastatic renal-cell carcinoma: is it really possible to define rational guidelines for post-treatment follow-up? Nat Clin Pract Oncol 2006;3:200-213.
4 Ravishankar D, Rajora AK, Greco F, Osborn HM: Flavonoids as prospective compounds for anti-cancer therapy. Int J Biochem Cell Biol 2013;45:2821-2831.
5 Meeran SM, Ahmed A, Tollefsbol TO: Epigenetic targets of bioactive dietary components for cancer prevention and therapy. Clin Epigenetics 2010;1:101-116.
6 Wang KC, Chang HY: Molecular mechanisms of long noncoding RNAs. Mol Cell 2011;43:904-914.
7 Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E, Chang HY: Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell 2007;129:1311-1323.
8 Ishibashi M, Kogo R, Shibata K, Sawada G, Takahashi Y, Kurashige J, Akiyoshi S, Sasaki S, Iwaya T, Sudo T, Sugimachi K, Mimori K, Wakabayashi G, Mori M: Clinical significance of the expression of long non-coding RNA HOTAIR in primary hepatocellular carcinoma. Oncol Rep 2013;29:946-950.
9 Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S, Chang HY: Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 2010;464:1071-1076.
10 Kogo R, Shimamura T, Mimori K, Kawahara K, Imoto S, Sudo T, Tanaka F, Shibata K, Suzuki A, Komune S, Miyano S, Mori M: Long noncoding RNA HOTAIR regulates polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers. Cancer Res 2011;71:6320-6326.
11 Kim K, Jutooru I, Chadalapaka G, Johnson G, Frank J, Burghardt R, Kim S, Safe S: HOTAIR is a negative prognostic factor and exhibits pro-oncogenic activity in pancreatic cancer. Oncogene 2013;32:1616-1625.
12 Hajjari M, Salavaty A: HOTAIR: an oncogenic long non-coding RNA in different cancers. Cancer Biol Med 2015;12:1-9.
13 Heubach J, Monsior J, Deenen R, Niegisch G, Szarvas T, Niedworok C, Schulz WA, Hoffmann MJ: The long noncoding RNA HOTAIR has tissue and cell type-dependent effects on HOX gene expression and phenotype of urothelial cancer cells. Mol Cancer 2015;14:108.
14 Bhan A, Mandal SS: LncRNA HOTAIR: A master regulator of chromatin dynamics and cancer. Biochim Biophys Acta 2015;1856:151-164.
15 Chiyomaru T, Fukuhara S, Saini S, Majid S, Deng G, Shahryari V, Chang I, Tanaka Y, Enokida H, Nakagawa M, Dahiya R, Yamamura S: Long non-coding RNA HOTAIR is targeted and regulated by miR-141 in human cancer cells. J Biol Chem 2014;289:12550-12565.
16 Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, Shi Y, Segal E, Chang HY: Long noncoding RNA as modular scaffold of histone modification complexes. Science 2010;329:689-693.
17 Chu C, Qu K, Zhong FL, Artandi SE, Chang HY: Genomic maps of long noncoding RNA occupancy reveal principles of RNA-chromatin interactions. Mol Cell 2011;44:667-678.
18 Li G, Margueron R, Ku M, Chambon P, Bernstein BE, Reinberg D: Jarid2 and PRC2, partners in regulating gene expression. Genes Dev 2010;24:368-380.
19 Pasini D, Cloos PA, Walfridsson J, Olsson L, Bukowski JP, Johansen JV, Bak M, Tommerup N, Rappsilber J, Helin K: JARID2 regulates binding of the Polycomb repressive complex 2 to target genes in ES cells. Nature 2010;464:306-310.
20 Peng JC, Valouev A, Swigut T, Zhang J, Zhao Y, Sidow A, Wysocka J: Jarid2/Jumonji coordinates control of PRC2 enzymatic activity and target gene occupancy in pluripotent cells. Cell 2009;139:1290-1302.
21 Masliah-Planchon J, Bieche I, Guinebretiere JM, Bourdeaut F, Delattre O: SWI/SNF chromatin remodeling and human malignancies. Annu Rev Pathol 2015;10:145-171.
22 Reisman D, Glaros S, Thompson EA: The SWI/SNF complex and cancer. Oncogene 2009;28:1653-1668.
23 Rao Q, Xia QY, Shen Q, Shi SS, Tu P, Shi QL, Zhou XJ: Coexistent loss of INI1 and BRG1 expression in a rhabdoid renal cell carcinoma (RCC): implications for a possible role of SWI/SNF complex in the pathogenesis of RCC. Int J Clin Exp Pathol 2014;7:1782-1787.
24 Cancer Genome Atlas Research N: Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature 2013;499:43-49.

25 Pal SK, Sonpavde G, Agarwal N, Vogelzang NJ, Srinivas S, Haas NB, Signoretti S, McGregor BA, Jones J, Lanman RB, Banks KC, Choueiri TK: Evolution of Circulating Tumor DNA Profile from First-line to Subsequent Therapy in Metastatic Renal Cell Carcinoma. Eur Urol 2017;72:557-564.
26 Park JH, Lee C, Suh JH, Chae JY, Kim HW, Moon KC: Decreased ARID1A expression correlates with poor prognosis of clear cell renal cell carcinoma. Hum Pathol 2015;46:454-460.
27 Jiang W, Dulaimi E, Devarajan K, Parsons T, Wang Q, O'Neill R, Solomides C, Peiper SC, Testa JR, Uzzo R, Yang H: Intratumoral heterogeneity analysis reveals hidden associations between protein expression losses and patient survival in clear cell renal cell carcinoma. Oncotarget 2017;8:37423-37434.
28 Imai-Sumida M, Chiyomaru T, Majid S, Saini S, Nip H, Dahiya R, Tanaka Y, Yamamura S: Silibinin suppresses bladder cancer through down-regulation of actin cytoskeleton and PI3K/Akt signaling pathways. Oncotarget 2017;8:92032-92042.
29 Bertrand E, Chartrand P, Schaefer M, Shenoy SM, Singer RH, Long RM: Localization of ASH1 mRNA particles in living yeast. Mol Cell 1998;2:437-445.
30 Macias S, Bragulat M, Tardiff DF, Vilardell J: L30 binds the nascent RPL30 transcript to repress U2 snRNP recruitment. Mol Cell 2008;30:732-742.
31 Bedi U, Mishra VK, Wasilewski D, Scheel C, Johnsen SA: Epigenetic plasticity: a central regulator of epithelial-to-mesenchymal transition in cancer. Oncotarget 2014;5:2016-2029.
32 Tam WL, Weinberg RA: The epigenetics of epithelial-mesenchymal plasticity in cancer. Nat Med 2013;19:1438-1449.
33 Helming KC, Wang X, Roberts CWM: Vulnerabilities of mutant SWI/SNF complexes in cancer. Cancer Cell 2014;26:309-317.
34 Armaos A, Cirillo D, Gaetano Tartaglia G: omiXcore: a web server for prediction of protein interactions with large RNA. Bioinformatics 2017;33:3104-3106.
35 Li S, Yamashita K, Amada KM, Standley DM: Quantifying sequence and structural features of protein-RNA interactions. Nucleic Acids Res 2014;42:10086-10098.
36 Hirata H, Ueno K, Nakajima K, Tabatabai ZL, Hinoda Y, Ishii N, Dahiya R: Genistein downregulates onco-miR-1260b and inhibits Wnt-signalling in renal cancer cells. Br J Cancer 2013;108:2070-2078.
37 Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, Thomas K, Presser A, Bernstein BE, van Oudenaarden A, Regev A, Lander ES, Rinn JL: Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proc Natl Acad Sci U S A 2009;106:11667-11672.
38 Koziol MJ, Rinn JL: RNA traffic control of chromatin complexes. Curr Opin Genet Dev 2010;20:142-148.
39 Davidovich C, Zheng L, Goodrich KJ, Cech TR: Promiscuous RNA binding by Polycomb repressive complex 2. Nat Struct Mol Biol 2013;20:1250-1257.
40 Furuse M, Itoh M, Hirase T, Nagafuchi A, Yonemura S, Tsukita S, Tsukita S: Direct association of occludin with ZO-1 and its possible involvement in the localization of occludin at tight junctions. J Cell Biol 1994;127:1617-1626.
41 Itoh M, Nagafuchi A, Moroi S, Tsukita S: Involvement of ZO-1 in cadherin-based cell adhesion through its direct binding to alpha catenin and actin filaments. J Cell Biol 1997;138:181-192.
42 Wu L, Murat P, Matak-Vinkovic D, Murrell A, Balasubramanian S: Binding interactions between long noncoding RNA HOTAIR and PRC2 proteins. Biochemistry 2013;52:9519-9527.
43 Hodges C, Kirkland JG, Crabtree GR: The Many Roles of BAF (mSWI/SNF) and PBAF Complexes in Cancer. Cold Spring Harb Perspect Med 2016;6:pii:a026930.
44 Cheng SW, Davies KP, Yung E, Beltran RJ, Yu J, Kalpana GV: c-MYC interacts with INI1/hSNF5 and requires the SWI/SNF complex for transactivation function. Nat Genet 1999;22:102-105.
45 Stojanova A, Tu WB, Ponzielli R, Kotlyar M, Chan PK, Boutros PC, Khosravi F, Jurisica I, Raught B, Penn LZ: MYC interaction with the tumor suppressive SWI/SNF complex member INI1 regulates transcription and cellular transformation. Cell Cycle 2016;15:1693-1705.
46 Prensner JR, Iyer MK, Sahu A, Asangani IA, Cao Q, Patel L, Vergara IA, Davicioni E, Erho N, Ghadessi M, Jenkins RB, Triche TJ, Malik R, Bedenis R, McGregor N, Ma T, Chen W, Han S, Jing X, Cao X, et al.: The long noncoding RNA SChLAP1 promotes aggressive prostate cancer and antagonizes the SWI/SNF complex. Nat Genet 2013;45:1392-1398.
47 Wang X, Gong Y, Jin B, Wu C, Yang J, Wang L, Zhang Z, Mao Z: Long non-coding RNA urothelial carcinoma associated 1 induces cell replication by inhibiting BRG1 in 5637 cells. Oncol Rep 2014;32:1281-1290.

48 Kawaguchi T, Tanigawa A, Naganuma T, Ohkawa Y, Souquere S, Pierron G, Hirose T: SWI/SNF chromatin-remodeling complexes function in noncoding RNA-dependent assembly of nuclear bodies. Proc Natl Acad Sci U S A 2015;112:4304-4309.

49 Cajigas I, Leib DE, Cochrane J, Luo H, Swyter KR, Chen S, Clark BS, Thompson J, Yates JR, 3rd, Kingston RE, Kohtz JD: Evf2 lncRNA/BRG1/DLX1 interactions reveal RNA-dependent inhibition of chromatin remodeling. Development 2015;142:2641-2652.

50 Chen Z, Gao Y, Yao L, Liu Y, Huang L, Yan Z, Zhao W, Zhu P, Weng H: LncFZD6 initiates Wnt/beta-catenin and liver TIC self-renewal through BRG1-mediated FZD6 transcriptional activation. Oncogene 2018;37:3098-3112.